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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ABSTRACT:

Sympatric species are often locally adapted to distinct microhabitats. However, temporal variation may cause local maladaptation and species boundary breakdown, especially during extreme climatic events leading to episodic selection. Repeated reciprocal transplants can reveal the interplay between 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 drastically differing snowpack. If there is ecological isolation, then we predicted divergent phenotypic selection between habitats in line with species' differences and local adaptation. We found interannual fluctuations in phenotypic selection, often in unpredicted directions. However, a combined-year analysis detected longer-term divergent selection on flowering time, a key temporally isolating and adaptative trait, suggesting that selection may reinforce species boundaries despite short-term fluctuations. Finally, we found temporal variation in local adaptation with *M. laciniatus* locally adapted in low snowpack years, while an extremely high snowpack year contributed to average local maladaptation of *M. guttatus*.

INTRODUCTION

Closely related species often occupy distinct habitats within areas of geographic overlap (Coyne & Orr, 2004; Rundle & Nosil, 2005; Schluter, 2009). Species boundaries in sympatry can be maintained by divergent adaptation to differing environments that generate pre- and/or post-zygotic isolation via selection against immigrants (e.g. habitat isolation) and intermediate hybrids (e.g. extrinsic post-zygotic isolation; Rundle & Nosil, 2005; Coyne & Orr 2004). However, identifying the individual traits that contribute to reproductive isolation is difficult because as speciation progresses, multiple forms of reproductive isolation accumulate between divergent lineages which hinders the measurement of individual barriers (Coyne & Orr, 2004). Once species are partially isolated, neutral processes also cause the accumulation of phenotypic divergence. Therefore, 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 can identify whether divergent adaptation is important for reproductive isolation and speciation (Lowry *et al.*, 2008; Anderson *et al.*, 2015). 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.

Despite these predictions, asymmetry in local adaptation, or maladaptation to native habitats, is often observed in reciprocal transplants (Hereford, 2009; Kooyers *et al.*, 2019; Dittmar & Schemske, 2023). This could be due to the prevalence of temporal fluctuations in selection (Siepielski *et al.*, 2009) which can influence the strength of reproductive isolation and species boundaries (Tataru *et al.*, 2023). Additionally, current mean environmental conditions may be different than the conditions during original divergence (Coyne & Orr, 2004; Rundle & Nosil, 2005). If species are no longer adapted to their current 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.

Variation in selection in longitudinal studies is usually measured on a seasonal or inter-annual timescale, a short-term measurement of population evolution. This leads to the question of how short-term fluctuations affect long-term evolutionary change in species and populations (Wadgymar *et al.*, 2017; Dittmar & Schemske, 2023). 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 directional or stabilizing trend over a long enough period of time. For example, 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. Due to the arduousness of temporally replicating reciprocal transplant experiments, it is not currently well understood how

short versus longer-term patterns of natural selection compare and impact species' divergence and reproductive isolation.

Another understudied agent of evolutionary change is episodic selection resulting from sudden and extreme environmental events. Long-term studies which capture the evolutionary responses to these dramatic ecological changes are rare, yet critical for understanding the maintenance of species boundaries (Coltman *et al.*, 1999; Pemberton *et al.*, 2022). 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 can impact biodiversity by affecting the long-term evolutionary trajectory of species adaptation and reproductive isolation (Grant & Grant, 1993). Extreme climatic events are predicted to become more frequent with continuing anthropogenic climate change (Bailey & van de Pol, 2016).

The *Mimulus guttatus* species complex, a group of closely related wildflowers, is well-suited for studying the effect 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 nearby rocky granite outcrops (Ferris *et al.*, 2014; Ferris & Willis, 2018). Rocky habitats are harsh with shallow soils, high light intensity, extreme temperatures, and a short growing season (Ferris *et al.*, 2014). *Mimulus laciniatus* has adapted to its granite habitat with smaller plant size, lobed leaves, self-fertilizing mating system with reduced floral size, and early rapid flowering (DeMarche *et al.*, 2013; Ferris & Willis, 2018). Typical *M. guttatus* populations are later-flowering, predominantly outcrossing, round-leaved, and have larger plant sizes and flowers (Awadalla & Ritland, 1997; Ferris *et al.*, 2014). Early flowering time is a drought escape strategy and acts as a key pre-mating 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). Plant taxa adapted to marginal habitats often have self-fertilizing mating systems which can offer reproductive assurance in a harsh environment where pollinators may be scarce (Wright *et al.*,

2013). Additionally self-fertilizing plants often have small flowers meaning less water is lost through evapotranspiration in floral tissue (Galen, 2000). Lobed leaves may be able to better regulate temperature and water loss through a reduced boundary layer (Nicotra *et al.*, 2011) and are unique to *Mimulus* species found in rocky habitat (Ferris *et al.*, 2014; Ferris & Willis, 2018; Ferris, 2019).

Here, we investigate spatial and temporal variation in natural selection on interspecific divergence between *Mimulus guttatus* and *M. laciniatus* with data from five years of repeated reciprocal transplant experiments under dramatically varying climatic 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) Does the strength and direction of selection on adaptive and reproductively isolating phenotypes fluctuate? (2) How do short versus long-term patterns of divergence compare? (3) Are *M. guttatus* and *M. laciniatus* locally adapted? (4) 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 were able to elucidate the effects of a changing climate on the maintenance of biodiversity in this system.

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). Advanced generation hybrids were used because recombination over multiple generations breaks up parental haplotypes and enables us to make inferences about selection on individual phenotypes (Nagy, 1997; Hall & Willis, 2006; Groh & Coop, 2024). 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 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 was 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). Further, we recorded survival, herbivory (presence or absence), and adaptive phenotypes previously identified to be under divergent selection (Ferris *et al.*, 2014; Ferris & Willis, 2018; Tataru *et al.*, 2023). 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. We found that selection gradients β values derived from fruit number (2013) were similar to those from seed number (all other years) in both strength and direction (Table S5 and S6) likely due to a strong positive correlation between seed and fruit number (Table S7). Therefore we compare fruit and seed number derived selection gradients across years. 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 included previously published data from two additional years (Ferris & Willis, 2018; Tataru *et al.*, 2023) and averaged survival, fecundity, and total fitness across all

years (2013, 2019, 2021, 2022, 2023) for each habitat 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

Environmental variation causes spatial and temporal variation in survival

Fine-scale environmental variation was significantly tied to survival trends in both habitats across years and fluctuated both spatially between habitats as well as temporally with snowpack (Figure 2, Table S2). Soil moisture availability throughout the season varies significantly between *M. laciniatus's* granite and *M. guttatus's* meadow habitat in each year with moisture in granite outcrops declining rapidly after an early season plateau, while in meadows there is a gradual, linear decline throughout the growing season. The amount of soil moisture available and the exact shape of the soil moisture curve fluctuated from year to year with higher snowpack years (2019, 2023) showing shallower declines in soil moisture over time in each habitat (Figure

2). Light intensity and temperature are higher on average in *M. laciniatus's* outcrop environment except in the extreme high snowpack year of 2023 where temperatures were cooler than usual and similar between habitats throughout the growing season (Figure 2).

Across all years and habitats soil moisture and time (day) were significantly associated with survival, with decreasing soil moisture over time associated with a corresponding decrease in proportion of plants surviving ($p < 0.001$). In *Mimulus guttatus*'s meadows, light intensity ($p < 0.001$) also significantly affected survival in 2021 and 2022, while soil temperature was impactful in 2022 and 2023 ($p < 0.001$; Table S2). Higher temperatures and light intensities were associated with lower survival. In *M. laciniatus's* granite habitat time, soil moisture, light and temperature all impacted survival in the same directions as described for *M. guttatus's* meadow habitat in all three recent years ($p < 0.0001$; Table S2). In 2023, the exceptionally high snowpack year, temperature but not light significantly affected survival ($p = 0.5672$). Across habitats in 2023, light was not associated with survival. In summary, 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. Further, 2021–2023 differed from 2019 where soil moisture was the only significant environmental factor (Tataru *et al.*, 2023). Light and temperature were not measured in 2013 (Ferris & Willis, 2018). Similar to 2013 and 2019, herbivory pressure differed significantly between habitats with greater herbivory in *M. guttatus'*s meadows in 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). However, only in 2021 did herbivory and the interaction of herbivory x site significantly affect total fitness in the best-fit ANOVA model (Table S3). Overall, herbivore pressure varied spatially, but its effect on fitness varied temporally.

An extreme climatic event dramatically shifted the growing season

The extreme high snowpack year (2023) differed from other years in several aspects. 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 (Figure 2a,b). Soil moisture declined more gradually than usual in both habitats, while light and surface

temperatures were lower (Figure 2c,d). Light intensity was notably lower in both meadow habitats (Figure 2e) and Granite 2 (Figure 2f). In granite in 2023, there was no characteristic temperature spike usually coinciding with mortality (Figure 2h). 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 2a). Of the individuals still alive in Meadow 1, many had not flowered indicating a significant life-history delay due to this extreme environmental event (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 36-70 days longer than previous years in granite habitats (Granite 2013-2022: 33-87 days; 2023: 112-113 days) but comparable to other years in meadows (Meadow 2013-2022: 74-160 days; 2023: 119-146 days).

Interannually fluctuation versus long-term directional selection

We found temporal and spatial fluctuations in the strength and direction of selection on each quantitative trait in both our negative binomial (any versus zero seeds) and zero-truncated Poisson (seed number excluding zero values) selection analyses (Figure 3). Based on observed trait differences between species, we predicted selection in *M. laciniatus's* granite habitat for early flowering time, smaller flowers, increased leaf lobing, shorter plants, and decreased stigma-anther distance (herkogamy), with the inverse direction of selection in *M. guttatus's* meadows (Figure 3a-e). While there was divergent selection between habitats in line with the direction of species differences for all traits except flower size in the historic drought of 2013, this was not the case in the other years (Figure 3). Instead, phenotypic selection was frequently in the opposite direction of expectations. The exceptions to this were flowering time in our negative binomial analysis (Figure 3f) and herkogamy (stigma-anther distance) in our zero-truncated Poisson models (Figure 3o). As predicted, there was stronger selection for early flowering in *M. laciniatus's* granite habitat in every year but one (Figure 3f, Table 1) and divergent habitat selection on herkogamy with selection for reduced stigma-anther separation in granite in most years (Figure 3o) which matches the native *M. lacinaitus's* self-fertilizing mating system. Generally, we found that selection gradients (β) based on seed number (zero-truncated Poisson) were stronger than those derived in our negative binomial analyses (selection on qualitative seed

production; Figure 3; Tables 1, 2). For many traits (leaf shape, plant height, herkogamy) selection was more temporally stable in *M. guttatus's* meadow habitat than in *M. laciniatus's* harsh rocky outcrops (Figure 3m,n,o). Additionally, we found the strongest signatures of phenotypic selection in the extreme high snowpack year (2023) relative to other years which confirms our predictions that this year represents an episodic selection event (e.g. flower width, leaf lobing, plant height, herkogamy; Figure 3; Tables 1, 2).

We saw that temporal fluctuation in selection was associated with variation in snowpack for some traits. For example, in the high snowpack years (2019 and 2023), selection on flowering time was reversed from our predictions with later flowering increasing fitness in granite whereas earlier flowering was advantageous in meadows (Figure 3k, Table 2, S4). For other traits such as flower width, we found that selection was more variable and unpredictable based on snowpack level. There was generally selection for larger flowers with the strongest selection for larger flowers in granite habitat in 2023, contrary to predictions (Figures 3g, l). In fact, the high snowpack and delayed growing season of 2023 (Figure 2) seemed to cause not only atypically strong selection but also selection in unexpected directions, particularly in *M. laciniatus's* granite habitat where there was weak selection for later flowering (Figure 3k), strong selection against leaf lobing (Figure 3m), for taller plants (Figure 3n), and bigger flowers (Figure 3l) in that year.

Although we found interannual fluctuations in the strength and direction of selection on all traits, when we combined data across years we saw long term patterns of directional selection (Tables 1, 2; Figure 4, S5). In both zero-truncated Poisson and negative binomial analyses, we found stronger long-term selection for earlier flowering time and taller plants in granite, selection for smaller flowers in both habitats, and long-term selection for more lobed leaves in granite with negligible selection on leaf shape in meadows. Herkogamy was not included in the combined dataset due to its absence in 2013. Therefore, while inter-annual fluctuations in the strength and direction of selection are common in this system, we still find longer-term divergent selection in the direction of species' differences in both flowering time and leaf shape.

Phenotypes are adaptively plastic between habitats

Parental genotypes exhibited plasticity in phenotypic traits between habitats, with the non-native species exhibiting phenotypic plasticity in the direction of the local species' phenotype. 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 S8, S9, S10). These results are similar to those from 2013 with plasticity in every trait except *M. laciniatus*'s flower size, in the direction of the local species' phenotype (Ferris & Willis 2018). In its nonnative granite habitat, *M. guttatus* had significantly shorter mean flowering time and smaller plant height (2021 and 2023) and significantly smaller leaf area and increased leaf lobing like *M. laciniatus* (2022 and 2023; Tables S8, S9, S10). 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 the direction of the *M. laciniatus* (Tables S8, S9, S10). In 2019, hybrids in granite differed by having larger flowers and larger leaf area relative to meadow (Tataru et al. 2023). Overall, an earlier mean flowering time in granite is common throughout years (2013–2023) across hybrids and parents.

Local adaptation fluctuates temporally with long-term maladaptation of M. guttatus

We found temporal fluctuation in the signature of local adaptation between *M. laciniatus* and *M. guttatus* parental genotypes. In 2013 we found a clear signature of local adaptation in total fitness, but this was not true for other years in our study (Figure 5). There was also often a difference in local adaptation between the individual components of fitness: survival and fecundity. *Mimulus laciniatus* had higher survival to flowering than *M. guttatus* in both habitats in all years, except in 2019 when there was low overall survival of parental genotypes (Figure 5a-e). With all five years combined, *M. laciniatus* had higher average survival in both habitats (Figure 5f). 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 5g-k). However, in 2022, *M. laciniatus* had higher fecundity in both habitats (Figure 5j), and in 2023, we found reciprocal local maladaptation where *M. guttatus* had higher fecundity than *M. laciniatus* in granite and vice versa in meadows (Figure 5k). With all years combined, we found overall evidence of local maladaptation in mean fecundity (Figure 5l); 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. guttatus* did equally well in granite in high snowpack years (2019

and 2023; Figure 5n, q), but in low snowpack years *M. laciniatus* had higher fitness and home-site advantage (2013, 2021, 2022; Figure 5m, o, d). Total fitness in meadow was not as predictable based on snowpack with *M. laciniatus* having higher total fitness across recent years (2021-2023) but lower fitness in 2013 and 2019. Overall, *M. laciniatus* had higher total fitness in both habitats (Figure 5r, S1).

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. 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. Our results suggest that local adaptation fluctuates across years but on average, *M. guttatus* is maladapted to its meadows and that habitat isolation may therefore be asymmetric. Finally, we find that episodic selection, caused by an extreme snowfall event, may disrupt local adaptation and impact species boundaries.

Inter-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 3). 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 3). 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 3).

Annual selection fluctuated unpredictably for flower width with combined selection for smaller flowers (Figure 3; Tables 1, 2), 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 (Tables 1, 2). 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 S8, S9, S10), 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 (Tables 1, 2; Figures 4, 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).

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; Kawecki & Ebert 2004) 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 that local adaptation fluctuated temporally (Figure 5m-q) with evidence of average local maladaptation of *M. guttatus* (Figure 5r). Annual fluctuations in snowpack was predictive of relative fitness of the parent species in granite with *M. laciniatus* and *M. gutta*tus performing equally well in high snowpack years (5n,q), but in low snowpack years the drought adapted-*M. laciniatus* had the home-site advantage (Figure 5m,o,p).

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). For example, in his classic metanalysis Hereford (2009) found that 29% of reciprocal transplant studies failed to detect local adaptation. It is possible that our reciprocal transplant did not detect existing trade-offs due to logistical constraints or that there are weak to no fitness trade-offs associated with adaptation for *M. laciniatus* across environments (Fry, 1996; Leimu & Fischer, 2008). Alternatively, an increase in dramatic interannual environmental fluctuations due to anthropogenic climate change may mean that *M. guttatus* is no longer 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 fluctuating 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 lead to population extinction.

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). In a repeated reciprocal transplant between serpentine and sandstone adapted populations of *Leptosiphon parviflorus* Dittmar & Schemske (2023) found that while serpentine populations had a temporally consistent local fitness advantage, sandstone populations only had a local advantage in two out of four years. While stable spatial heterogeneity should favor the evolution of local adaptation, temporal fluctuation in the environment should select against local specialization and for phenotypic plasticity (Kawecki & Ebert 2004). The average fitness advantage of *M. laciniatus* across environments could be due to adaptive 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 S8, S9, S10). *Mimulus laciniatus's* occupation of a harsh, highly variable environment (Figures 2 & 3) 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, at least in the traits we measured, is unlikely to account for *M. laciniatus's* performance advantage across habitats and the lack of fitness trade-offs.

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 5j), 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 throughout the season 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. Although *M. guttatus* still had lower survival than *M. laciniatus*, it had remarkably high fecundity (Figure 5e, k) resulting in similar total fitness between the two species in granite which was unprecedented in previous years (Figure 5q). 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 3). 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; Dittmar & Schemske, 2023; Oakley *et al.*, 2023).

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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.

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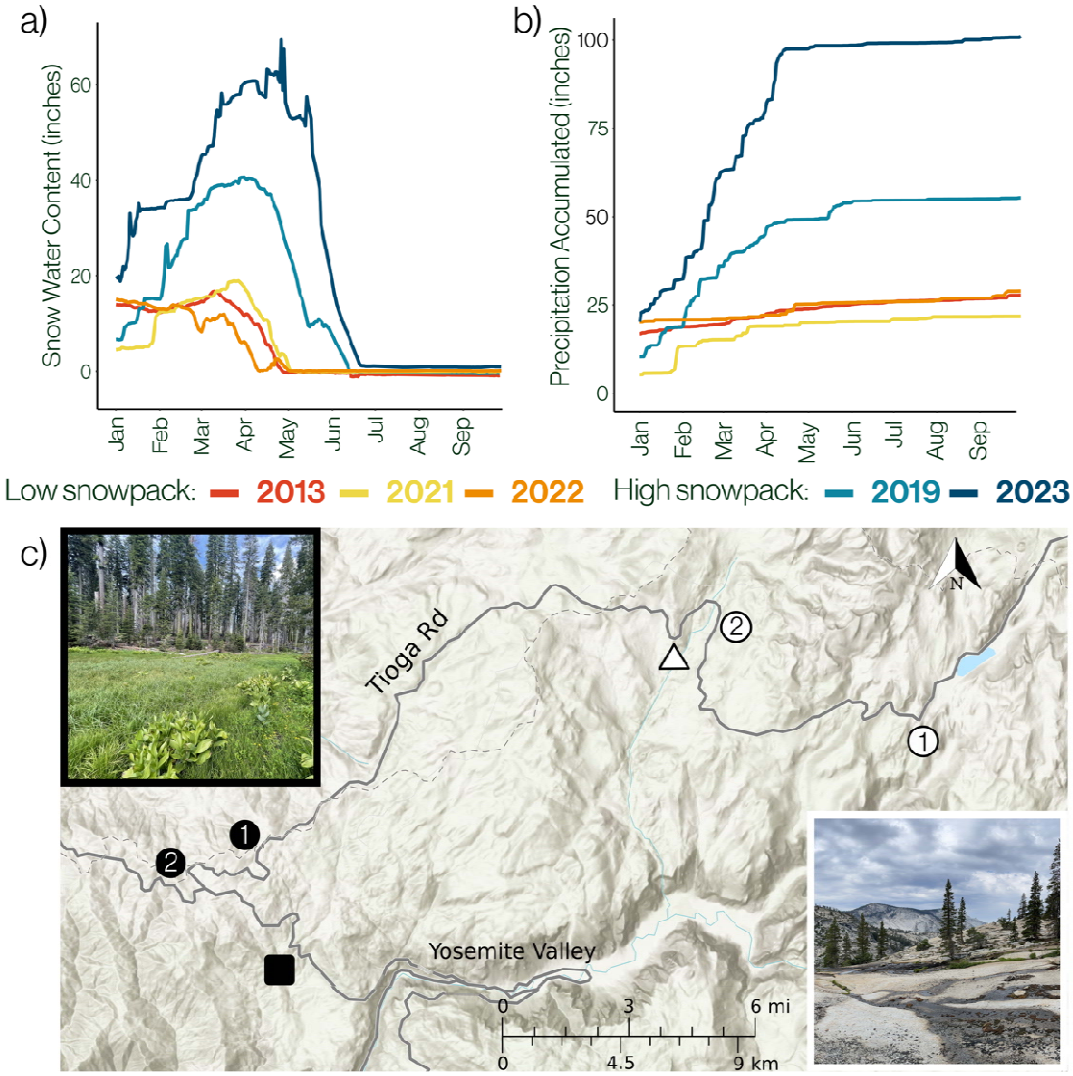


Figure 1. Temporal and spatial scale of the experimental design. Annual changes in a) snow water content and b) accumulated precipitation during experimental years of low snowpack (2013, 2021, 2022) and comparatively higher snowpack (2019 and 2023) with the c) spatial

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).

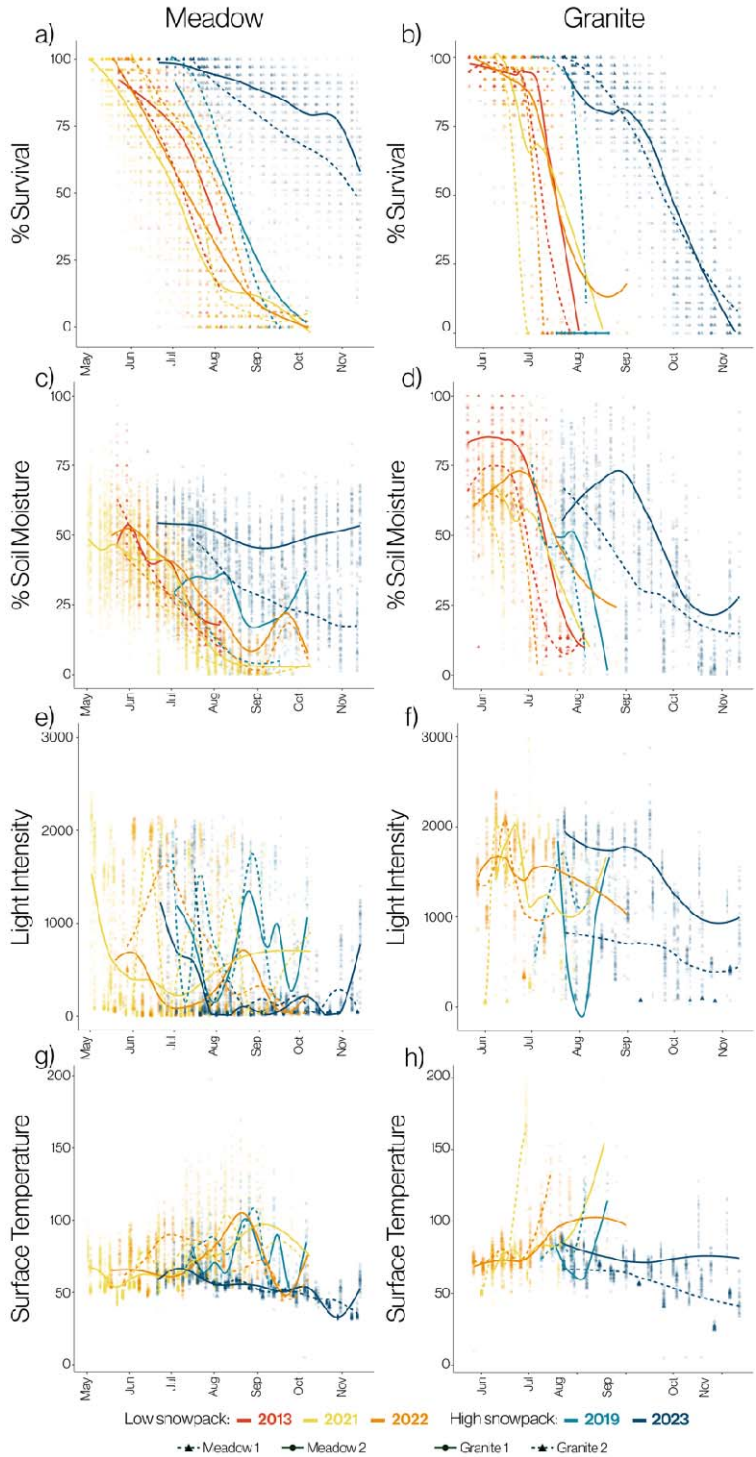


Figure 2. Seasonal changes in (a,b) survival (%), (c,d) soil moisture (%), (e,f) light intensity (µmol m-2 s-1), and (g,h) soil surface temperature (°F) in each block per site in meadow and granite habitats in low snowpack years (2013, 2021, 2022) and high snowpack years (2019, 2023). Data from 2013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

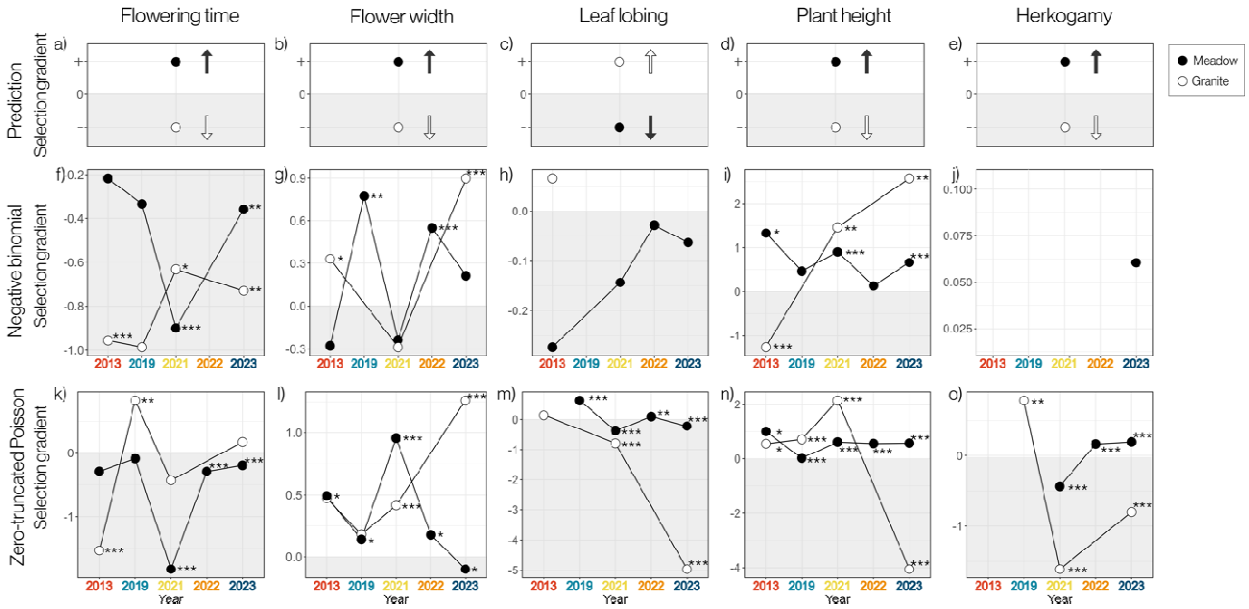


Figure 3. Visual representation of selection gradients (β) of phenotypic traits measured for hybrids during experimental years in granite (open circles) and meadow (filled circles) habitats. Shown are (a-e) predicted values in each habitat based on native species trait differences and estimated values from (f-j) negative binomial models and (k-o) 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 from Ferris and Willis 2018 and Tataru *et al.* 2023.

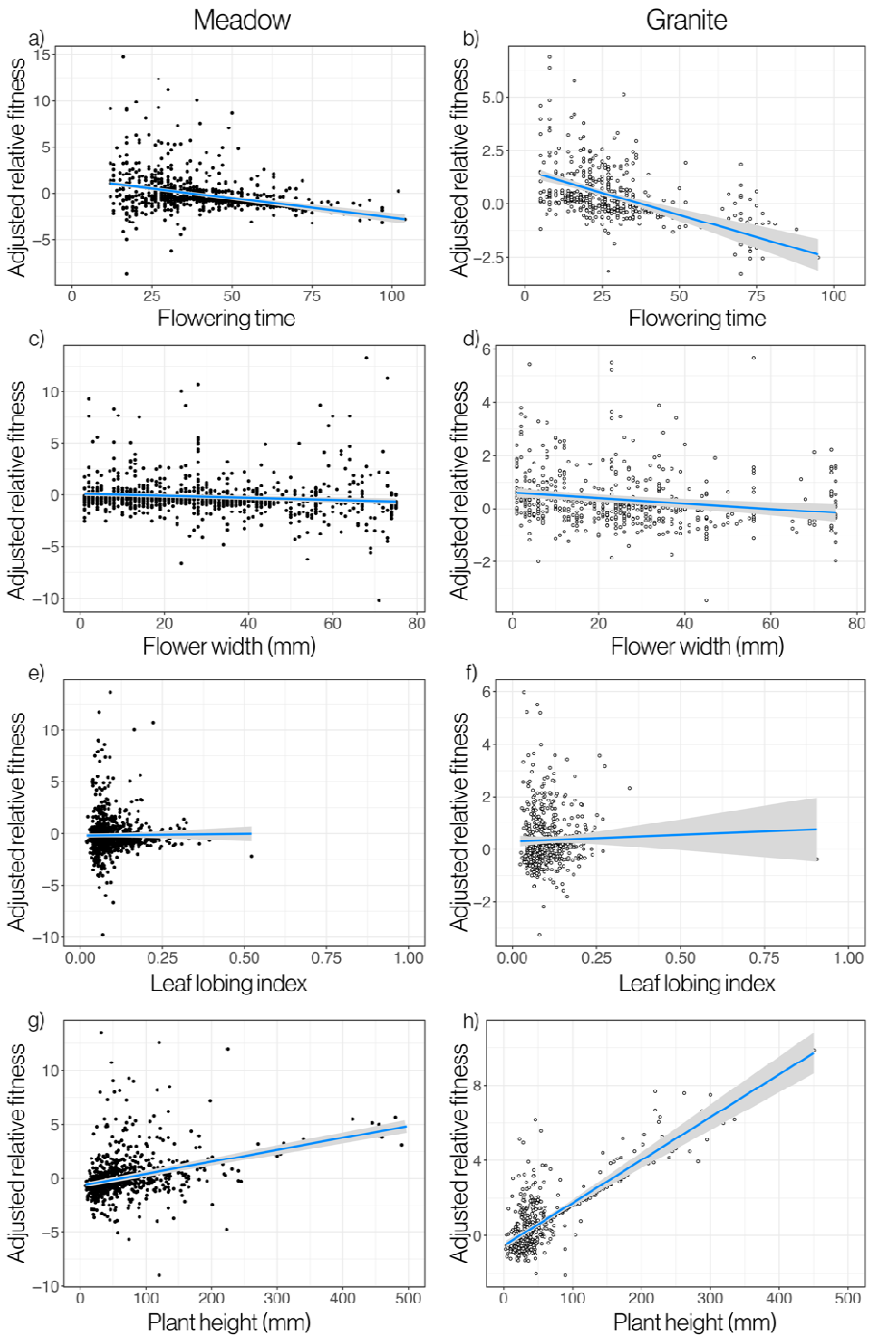


Figure 4. Patterns of cumulative selection with all years combined in meadow (left; filled circles) and granite (right; open circles) habitat in a zero-truncated Poisson analysis on (a,b) flowering time (days from planting), (c,d) width of first flower (mm), (e,f) leaf lobing index, and (g,h) plant height 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 from Ferris and Willis 2018 and Tataru *et al.* 2023 and re-analyzed here.

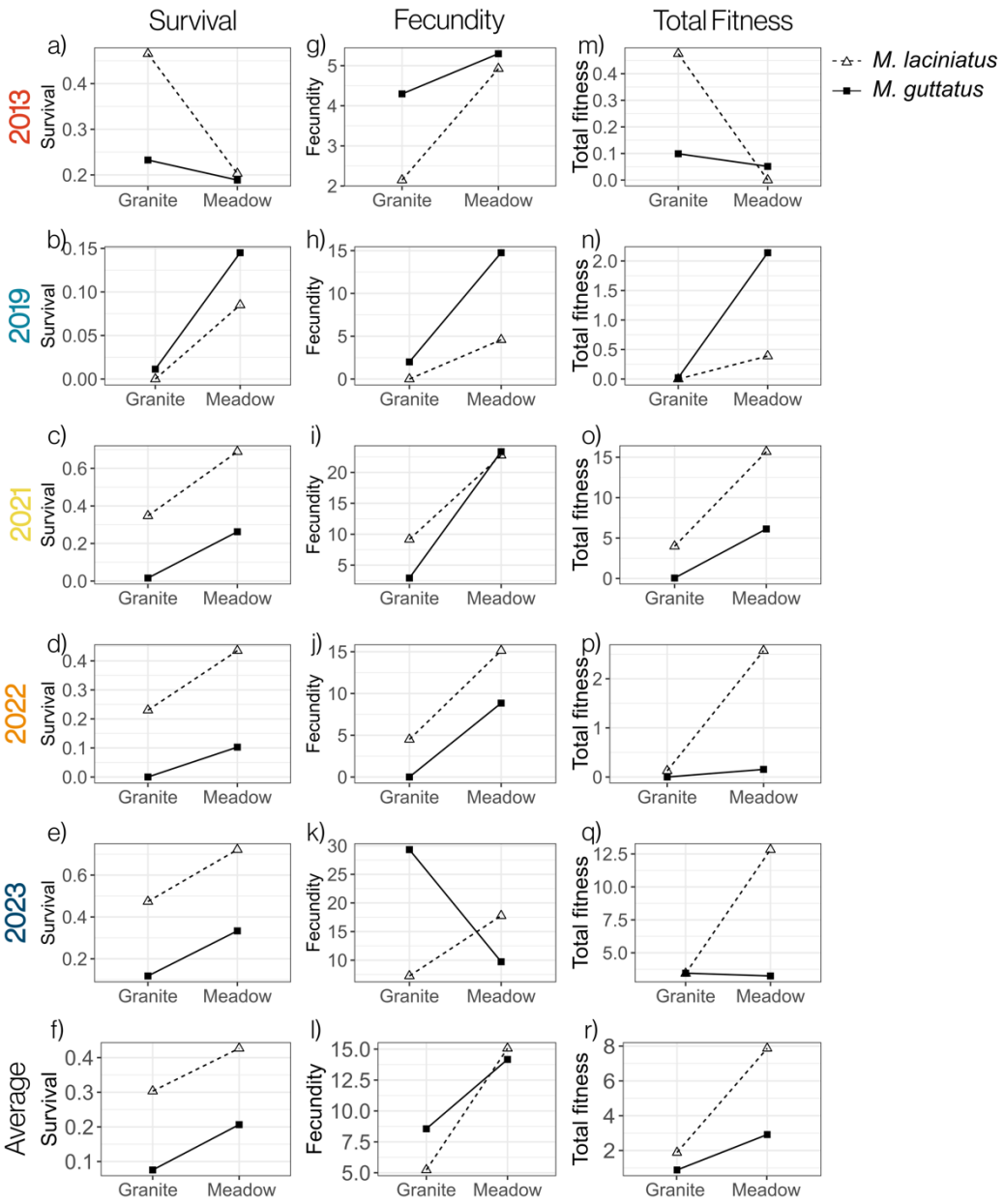


Figure 5. Survival to flowering (a-f), fecundity (mean seed number per reproductive individual; g-l), and total fitness (mean seed number per planted seed; m-r) of *Mimulus guttatus* (filled square, solid line) and *M. laciniatus* (open triangle, dashed line) in granite and meadow habitats, from each experimental year and averaged across years (f, l, r). 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 from Ferris and Willis 2018 and Tataru *et al*. 2023.

Trait	Meadow				Granite		
	2021	**2022**	**2023**	**Combined**	**2021**	**2023**	**Combined**
Flowering time (FT)	-0.900***	-	-0.35761**	-0.42795***	-0.6318*	-0.7304**	-0.80732***
Flower width (FW)	-0.2393	0.551***	0.21311	-0.06862*	-0.2882	0.8929***	-0.09141**
Leaf lobing (LL)	-0.143	-0.028	-0.06197	-	-	-	-
Herkogamy (H)	-	-	0.06031	-	-	-	-
Plant height (PH)	0.903***	0.137	0.66220***	0.60214***	1.461**	2.5706**	0.85300***
FW x PH	-0.291*	-	-0.25121*	-	-1.057	-	0.11827**
FT x H	-	-	0.23698*	-		-	-
LL x PH	-0.232	0.096*	-	-		-	-
FT x PH	-	-	-	-0.14995***	-	-	-0.56967***
FW x LL	-	-	-0.48341**	-	-	-	-
H x PH	-	-	-	-	-	-	-
FT x FW	-	-	-	-0.09303*	-	-	-
n =	200	258	775	2014	166	214	992

Table 1. Negative binomial analysis on whether or not a plant produced seeds on individual experimental years 2021-2023 and on a combined dataset of 2013, 2019, 2021, 2022, and 2023. 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. Herkogamy was excluded to from the combined analysis 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.

Trait	Meadow				Granite		
	2021	**2022**	**2023**	**Combined**	**2021**	**2023**	**Combined**
Flowering time (FT)	-1.827***	-0.289***	-0.19540***	-1.08156***	-0.432	0.1733	-1.12177***
Flower width (FW)	0.957***	0.178*	-0.09655*	-0.23893***	0.415***	1.2539***	-0.19218*
Leaf lobing (LL)	-0.374***	0.094**	-0.21639***	0.05601	-0.794***	-4.9779***	0.19775
Herkogamy (H)	-0.436***	0.167***	0.19504***	-	-1.610***	-0.8008***	-
Plant height (PH)	0.615***	0.563***	0.57737***	0.96239***	2.15***	-4.0555***	1.05603***
FT x FW	1.964***	0.542***	0.31703***	-0.21770***	-	-0.8541***	-0.16277
FT x H	-0.956***	-0.314***	0.31146***	-	-2.022***	-	-
FT x LL	-	-	-	-	-	-	-0.30463**
FT x PH	-0.651***	-0.420***	-0.09749*	-0.11394	1.110*	-	-0.34790**
FW x LL	0.715***	-	-0.42251***	-0.05836	-	1.6669***	0.09612
FW x PH	-0.601***	-0.154***	-0.12595***	0.07574	-	-1.6953***	0.19489*
LL x PH	-0.186***	-	0.24088***	0.03987	-1.517***	-8.0169***	0.47229
H x PH	0.401***	0.072**	-0.07153***	-	0.769***	-1.9776***	-
FT x FW x LL	-	-	-	-	-	-	0.27679**
FT x LL x PH	-	-	-	-	-	-	-0.22341*
FT x FW x PH	-	-	-	0.10875*	-	-	-
FW x LL x PH	-	-	-	-0.16163*	-	-	-0.30395***
n =	128	134	202	998	87	51	547

Table 2. Zero-truncated Poisson analysis, using number of seeds produced if any, on individual experimental years 2021-2023 and on a combined dataset of 2013, 2019, 2021, 2022, and 2023. 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 to from the combined analysis 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.

Table S8. Trait means, standard error, standard deviation, and coefficient of variation from 2021.

Table S9. Trait means, standard error, standard deviation, and coefficient of variation from 2022.

Table S10. Trait means, standard error, standard deviation, and coefficient of variation from 2023.

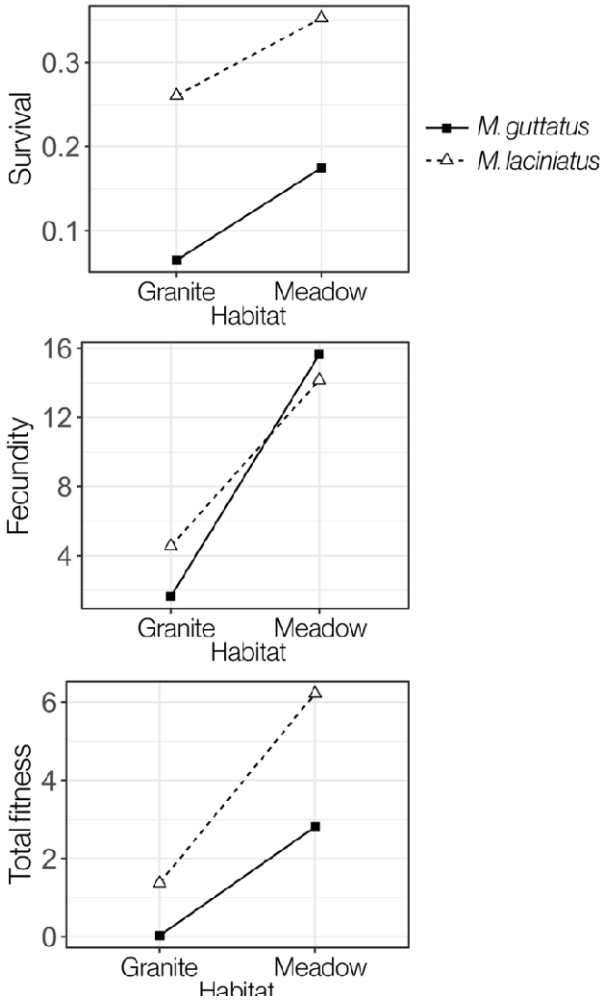


Figure S1. Combined survival to flowering, fecundity (mean seed number per reproductive individual), and total fitness (mean seed number per planted seed) of *Mimulus guttatus* (filled square, solid line) and *M. laciniatus* (open triangle, dashed line) in granite and meadow habitat from 2013-2022 (excluding 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 from Ferris and Willis 2018 and Tataru *et al.* 2023.

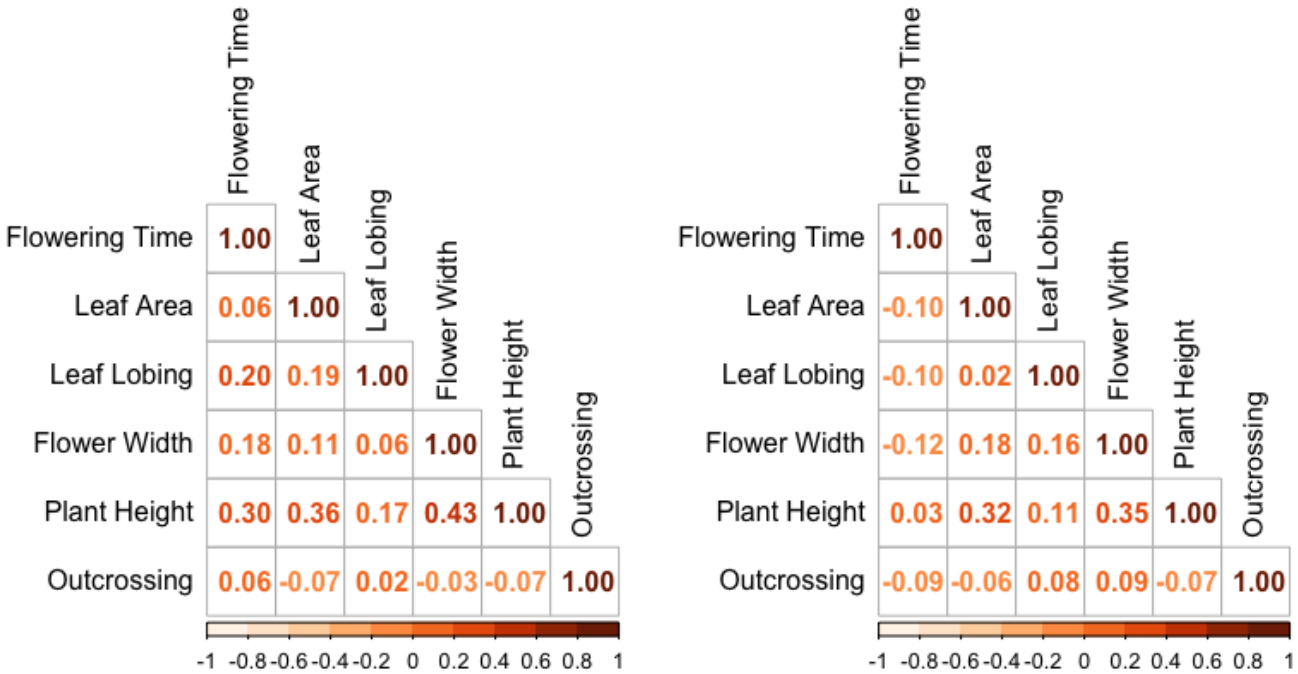
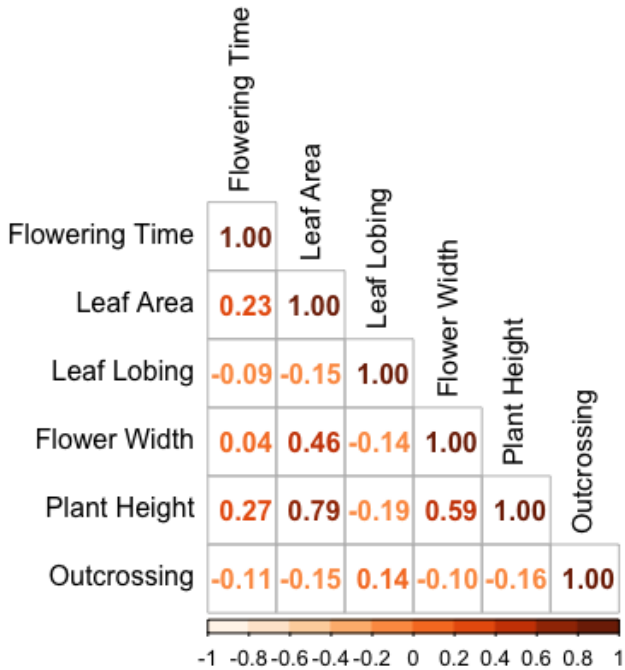


Figure S2. Trait correlation matrices for F2 hybrids in 2021 in meadow (left) and granite (right) habitats.



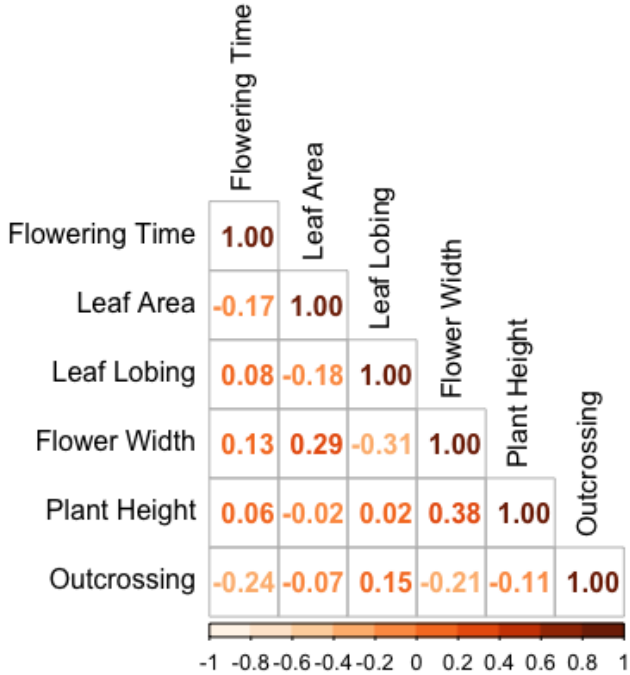


Figure S3. Trait correlation matrices for F3 hybrids in 2022 in meadow (left) and granite (right) habitats.

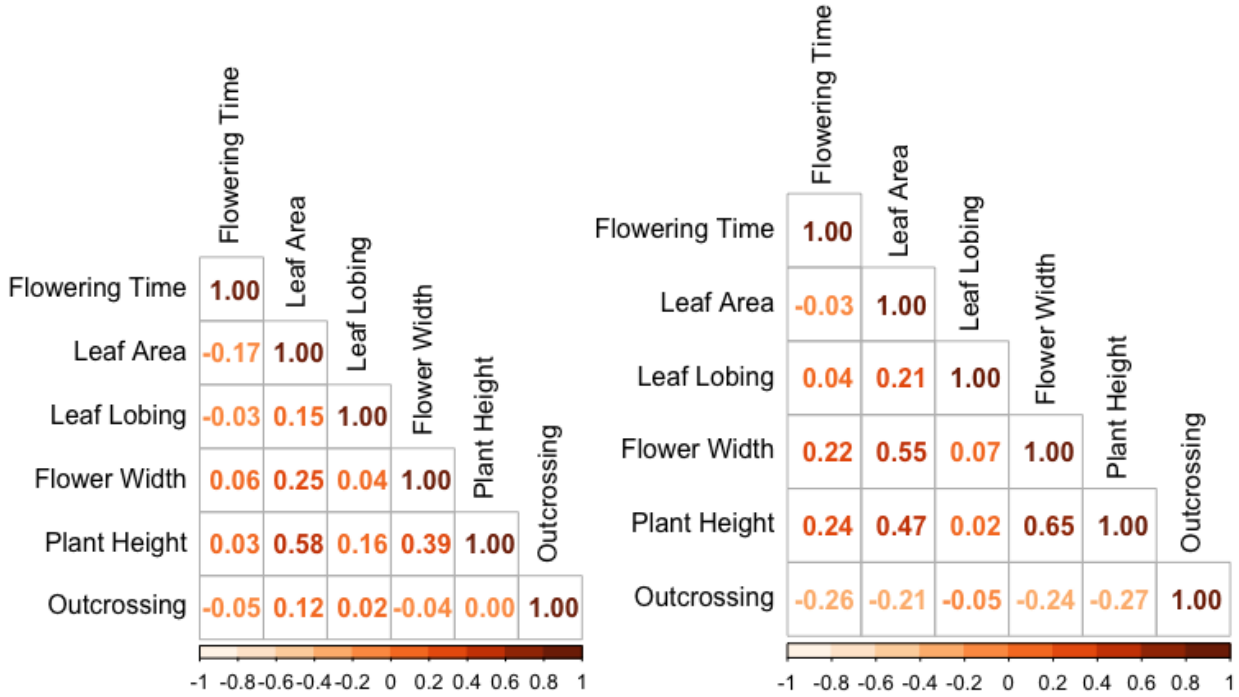


Figure S4. Trait correlation matrices for F6 hybrids in 2023 in meadow (left) and granite (right) habitats.

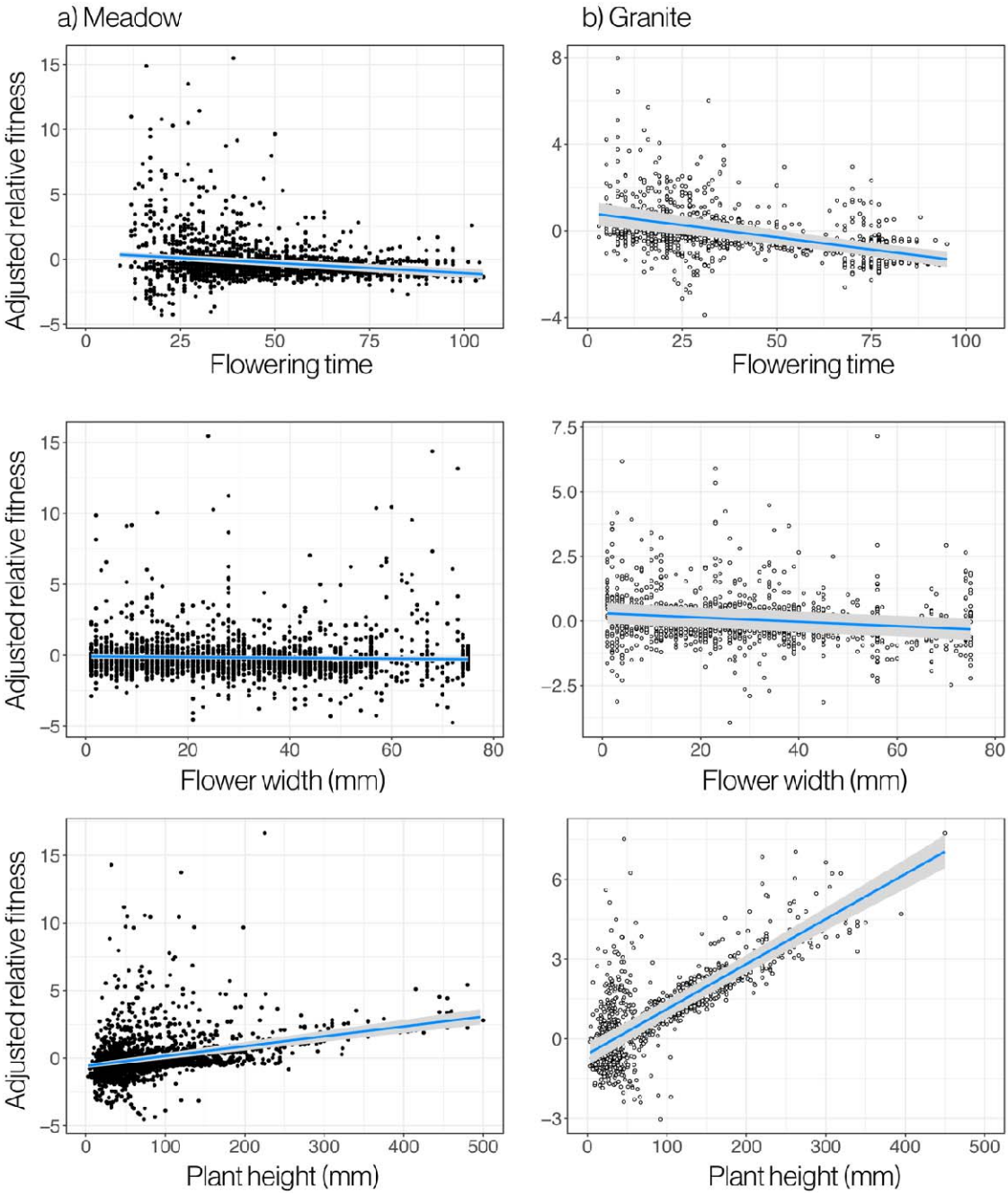


Figure S5. Patterns of cumulative selection with all years combined in a) meadow (filled circles) and b) granite habitats (open circles) in a negative binomial analysis on flowering time (days from planting), width of first flower (mm), leaf lobing index, and plant height at flowering (mm to apical meristem). 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 from 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.

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.

		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 S8. 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 S9. 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 S10. 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*)