Title: The genetic architecture and evolution of life history divergence among perennials in the *Mimulus guttatus* species complex

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Abstract

Ecological divergence is a main source of trait differences between closely related species. Despite its importance in generating biodiversity, the genetic architecture of most ecologically relevant traits is poorly understood. In plants, differences in elevation can impose substantial selection for phenotypic divergence of both complex, correlated suites of traits (such as life history), as well as novel adaptations. Here, we use the *Mimulus guttatus* species complex to assess if divergence in elevation is associated with trait divergence in a group of closely related perennial species, and determine the genetic architecture of this divergence. We find that divergence in elevation is associated with both differences in multivariate quantitative life history traits, as well as a unique trait; the production of belowground stolons, but the extent of phenotypic divergence among species depends on ontogeny. We show that the genetic architecture of this divergence between two perennial species is simple, involving few mid to large effect Quantitative Trait Loci (QTLs). Lastly, we discover that the genetic architecture of the ability to produce belowground stolons changes through development. In sum, perennials of the *M. guttatus* species complex exhibit substantial phenotypic divergence, which is associated with elevational differences, and is controlled by few genetic changes.

Introduction

Closely related species often vary ecologically, and this divergence is a major source of phenotypic differentiation [1]. Phenotypic differentiation can promote species coexistence [2], and contribute to reproductive isolation [3]. Yet, the ease at which ecological divergence evolves depends, in part, on the genetic architecture of ecologically adaptive traits. The number of alleles controlling traits, as well as the distribution of their effect sizes, and gene action (e.g. dominance or epistasis) can impact how easily evolution can proceed [4]. If species diverge in suites of correlated traits, the extent of pleiotropy or close linkage between loci controlling multiple traits may restrict multivariate trait evolution to certain phenotypic axes [5]. Alternatively, if traits are controlled by many loci, only some of which have correlated effects on multiple traits, then evolution may be more flexible [6,7].

One cause of phenotypic divergence among closely related plant species is elevation [8,9]. Plant populations differ predictably in phenotypes along elevational gradients; both within species [10–14], and between close relatives [15–17]. Differences in water availability, herbivory, climate, growing season, or a combination of factors may shape phenotypic evolution along elevational gradients [11,12,14,18,19]. Adaptations to high elevation environments often involve both changes in complex suites of traits- such as life history divergence, including correlated changes in the timing of reproduction, investment in vegetative biomass, and the timing of senescence [10–12,16,20], as well as unique phenotypes, like shorter stature [21], changes in leaf physiology [22], or production of belowground morphological structures [23,24].

Investment in elevation associated traits may also change with ontogeny. This can occur simply because more time has passed for particular morphological features to develop [25], and/or because the importance of different selective pressures may change throughout development [26]. If species differ in the timing of production of ecologically important traits, then the genetic architecture of such traits may also change across development. However, few studies have quantified the extent of trait divergence between closely related species throughout ontogeny or tracked the genetic architecture of ecologically relevant traits across development (but see [27]).

The *Mimulus guttatus* species complex (Section *Simiolus*, DC, Phrymaceae) comprises about a dozen closely related species that exhibit substantial phenotypic variation, and inhabit a broad altitudinal range across the Pacific Northwest [28]. A major axis of ecologically associated phenotypic variation is life history. Considerable effort has been made to characterize life history in this group, though most studies have focused on variation within individual annual species [11,18,29–31], between closely related annual species [32,33], or between annuals and perennials [5,19,34–43]. Very little is known about the variation in ecologically associated trait divergence among perennial taxa in this group, although they account for a sizable fraction of species.

Here we characterize the geographic and ecological distributions of five perennial taxa within the *M. guttatus* species complex, assess the extent of variation in life history across development using a common garden experiment, and determine if life history variation within and among perennial taxa is related to differences in elevational niche. Additionally, we map the genetic architecture of life history divergence between two perennial species. We assess the extent to which loci affect multiple traits, and also characterize the genetic architecture of a novel life history trait through development. Overall, our findings contribute to our understanding of ecological and trait divergence between closely related species, and shed light on the genetic architecture of both correlated and novel traits.

Materials & Methods

Study species

Although the taxonomy of the *M. guttatus* species complex is debated [44,45], at least five distinct perennial taxa are recognized: *M. tilingii* (*sensu lato*)*, M. decorus, M. corallinus,* and two ecotypes of perennial *M. guttatus;* coastal perennials (AKA *M. grandis*; [44]) and inland perennials (AKA *M. guttatus sensu stricto*; [44]). *Mimulus decorus* and *M. tilingii* both comprise multiple genetic lineages [44,46], but as these lineages form monophyletic groups, we include each as a single taxa.

Perenniality in the *M. guttatus* species complex is defined by the production of stolons. Stolons are horizontally growing, vegetative stems that develop from axillary meristems. Although vegetative at first, stolons have the ability to become reproductive.

In addition to above ground stolons, some perennials produce stolons that grow underground (e.g. rhizomes; [47,48]). Belowground stolons, like their above ground counterparts, are horizontally growing, vegetative stems that originate above ground. However, once underground, their morphology changes; they become high branched, white, and repress leaf growth (Fig. S1, Fig. S7). Belowground stolons can eventually re-emerge above ground, wherein, their morphology reverts to that of a typical above ground stolon, including the ability to become reproductive. Belowground stolons thus represent a unique axis of investment in vegetative biomass, but little is known about their ecological or phylogenetic distribution or their genetic basis.

Estimating ecological distributions

To categorize the climate niche, we compiled occurrence records from Global Biodiversity Information Facility (GBIF) for each species within the native range and combined these with known collection records (available: http://mimubase.org/). These ranges largely agree with previously published ranges [47], although we include more northerly occurrences for *M. decorus* compared to [47]. For records in which elevation was not recorded, we estimated elevation using the USGS National elevation map (https://viewer.nationalmap.gov/theme/elevation/##bottom) or the R *raster* package [49]. We extracted BIOCLIM variables for all unique locations for each species at a resolution of 10 arc-degrees using the *raster* package [49]. We performed a PCA with all BIOCLIM variables plus elevation with variables scaled to have unit variance. A broken stick model determined that PCs 1 and 2 explained significantly more variation than expected. We assessed if species differed in climate niche by performing an ANOVA for each significant PC with species as the independent variable, then pairwise T-tests with Holm correction for multiple testing to determine which species varied. All statistical analyses were carried out in R [50].

Quantifying life history variation

In order to assess trait divergence among perennials of the *M. guttatus* species complex, we performed two common garden experiments. Seeds were cold stratified on moist Fafard 4P potting soil for one week at 4°C to break dormancy and promote germination synchrony, then transferred to long day conditions in the Duke University greenhouses (18h days, 21ºC days/18ºC nights). On the day of germination, seedlings were transplanted to individual 4” pots and monitored daily.

I. Population survey

To study phenotypic differences among perennial taxa, we performed a common garden survey of 40 populations from five perennial species (average of 8 populations per species and two maternal families per population; Table S1). For each maternal family, we grew three replicate individuals; one for each developmental time point surveyed. In total, our population survey involved 199 plants.

We surveyed plants at three developmental time points; three weeks post stratification (a vegetative period), the day of first flower, and six weeks post stratification (a mid/late-reproductive period). At each time point, one replicate individual per maternal family was unpotted, and belowground biomass was cleaned. Plants were then measured for the number of stolons produced, the length and width of the longest stolon, the length of the first stolon leaf, the number of branches per node, the highest node at which stolons emerged, and the presence/absence of belowground stolons. If belowground stolons were present, both above and belowground stolons were measured for each stolon trait (i.e. length, width, stolon leaf length, branches per node, highest node of emergence). On the day of first-flower, we scored the date and node of first-flower, corolla length and width, and the length and width of the first true leaf. As the phenotyping was destructive, we discarded individuals after phenotyping. In the few cases in which not all maternal families produced three replicates, we preferentially measured replicates at later developmental times.

II. Analysis of population survey

We aimed to assess whether life history differed among perennials of the *M. guttatus* species complex in a common garden. We used PCA to summarize life history variation across multi-trait space. As PCA is sensitive to missing data, and some perennial taxa did not make belowground stolons, we restricted the PCA to include the total number of stolons, the highest node of stolon emergence, the number and proportion of belowground stolons, and the length, width, and leaf length of above ground stolons. PC1 explained 49.82% of the variation and was the only significant PC according to a broken stick model. We performed linear mixed models using the *lme4* and *car* packages in R [51,52] with PC1 as the dependent variable, species, time point, and their interaction as fixed effects and population as a random effect. We found a significant species by time point interaction (^{2}=46.041, *df*=4, *p*<0.00001), and therefore performed linear mixed models for each time point separately with species as a fixed effect and population as a random effect. Significance was assessed using a Type III Anova in the R *car* package [51,52].

To determine whether variation in life history among populations was related to a population's source environment we performed additional linear mixed models with PC1 from the morphological PCA as the dependent variable, elevation, species, time point, and all possible interactions as fixed effects. Population was included as a random effect. Again, we find a significant species by time point interaction (^{2}=11.6708, *df*=4, *p*=0.02). We thus perform submodels for each developmental time point, with PC1 as the dependent variable, species and elevation as fixed effects, and population as a random effect. We did not include a species by elevation interaction in the final models, as it was not significant, these models had higher AIC scores and models were not significantly improved by the addition of this interaction term.

Lastly, we evaluated the extent to which life history traits covary within perennial taxa. Strong associations between the timing of reproduction and investment in vegetative biomass have been previously described in *Mimulus [5,36,37]*. To determine if perennials exhibited an association between flowering time and stolon number, we performed a linear mixed model with node of first-flower as the dependent variable, and the number of stolons and species as fixed effects, and population as a random effect. In the final model, the species by stolon number interaction term was dropped, as it was

234 not significant, a model which included it had a higher AIC and the model was not
235 significantly improved.
236
237 *Determining the genetic basis of life history divergence*
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239 *I. F2 grow out*
240
241 We concurrently grew 429 F2s derived from a single self-fertilized F1 between *M.*
242 *decorus* (IMP) and coastal perennial *M. guttatus* (OPB). These parents vary in several
243 perennial traits, including the total number of stolons, the presence of belowground
244 stolons, and flowering time (Table S3; Fig. S6). We additionally grew 24 replicates of
245 each inbred parental line and F1s. Due to an asymmetric seed barrier between *M.*
246 *decorus* and *M. guttatus* [46], *M. guttatus* served as the maternal donor for all F1s.
247 Individuals were surveyed at the same developmental time points as above, but
248 were not destructively surveyed. Therefore, at the three and six week post stratification
249 individuals were assessed only for the number of above and belowground stolons. On
250 the day of first flower, individuals were scored for flowering time (date and node of first
251 flower), the length and width of the corolla and first true leaf, the highest node of stolon
252 emergence, and above ground stolons were measured for several traits (length, width,
253 leaf length, number of branches per node, highest node of emergence). A total of 410
254 F2 individuals were phenotyped, as well as 24 of each parental line and F1.
255
256 *II. Library construction and sequencing*
257
258 We extracted DNA from F2 individuals using a modified CTAB protocol ([53];
259 dx.doi.org/10.17504/protocols.io.bgv6jw9e). We created libraries for 384 F2s and each
260 parental line using a modified Multiplexed Shotgun Genotyping approach with the
261 enzyme Csp6I ([54]; dx.doi.org/10.17504/protocols.io.bbjbikin). All eight libraries were
262 split across 2 lanes of Illumina 4000 High Seq at the Duke Sequencing Facility, for an
263 average of 97.4 million reads per library (~2 million reads per F2 individual).
264

265 *III. Read processing, linkage map construction, genotype estimation & QTL mapping*
266
267 Reads were demultiplexed using Stacks 2.2 [55,56]. Each file was aligned to the V2.0
268 *M.guttatus* reference genome (https://phytozome.jgi.doe.gov/) using BWA [57], then
269 cleaned and sorted using Picard tools (http://broadinstitute.github.io/picard/). We used
270 GATK to call SNPs for each sample individually, then jointly genotype all individuals
271 [58]. We removed indels and included sites with a minimum genotype quality of 30
272 using VCFtools [59]. We polarized SNPs in the F2s based on sites that were alternative
273 homozygotes in the parental inbred lines, and determined genotype markers by using
274 the number of counts for each parental allele across 100kb windows. A window was
275 determined to be homozygous if >95% of reads were of one parent, and heterozygous if
276 the percent of reads were between 0.1 and 0.9. We filtered the dataset to remove
277 individuals with very low genotyping (<50 markers across the genome) and markers
278 with genotype information for <10% of individuals.
279 We implemented GOOGA [60] to estimate individual genotyping error rates,
280 construct a linkage map, and estimate genotype posterior probabilities for each
281 individual at each marker. We defined genotypes using a posterior probability >/=0.95.
282 We removed individuals with substantial missing data (>90% missing data) or severely
283 distorted overall genotypic compositions (<20% or >80% heterozygous calls). The
284 remaining dataset comprised 213 individuals with 1,311 markers.
285 We used Haley-Knott regressions in the R/*qtl [61,62]* to identify LOD scores, and
286 set significance thresholds using 1,000 permutations. For one QTL on LG4 we find a
287 modest effect on the number of stolons, although this did not pass the genome-wide
288 permutation threshold. We include this QTL in our final map, as single marker analysis
289 using the *fitqtl* function determined there was a significant association (F=2.33,
290 p=0.033). For traits in which more than one QTL reached significance, we assessed
291 non-additive interactions among QTLs using the *addint* function in R/qtl. Except in one
292 case, we do not recover any significant interactions among QTLs. We calculated effect
293 sizes of each QTL as the difference between alternate homozygotes, then calculated
294 the percentage of the parental difference explained. We also estimated how much
295 phenotypic variance in F2s was explained by each QTL in two ways. First, we

296 calculated how much of the range of F2 phenotypes was explained by each QTL.
297 Second, we estimated the proportion of Vg (the amount of phenotypic variation that can
298 be explained by genetic variation) explained by each QTL. To estimate Vg in units
299 comparable to the effect sizes, we subtracted the variance observed in F1s from the
300 variance in F2s for each trait then square-root transformed this value so it was in the
301 same unit of measurement as the trait.
302
303 **Results:**
304 *Perennial taxa vary in both climate niche and life history*
305
306 Perennial taxa of the *M. guttatus* species complex differ in geographic and
307 environmental distributions (Fig. 1). Four of five taxa are relatively restricted
308 geographically and ecologically, while inland perennial *M. guttatus* is widespread. Of the
309 more restricted taxa, three inhabit almost exclusively mid to high elevation habitats, with
310 *M. tilingii* exhibiting a more broad distribution across the Sierras, Cascades, Rockies,
311 and Pacific Coastal ranges (Fig. S2; Fig. S3) In contrast, *M. corallinus* exclusively
312 inhabits mid to high elevation sites in the Sierras, and coastal perennial *M. guttatus*
313 inhabits low elevation sites along the Pacific coast. The climate niches of these habitats
314 are summarized by PC1 and PC2, which explain 44.41% and 30.98% of the variance,
315 respectively, and for which species show significant differences (PC1: F=96.2, df=4,
316 p<0.0001; PC2: F=189.1, df=4, p<0.0001; Fig. 1C; Fig. S3). Higher values of PC1
317 represent habitats at higher elevation, with more precipitation in the hottest and driest
318 quarters, and higher seasonality, while higher values of PC2 represent habitats with
319 greater precipitation overall and in the wettest month and quarter (Fig. S3). Thus *M.*
320 *decorus, M. corallinus,* and *M. tilingii* tend to live in more seasonal, high elevation
321 habitats.
322

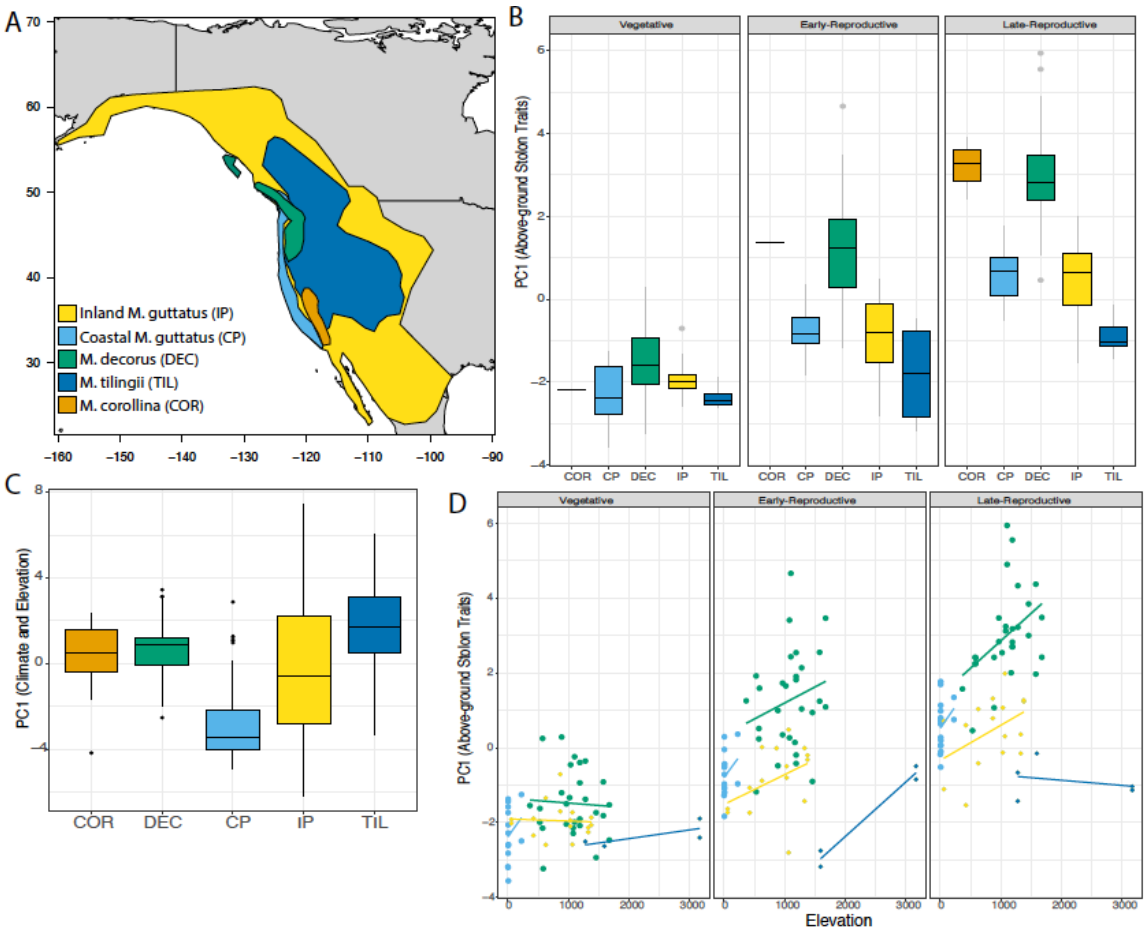


Fig.1: Perennials vary in niche and life history. (A) Geographic ranges of different perennials based on herbarium records. (B) Perennials vary in life history, and these differences extenuate with developmental time (panels). Greater values of PC1 indicate more investment in vegetative biomass. (C) PC1 of climate and elevation across species. Greater values indicate more seasonal environments at higher elevation. (D) Life history in a common garden as a function of elevation of the source population for all perennial taxa. Each panel represents a developmental sampling time. Lines represent significant regressions.

Perennial taxa exhibit substantial variation in life history (as measured by PC1 which explained 49.82% of the variance), but this difference extenuates with development (Fig. 1B; significant species by time point interaction in the full model; ^{2}=46.04, *df*= 4, *p*<0.0001. Subsequent submodels for each time point yielded a significant species effect: vegetative: ^{2}=12.673, *df*=4, *p*=0.013; early-reproduction: ^{2}=55.249, *df*=4, *p*<0.0001; late-reproduction: ^{2}=97.314, *df*=4, *p*<0.0001). Greater values of PC1 are associated with the production of more stolons, stolons that emerge

at higher nodes, and above ground stolons that are more branched (Fig. S4). Thus, *M. corallinus* and *M. decorus* tend to make the greatest number of stolons, which emerge at higher nodes and are more branched, while inland perennial *M. guttatus,* coastal perennial *M. guttatus, and M. tilingii* tend to make fewer stolons. A PCA that included reproductive traits (e.g. flowering time, floral size), as well as stolon traits agreed with these results and indicated that perennial taxa that invested more in stolon development also flowered later (Fig. S5).

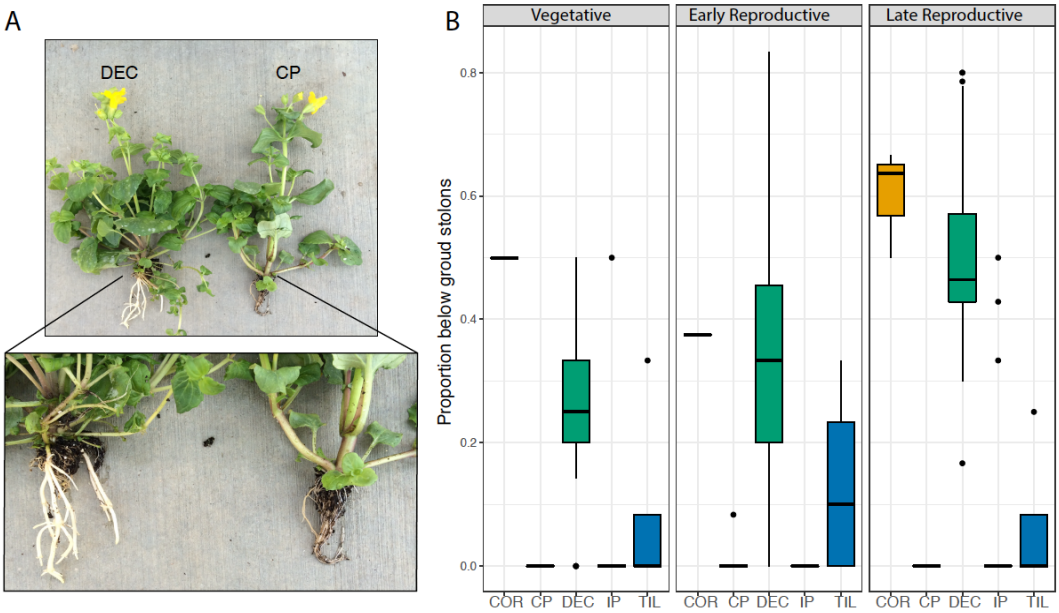


Fig.2: Mid and high elevation perennials make belowground stolons. (A) Representative *M. decorus* and coastal perennial *M. guttatus*. Both plants make above ground stolons, but only *M. decorus* produces belowground stolons. (B) Proportion of belowground stolons across development by species. COR= *M. corallinus,* CP= Coastal Perennial *M. guttatus,* DEC= *M. decorus*, IP= Inland Perennial *M. guttatus*, TIL= *M. tilingii.*

We aimed to determine if variation in life history in a common garden was related to the environment of the source population. We find that life history covaries with elevation, but only at later developmental time points (Fig 1D; full model: significant species by time point interaction: ^{2}=11.671 , *df*=4, *p*=0.02. Submodels by developmental time point showed variable effects of elevation. Vegetative: ^{2}= 0.044, *df*=1, p=0.83; early-reproductive: ^{2}= 8.324, *df*=1, *p*=0.0039; late-reproductive:

362 ^{2}=4.969, *df*=1, *p*=0.026). Populations that exist at higher elevations invest more in
363 vegetative biomass; they produce more stolons, and stolons that emerge at higher
364 nodes and are more branched (Fig. 1D; Fig. S4). For species that produce belowground
365 stolons, higher elevation populations also tend to produce more belowground stolons.
366 We scored the presence of a unique life history trait; the production of
367 belowground stolons. Belowground stolons are thinner, shorter, and more branched
368 than their above ground counterparts (Fig. S1). They also lack chlorophyll, are
369 substantially more prone to breakage, and repress the growth of vegetative structures
370 relative to above ground stolons (Fig. S1; personal obs). Three of five perennial taxa
371 produced belowground stolons (Fig. 2), all of which live exclusively in mid to high
372 elevation habitats (Fig. 1C).
373
374 *The genetic basis of life history variation among perennials is relatively simple*
375
376 We constructed an F2 mapping population between *M. decorus* and coastal perennial
377 *M. guttatus* in order to dissect the genetic architecture of life history differences. These
378 two parents vary in a number of life history traits (Table S3; Fig. S6), including flowering
379 time and size, leaf size, investment in stolons, and the presence of belowground
380 stolons, and this is representative of differences among species (Fig. 1). Using reduced
381 representation sequencing, we constructed a linkage map using 1,311 markers across
382 14 chromosomes. The total map length was 1,232.3cM (range: 52.1-125.8cM per
383 linkage group), with an average marker spacing of 1cM.

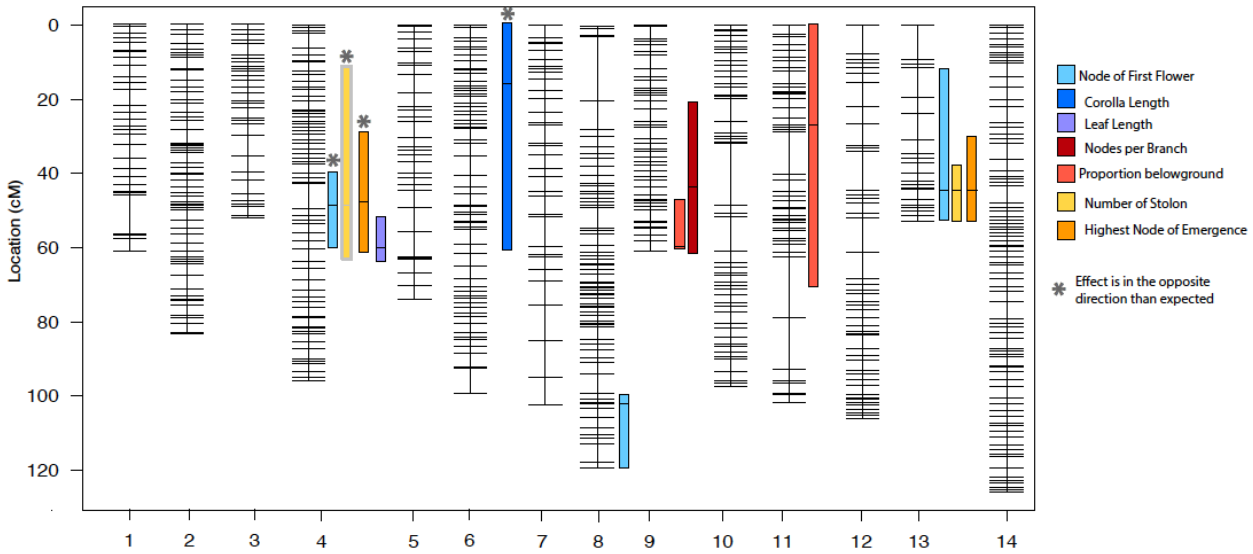
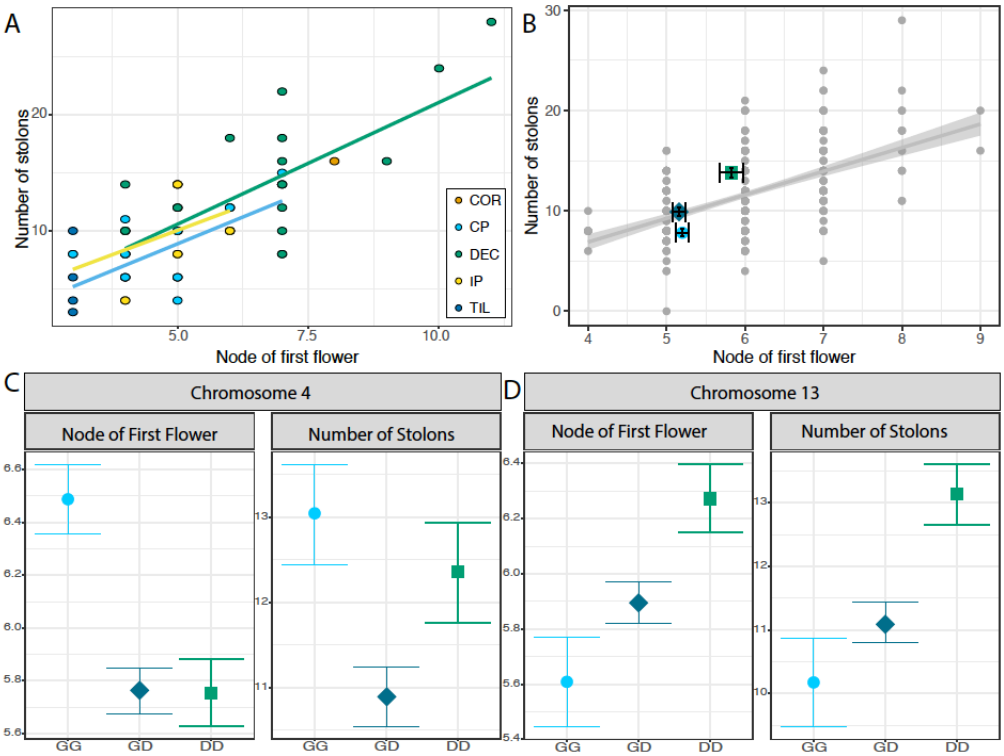


Fig. 3: QTL map of life history traits between coastal perennial *M. guttatus* and *M. decorus*. Each colored box represents the 1.5 LOD interval surrounding significant associations (thick horizontal line). Asterisks indicate that the effect of the QTL is opposite of expected. QTL highlighted in grey did not pass a genome-wide significance threshold, but was significantly associated based on individual marker analysis.

The genetic architecture of all traits was fairly simple (Fig. 3, Fig. S8). For each of seven traits that differed significantly between parents, we identified one to three significant QTLs with mid to large effects. Individual QTL explained 11.6% of the trait range within F2s (range: 1.5-19.5%), 87.8% of the heritable variation among F2s (range: 14->100%), and 83.6% of trait difference among parents (range: 7.4->100%; Table S4) on average. The average cumulative effect of all QTLs across traits was 21% of the trait range within F2s, and >100% for both the heritable variation among F2s and the trait difference among parents (Table S4). The observation that the cumulative effects of all QTLs for each trait often surpass the heritable variation among F2s and/or the difference between parents is largely due to the fact that several large effect QTL influenced traits in the opposite direction than predicted from the parental difference (Fig. 3). Of the 12 QTLs we identified, four showed effects opposite to the parental difference, three of which co-localize to LG4.

We find evidence that two life history traits- flowering time and number of stolons- strongly covary within perennial taxa in the *M. guttatus* species complex (Fig. 4;

407 ^{2}=47.4; *df*=1; *p*<0.0001, with a marginally significant species effect: ^{2}=9.11; *df*=4;
408 *p*=0.06). Correlations between these traits persisted among F2s, suggesting that they
409 are genetic (Fig. 4; r^2=0.54, *p*<0.0001; Table S2). In line with this, QTLs on LG4 and
410 LG13 affect flowering time, highest node of emergence, and stolon number (Fig. 3;
411 Table S4). The allelic effects of these two QTL are opposite; for LG4 the *M. guttatus*
412 allele is associated with later flowering and more stolons (typically *M. decorus* traits),
413 while the LG13 QTL shows effects in the predicted direction (Fig. 4; Table S4). We also
414 observe transgressive segregation for both of these traits among F2s (Fig. 4).
415



416

417 **Fig. 4: Life history traits covary among perennial species, and share a genetic**
418 **basis. (A) Phenotypic correlation between flowering time and stolon number**
419 **across 40 populations of 5 perennial taxa. COR= *M. corallinus,* DEC= *M. decorus,***
420 **TIL= *M. tilingii,* IP= inland perennial *M. guttatus,* CP= coastal perennial *M.***
421 ***guttatus.* Lines represent linear regressions for each species. (B) Genetic**
422 **correlations between flowering time and number of stolons in F2s derived**
423 **between *M. decorus* and coastal perennial *M. guttatus.* Grey circles= individual**
424 **F2s, blue circle = the coastal perennial *M. guttatus* parent (OPB), turquoise**
425 **diamond = F1s, and green square = the *M. decorus* parent (IMP). (C,D) Phenotypic**
426 **effects of two QTL on flowering time and stolon number. Error bars are standard**
427 **errors.**

Lastly, we find that the production of belowground stolons changes through development in hybrids and consequently, so does the genetic architecture. Early on, few F1s or F2s make belowground stolons, and we find no significant QTL while plants are vegetative (Fig. 5). At reproduction, more hybrids produce belowground stolons, but the trait remains recessive. At this point in development, we identify two significant QTLs which exhibit significant non-additive effects (F=4.24, p=0.0026), such that trait expression in F2s requires the *M. decorus* allele at both loci. However, later in reproduction, most F1s and F2s make belowground stolons. At this point, no interactive effects are detected (p=0.89), and a single locus is sufficient to explain ~31% of the parental difference (26% of the F2 range). Although belowground stolons differ from their above ground counter parts in many morphological traits (Fig. S1), these traits do not appear to segregate independently in F2s (personal obs; Fig. S7).

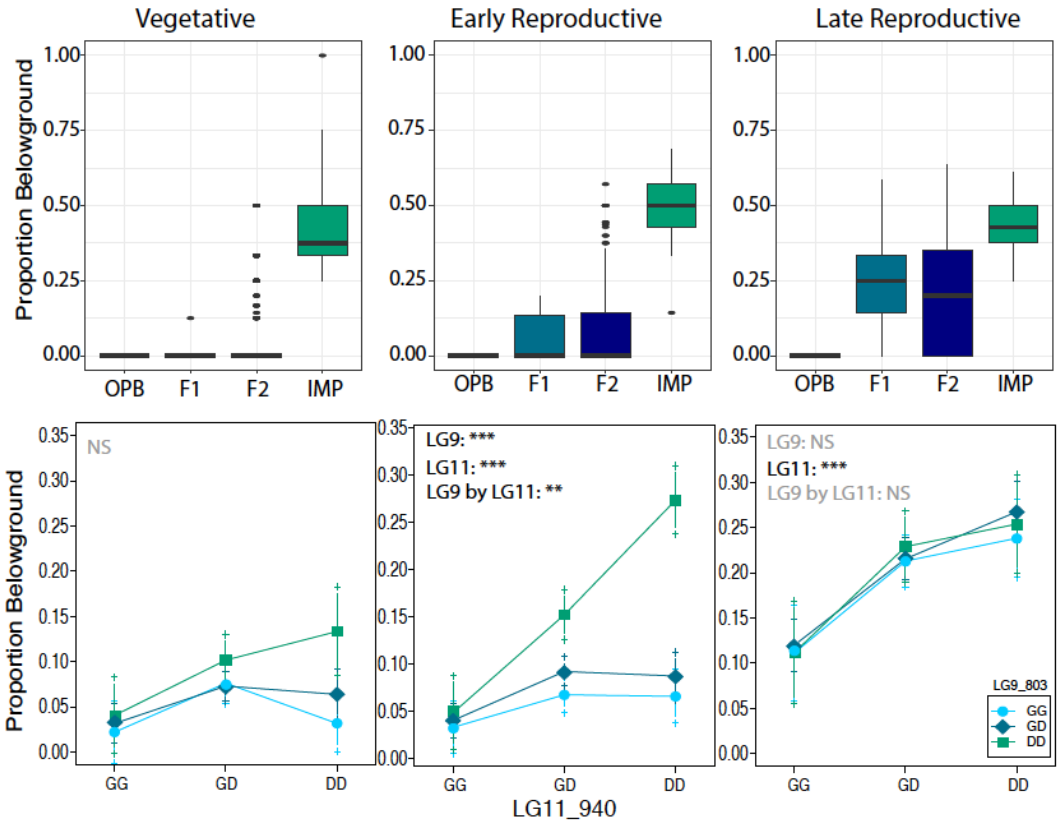


Fig. 5: The genetic architecture of belowground stolons changes across development. Top row: trait distributions for each parent (OPB= coastal perennial

***M. guttatus* and IMP= *M. decorus*), F1s, and F2s. Bottom row: Effect of two markers on the proportion of belowground stolons. The significance of each marker and their interaction is listed in the top left corner. G= the coastal perennial *M. guttatus* allele, D= the *M. decorus* allele.**

Discussion

We assessed the extent of divergence in elevational niche and life history among perennial taxa of the *M. guttatus* species complex, and determined the genetic basis of life history differences between two perennial species. We find that perennials live in a variety of habitats, though many are restricted to mid to high elevations. Perennials also vary in life history, but the extent of differences between species depends on ontogeny. We find that the presence of belowground stolons is largely restricted to species that occur in mid to high elevation habitats, and within species, investment in vegetative biomass is associated with elevation. Together, these suggest that elevation- and covarying climatic factors- may shape life history evolution within and among perennials of this group. Lastly, we find that the genetic architecture of life history traits is fairly simple among two closely related perennial species. Most traits are largely explained by few mid to large effect QTLs, including the ability to produce belowground stolons.

Elevation may shape life history variation within and among perennials

Perennials of the *M. guttatus* species complex vary in both life history and climate niche. In a common garden, species exhibit clines in life history along elevational gradients, suggesting that elevation- and its associated environmental factors- may shape life history. Individuals from higher elevations produce more stolons, stolons at higher nodes, and stolons that were more branched. Increased investment in vegetative biomass at higher elevations may increase survival over long winter seasons and promote greater seed production in later years, as each stolon has the potential to become reproductive. In line with this, perennials have higher fitness than annuals in a high elevation common garden [17]. Similarly, adaptive clines in investment in growth and delayed reproduction exist along elevational gradients of annual *M. guttatus* [11,18], as well as other perennial taxa [10,12,13,15,20].

Curiously, *M. tilingii* invested the least in vegetative biomass in our common garden experiment (as well as a previous common garden [46,63]), despite inhabiting the highest elevations in nature. *Mimulus tilingii* invests substantially in vegetative biomass in nature [48]. Differences between wild and greenhouse plants likely stem from differences in age, wherein naturally collected plants tend to be older and therefore have had more time to invest in vegetative biomass.

We find the taxa that are restricted to mid to high elevation habitats exhibit a novel trait relative to most *M. guttatus* populations; the presence of belowground stolons. Belowground stolons may afford perennials a greater chance of survival in environments with extreme cold temperatures and sudden freezing. While uncommon in our experiment, 4/48 inland perennial *M. guttatus* individuals produced belowground stolons. These individuals hailed from 2 mid-elevation sites from the Coastal Range and Cascades (1,065m and 866m, respectively). *Mimulus guttatus* can co-occur with *M. decorus* and/or *M. tilingii* at high elevations, and this trait may have introgressed between species. Belowground stolons may also have evolved independently in each species or existed as a shared polymorphism in their ancestor (as discussed in [3,64]). Alternatively, belowground stolons may represent an ancestral state, wherein a loss of this character accompanied colonization of low elevation habitats in *M. guttatus*. Further population genetics studies will be required to test these potential scenarios.

The genetic architecture of life history divergence is relatively simple

We find that life history divergence between two perennial species has a relatively simple genetic basis. One to three QTLs explain a significant proportion of the parental difference in most life history traits, and the effects of these QTLs are largely additive. This is simpler than the genetic architecture of life history divergence in other systems [5,30,32,35,65–67]. One explanation for why some genetic architectures are complex versus simple is how natural selection acts on the trait in question [68,69]. Strong, divergent natural selection between populations is expected to produce more simple genetic architectures than stabilizing or balancing selection within populations [68–70]. This is true for a number of examples of adaptations to discrete habitats [3,71]. In the

508 case of life history divergence between coastal perennial *M. guttatus* and *M. decorus*,
509 trait differences may have arisen due to divergent natural selection via elevation.
510 Further reciprocal transplants and manipulative common gardens will be needed to
511 assess the agents, strength, and targets of natural selection in this system.
512 We uncover some curious features of the genetic architecture of life history
513 divergence between coastal perennial *M. guttatus* and *M. decorus.* First, we find two
514 QTLs that are associated with three highly correlated traits; stolon number, flowering
515 time, and the highest node of stolon emergence. QTLs that affect both flowering time
516 and stolon number have been described between annual and perennial *M. guttatus*
517 *[5,35,37]*, and may represent a genetic control of early meristem commitment (e.g.
518 production of floral buds versus stolons; [5,41,72]). Interestingly, the LG4 QTL exhibits
519 opposite allelic effects than predicted by the parental difference. Consequently, some
520 F2s exhibit transgressive phenotypes- delayed flowering and substantially more stolons
521 than either parent. Such extreme phenotypes may have negative fitness consequences
522 in nature, highlighting a potential for reproductive isolation among perennials.
523 Second, we find that the genetic architecture of the proportion of belowground
524 stolons changes with development, in part, because the dominance changes through
525 development. Early in reproduction, the ability to produce belowground stolons is
526 recessive, and is genetically controlled by two interacting loci. However, later in
527 development, the ability to produce belowground stolons becomes dominant (with the
528 proportion of belowground stolons showing no dominance), and only one of the
529 previously determined QTL continues to influence this trait. One potential genetic model
530 for the change in genetic architecture through development is if the LG11 QTL largely
531 controls the ability to produce belowground stolons, but timing of expression of this QTL
532 is modulated by the LG9 QTL. Previous work in *Arabidopsis* suggests that the genetic
533 basis of trichome density is largely controlled by a single large effect QTL in both
534 juvenile and adult leaves [27]. Although much less studied, an understanding of how the
535 genetic architecture of traits may change through development may also provide details
536 of the molecular genetic basis of development in ecologically important traits.
537 Lastly, although belowground stolons differ from above ground stolons in a
538 number of traits, these traits do not appear to segregate independently in F2s. Thus,

although repurposing stolons to grow below ground involves many phenotypic changes, the evolution of this trait may be accomplished by few genetic steps. Similarly, complex behavioral shifts in *Drosophila* are largely attributable to a single retrotransposon insertion [73], and in *Penstamon* single QTL influence several floral phenotypes [7]. Alternatively, this complex trait may be controlled by many closely linked alleles (such as [74]). Further work determining if and how few genetic changes has allowed *Mimulus* to inhabit high elevation habitats can provide insights into the genetics of adaptation.

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Author Contribution Statement

JMC conceived of the idea for this project, performed all experiments and data analyses, and wrote the paper. MWB helped with phenotyping and DNA extractions. JW contributed to the intellectual development of this project.

Data Accessibility Statement

Data for the common garden experiments and sequencing will be made available on Dryad and the NCBI SRA upon acceptance of this manuscript.

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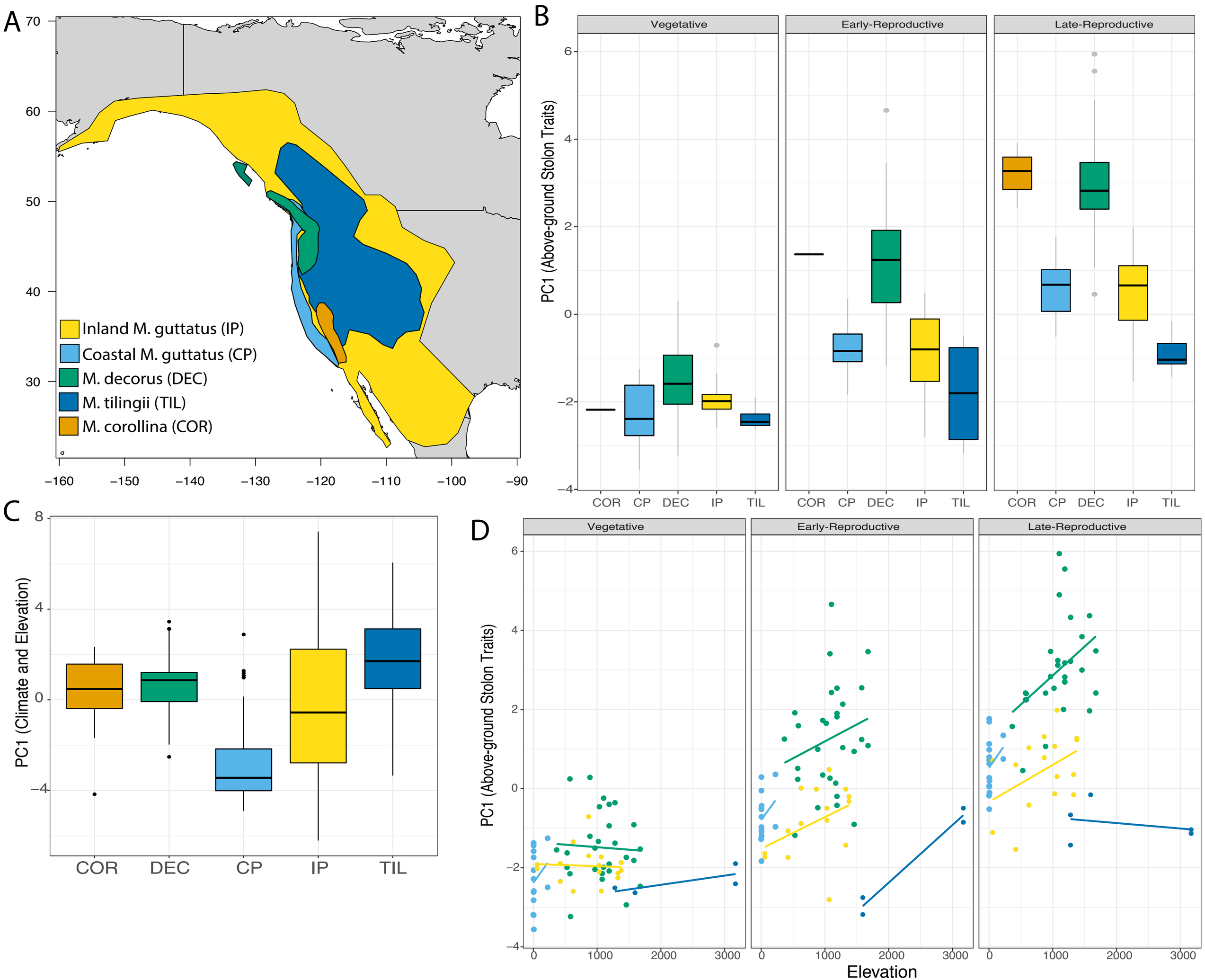
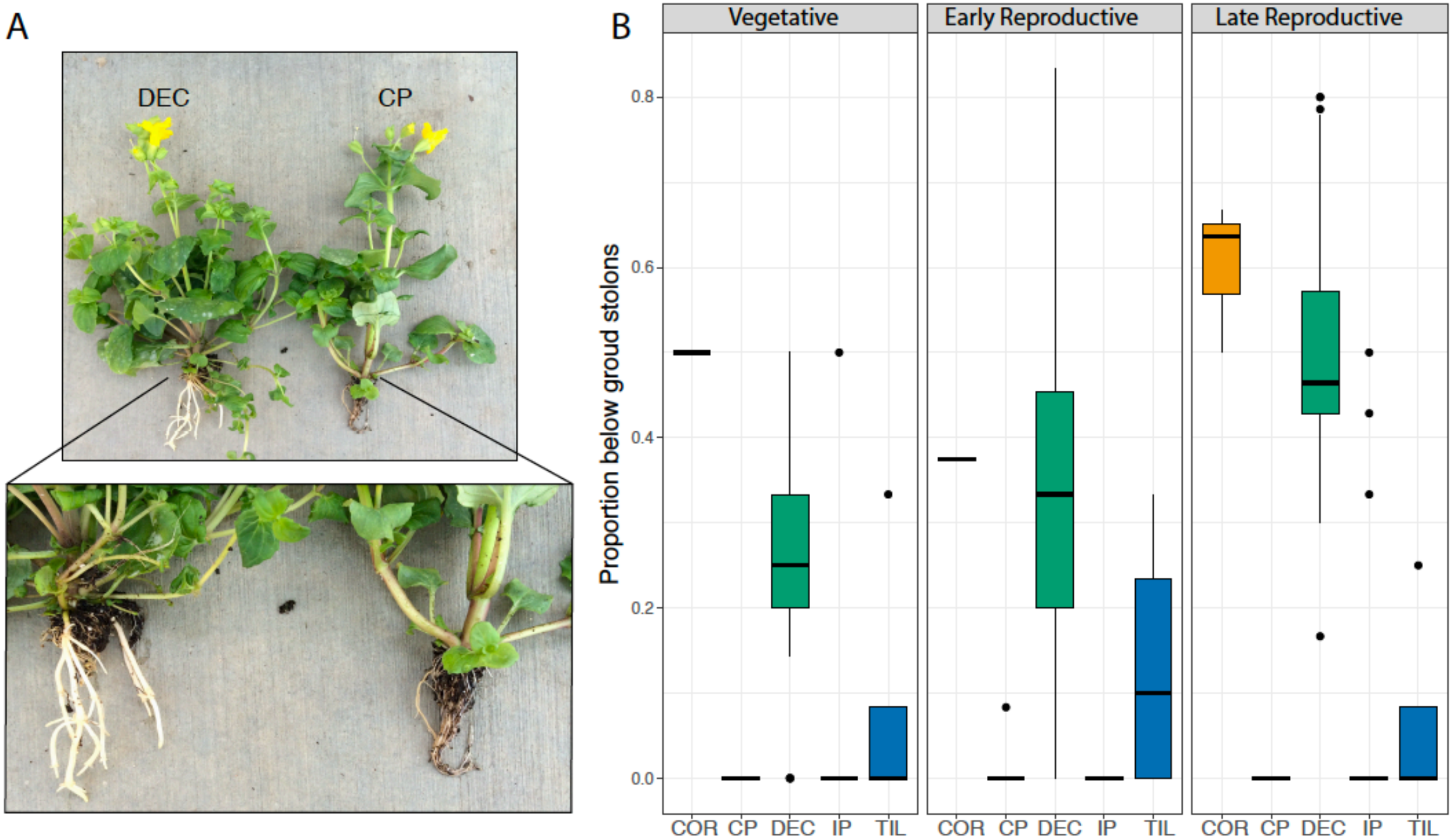
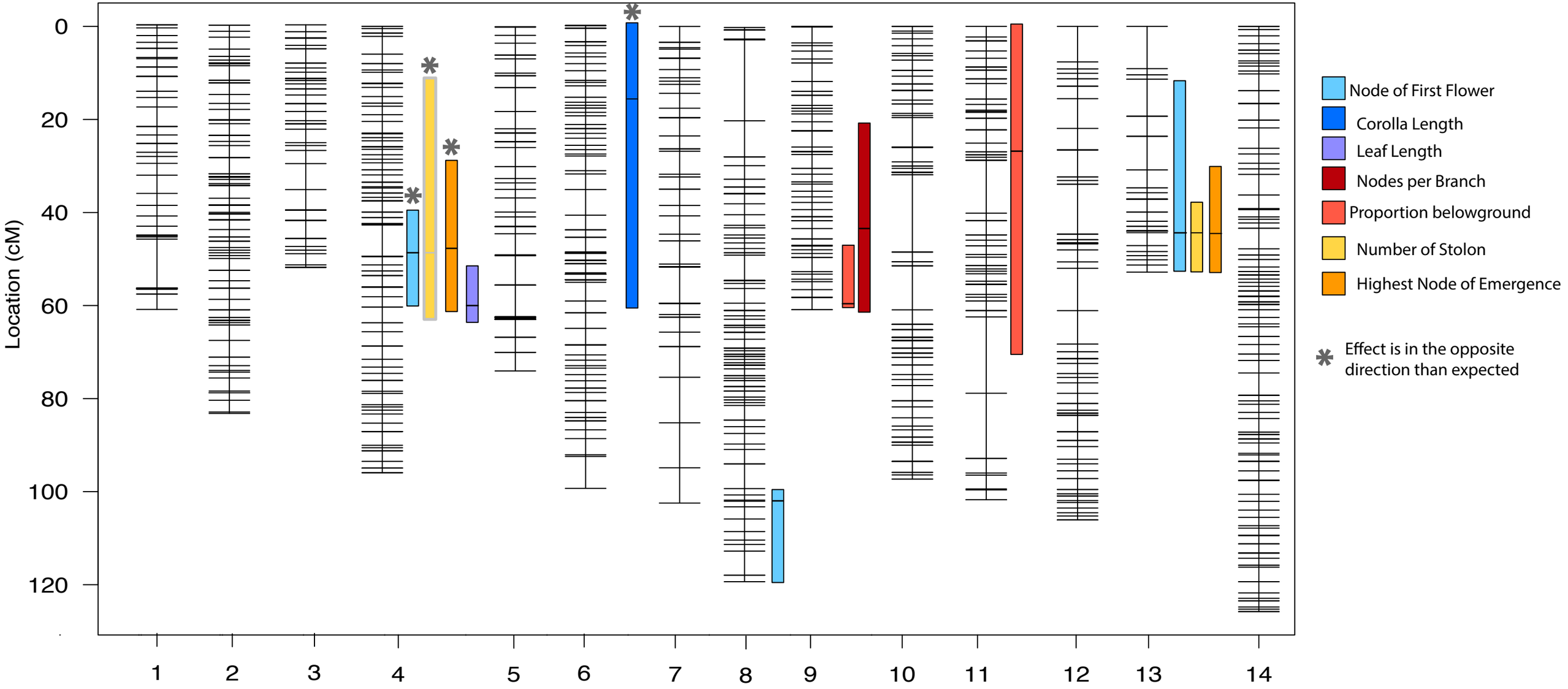

A
Inland M. guttatus (IP)
Coastal M. guttatus (CP)
M. decorus (DEC)
M. tilingii (TIL)
M. corollina (COR)
70
60
50
40
30
−160
−150
−140
−130
−120
−110
−100
−90
B
Vegetative
Early-Reproductive
Late-Reproductive
PC1 (Above-ground Stolon Traits)
6
4
2
0
−2
−4
COR
CP
DEC
IP
TIL
C
PC1 (Climate and Elevation)
8
4
0
−4
COR
DEC
CP
IP
TIL
D
Vegetative
Early-Reproductive
Late-Reproductive
PC1 (Above-ground Stolon Traits)
0
1000
2000
3000
Elevation


A
DEC
CP
B
Vegetative
Early Reproductive
Late Reproductive
Proportion below groud stolons
0.0
0.2
0.4
0.6
0.8
COR
CP
DEC
IP
TIL


Location (cM)
0
20
40
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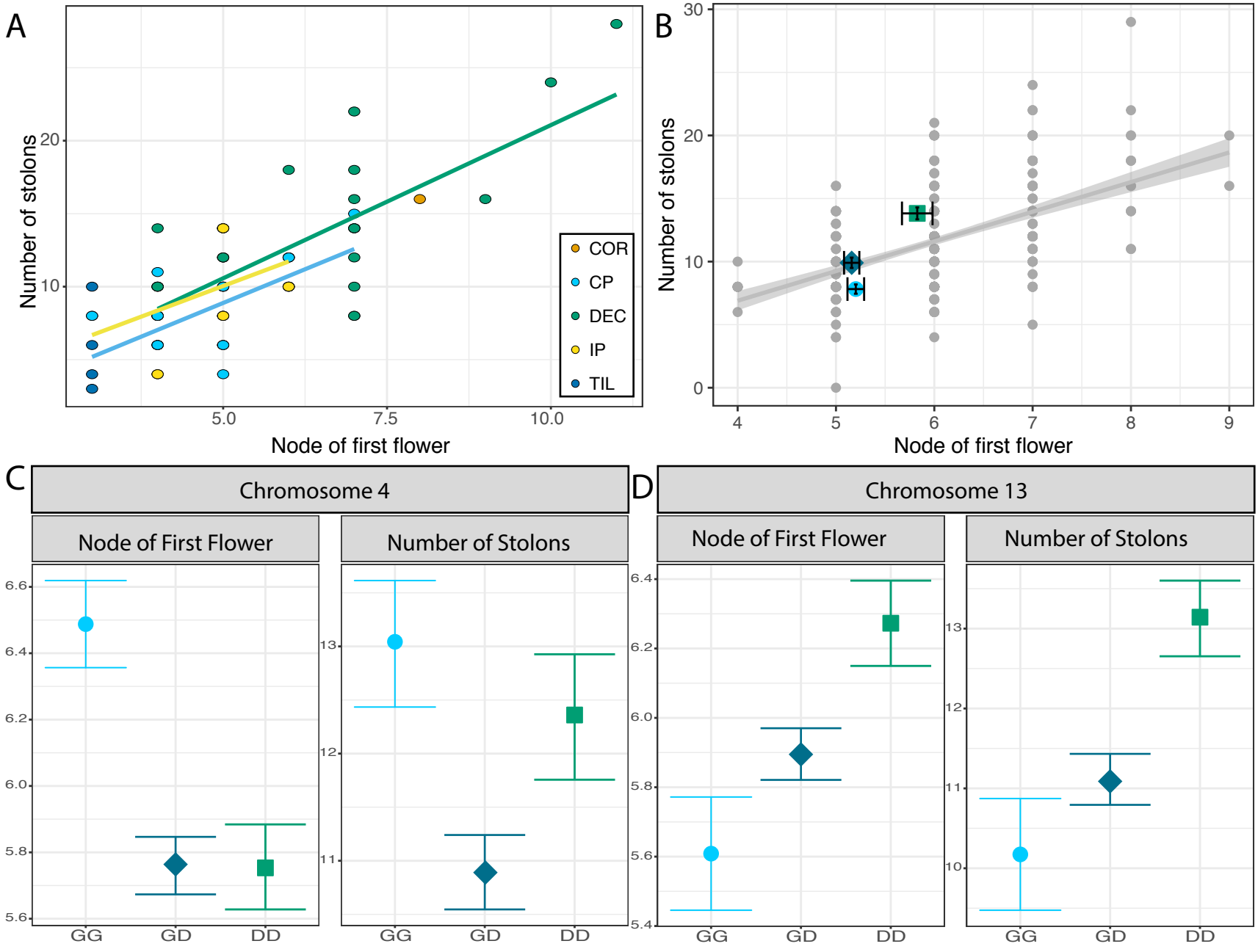
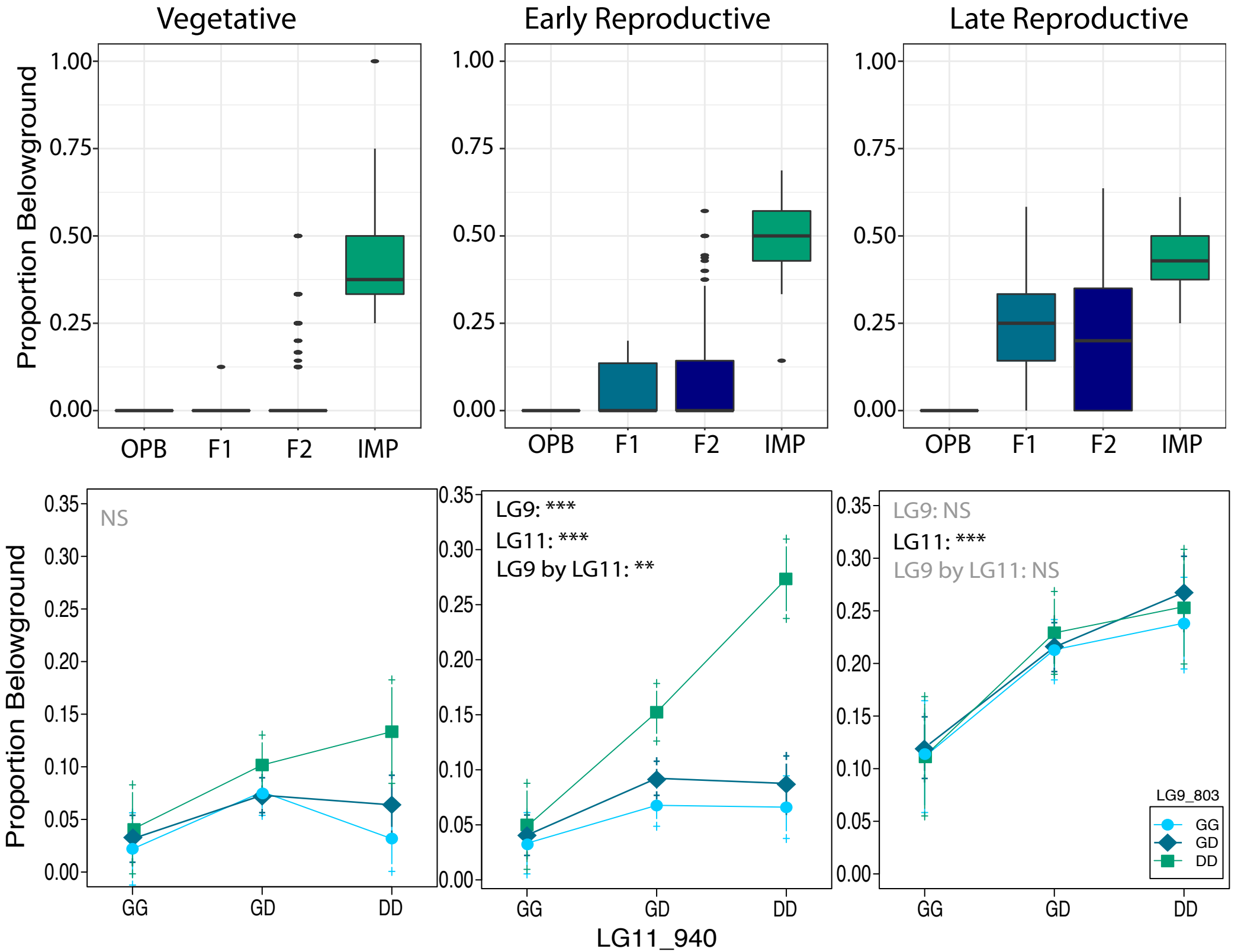
80
100
120
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Node of First Flower
Corolla Length
Leaf Length
Nodes per Branch
Proportion belowground
Number of Stolon
Highest Node of Emergence
Effect is in the opposite direction than expected


A
Number of stolons
Node of first flower
COR
CP
DEC
IP
TIL
B
Number of stolons
Node of first flower
C
Chromosome 4
Node of First Flower
Number of Stolons
GG
GD
DD
D
Chromosome 13
Node of First Flower
Number of Stolons
GG
GD
DD


Vegetative
Early Reproductive
Late Reproductive
Proportion Belowground
1.00
0.75
0.50
0.25
0.00
OPB
F1
F2
IMP
NS
LG9: ***
LG11: ***
LG9 by LG11: **
LG9: NS
LG11: ***
LG9 by LG11: NS
0.35
0.30
0.25
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GG
GD
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LG11_940
LG9_803
GG
GD
DD

Supplemental Figures

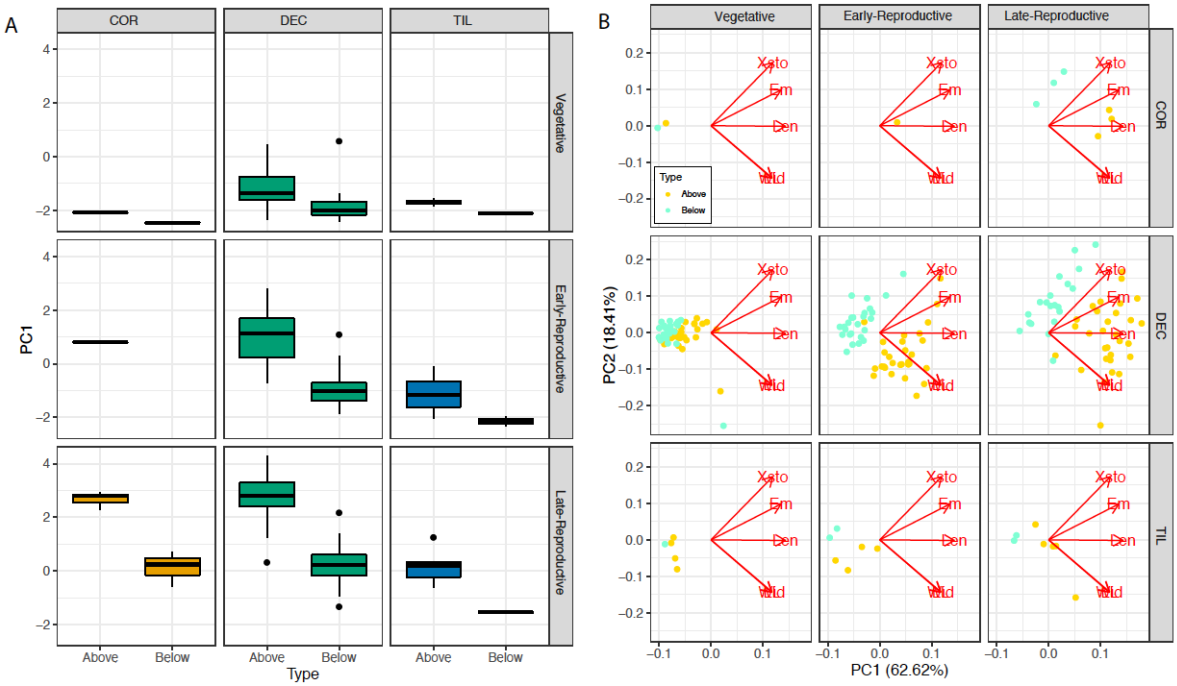


Figure S1: Above and Below ground stolons differ, and these differences grow through development. Analyses were restricted to the three species that made both above and below ground stolons (*M. decorus, M. tilingii,* and *M. corallinus*). We performed a PCA of all stolon traits measured for both above and below ground stolons (i.e. the highest node of emergence, average branches per node, and the length, width and leaf length of the longest stolon). PC1 explained a significant proportion of the variation (62.62%). We find a significant species by time point (^{2}=16.627, *df*=2, *p*=0.0002) and marginally significant type by time point interaction (^{2}=3.109, *df*=1, *p*=0.077), suggesting that the difference between species and between above and below ground stolons changed across development. Early in development, above and below ground stolons are similar and also similar across species (no significant effect of species (*p*=0.17) or type (*p*=0.65)), but differences- both between species and between types- grow as plants develop (early reproductive: significant species effect: ^{2}=18.3, *df*=2, *p*=0.0001; significant type effect: ^{2}=5.695, *df*=1, *p*=0.017; late reproductive: significant species effect: ^{2}=25.079, *df*=2, *p*<0.0001; significant type effect: ^{2}=31.007, *df*=1, *p*<0.0001). For all species, below ground stolons tend to be thinner, shorter, more branched, emerge at lower nodes, and have small, embryonic leaves. Qualitatively, they are also achlorotic (i.e. white), and far more prone to breakage than aboveground stolons. (A) Differences among species across development for the first PC of stolon morphology. (B) A biplot of stolon morphological differences between species across development, with the loading of each variable denoted by a red arrow. Traits: Xsto= Number of

stolons, Em= highest node of stolon emergence, Len= length, Wid= width, LL= stolon leaf length.

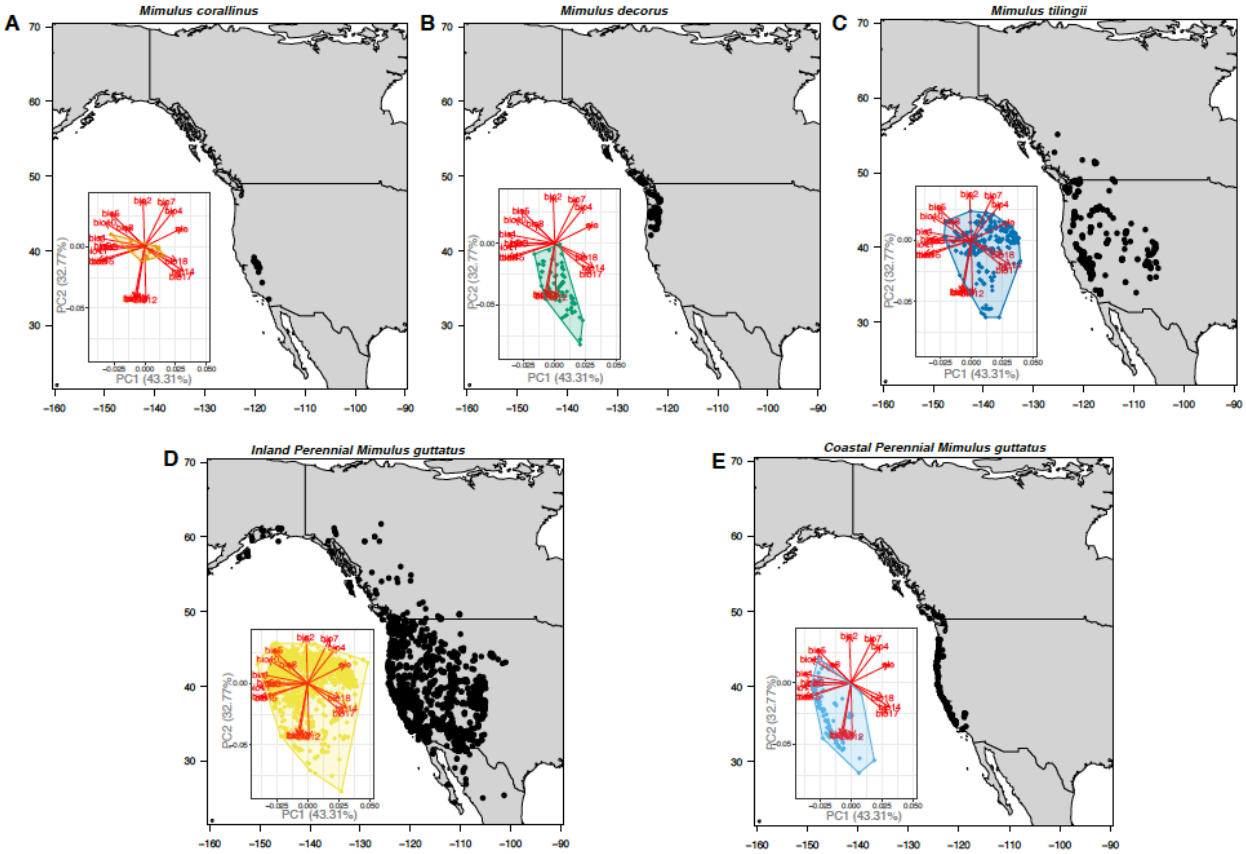


Figure S2: Raw geographic distributions and ecological niche for five perennial *Mimulus* taxa: *M. corallinus, M. decorus,* inland perennial *M. guttatus* (*M. guttatus sensu stricto,* according to the taxonomic revision prevented in Baker *et al.* 2012), coastal perennial *M. guttatus* (*M. grandis,* according to the taxonomic revision prevented in Baker *et al.* 2012), and *M. tilingii sensu lato*. Each point is a unique latitude/longitude combination based on concatenated herbarium records from Global Biodiversity Information Facility. Ecological niche was determined based on a PCA of 19 BioClim variables plus elevation. PCs 1 and 2 are plotted for each species in the inset.

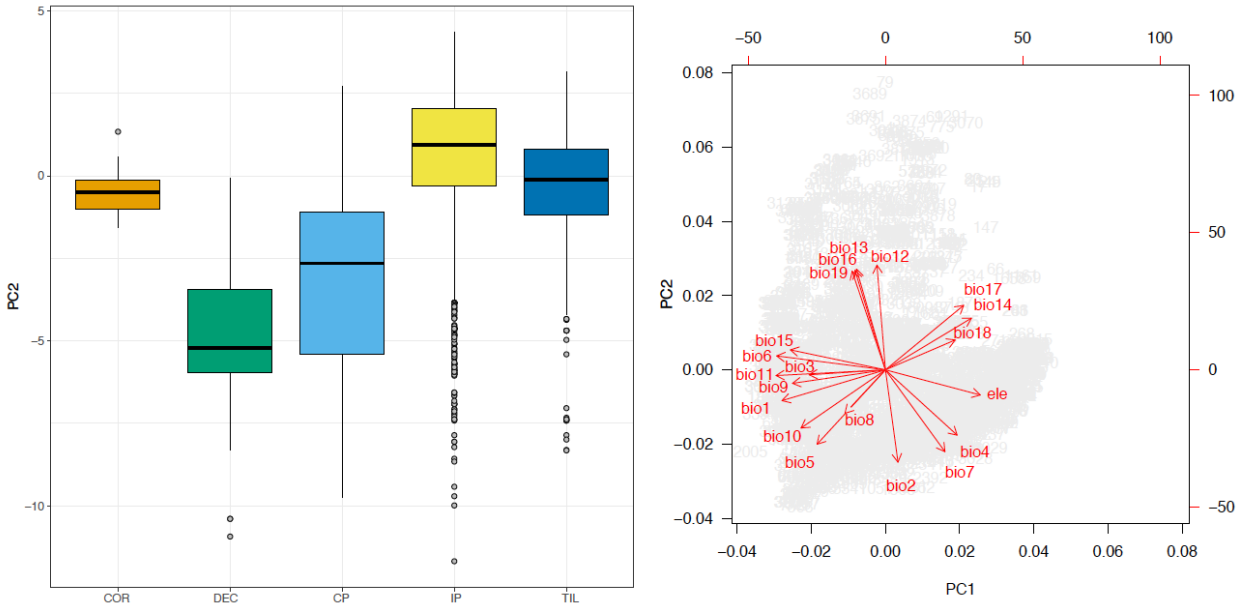


Figure S3: Climate PC2 (30.98% of the variation; Left) as a function of species. Right: Biplot for all 19 BIOCLIM variables and elevation for the climate PC.

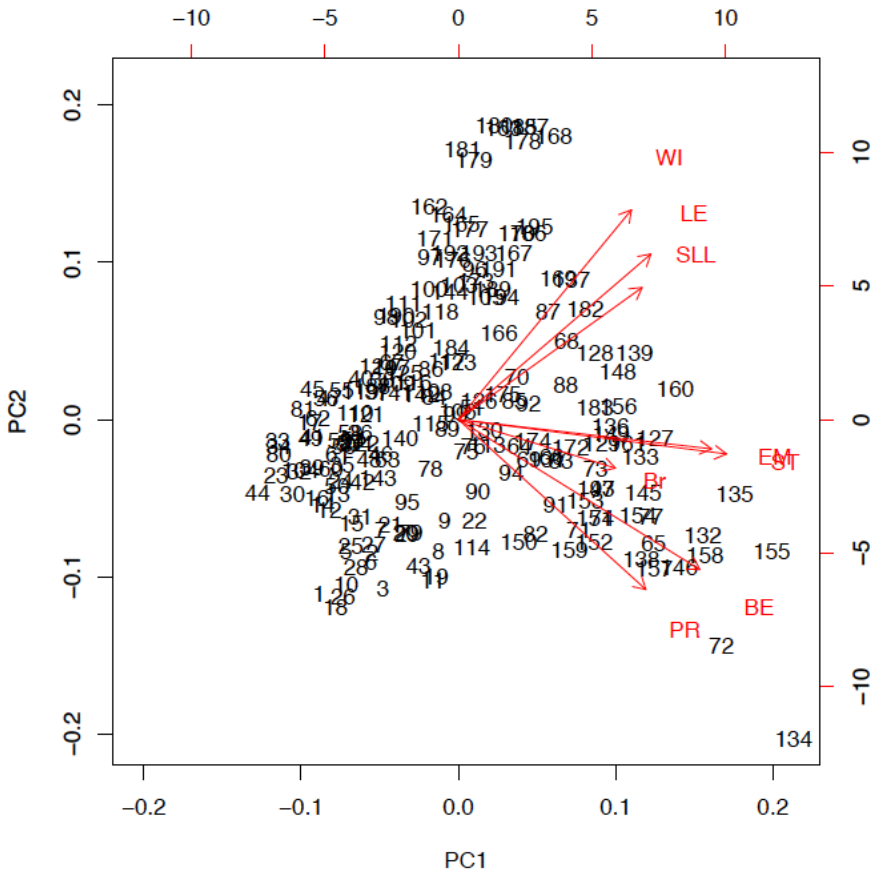


Figure S4: Biplot for a PCA involving life history traits across 40 populations of five perennial taxa across three developmental times. LE= stolon length, WI: stolon width, SLL= stolon leaf length, EM= highest node of emergence, ST= the number of stolons, BE= average number of branches per node, and PR= proportion belowground.

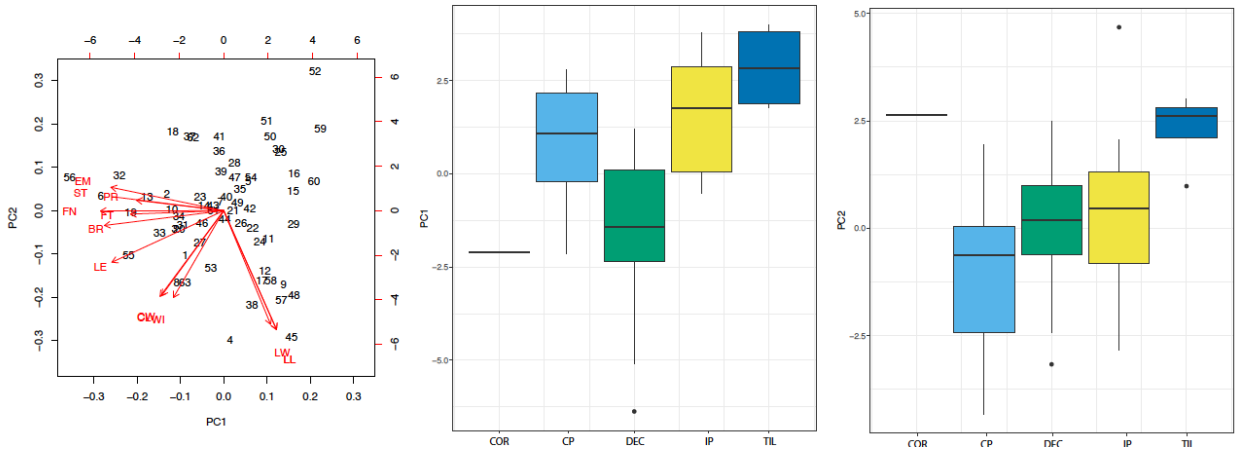


Figure S5: An additional PCA of both stolon traits and reproductive traits at only the day of first flower. Far Left: Biplot of the PCA, LE= stolon length, WI: stolon width, SLL= stolon leaf length, EM= highest node of emergence, ST= the number of stolons, BR= average number of branches per node, and PR= proportion belowground, CL= corolla length, CW= corolla width, LL= leaf length, LW= leaf width, FT= days til flower, FN= node of first flower. Middle: Values of PC1 across species (39.13% of the variation; Species effect PC1: ^{2}=40.266, *df*=4, *p*<0.0001). Right: values of PC2 across species (25.77% of the variation; species effect: PC2: ^{2}=18.764, *df*=4, *p*=0.00087).

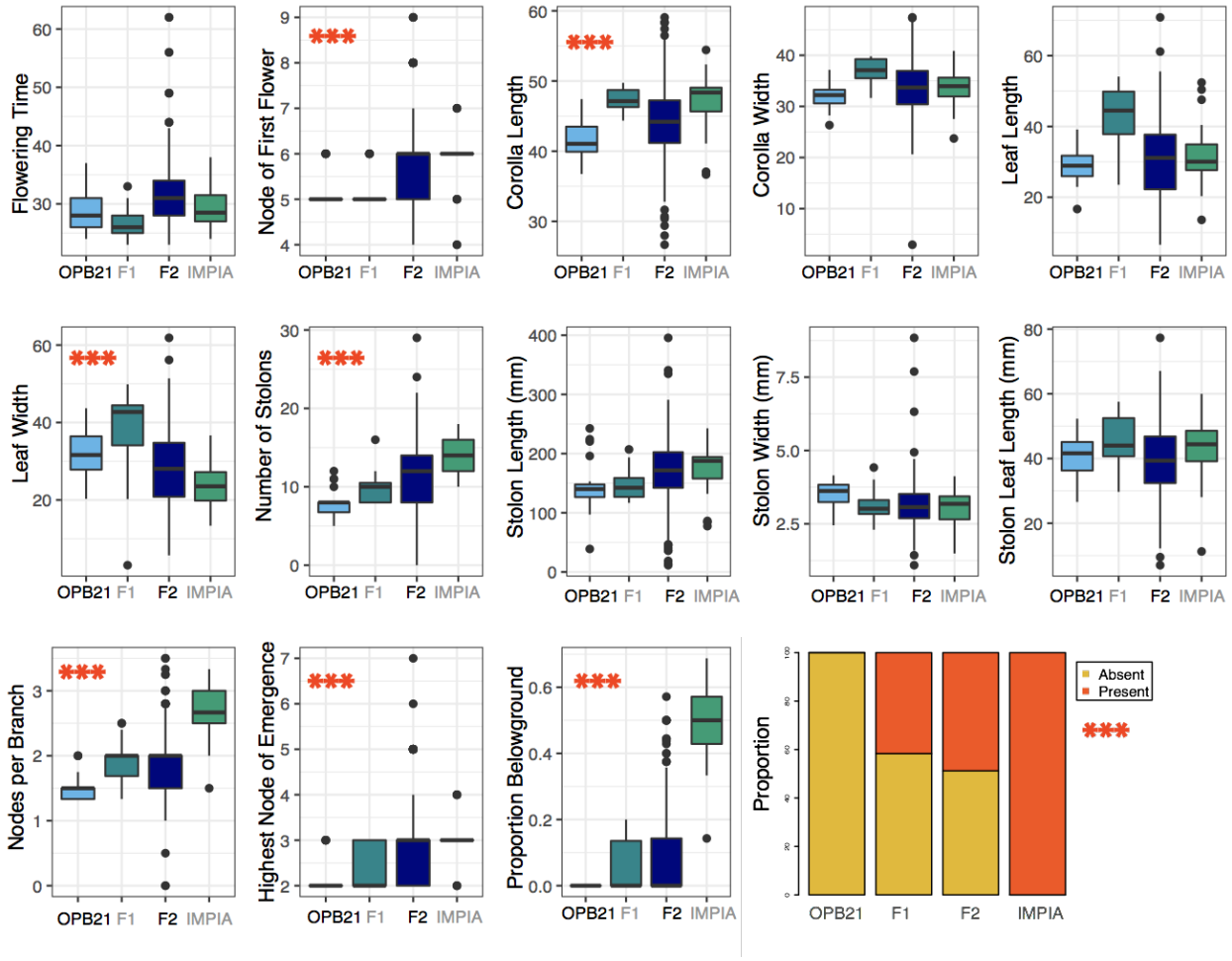
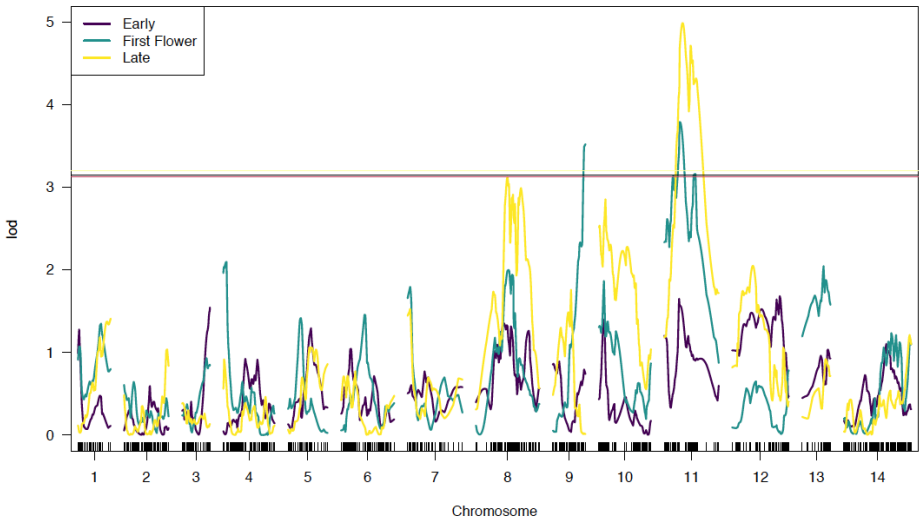
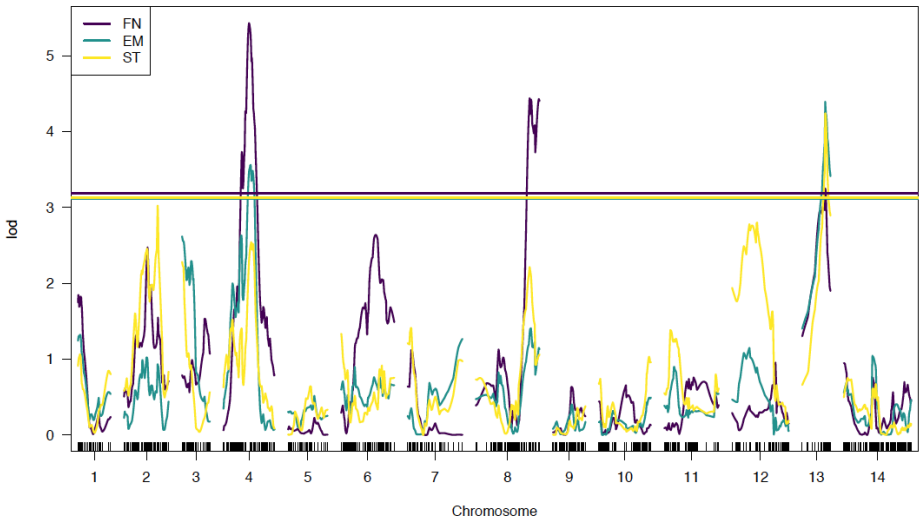


Figure S6: Trait distributions for each parental line and their hybrids. Significant differences between parental lines denoted by three red asterisks.

Figure S7: Representative pictures of parents and hybrids for the presence of below ground stolons. Top left: Coastal perennial *M. guttatus* (OPB; L), an F1 hybrid (middle), and *M. decorus* (IMP; R). Bottom Left: Three representative F2 individuals. The first produced no belowground stolons (L), only side branches that delve underground (middle; counted as not producing belowground stolons), and produces belowground stolons (R). Right; close up of belowground stolons in a representative F2: note that stolons still produce embryonic leaves, are white, and branched.


Early
First Flower
Late
lod
Chromosome


FN
EM
ST
lod
Chromosome

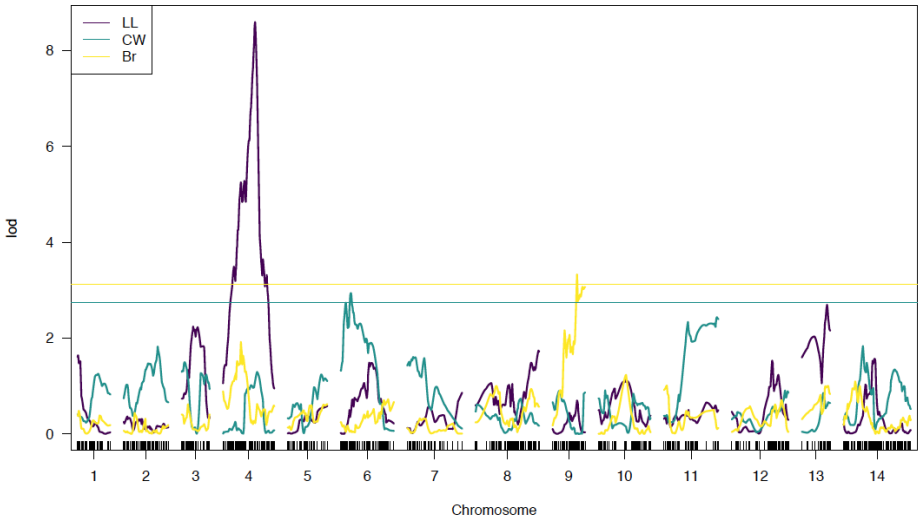

LL
CW
Br
lod
Chromosome

Figure S8: Likelihood of Odds (LOD) scores across all chromosomes for all traits that significantly differed between parental lines. Top panel: the proportion of below ground stolons across three developmental time periods: early (3 weeks post stratification), First Flower (the day of first flower), late (6 weeks post stratification). Middle panel: FN (node of first flower), EM (highest node of stolon emergence), ST (number of stolons). Bottom panel: LL (leaf length), CW (corolla width), Br (average branches per node on the longest stolon). Horizontal lines represent that significance threshold as determined by permutation for each trait.

Supplemental Tables

Table S1: Seed collections used in a common garden experiment.

Population	Number of Maternal Families	Species	Latitude	Longitude	Elevation (m)	Collector
EAGLEMEADOW	1	*M. corallinus*	38.32	-119.92	2046	Megan Peterson
SILVERCREEK	1	*M. corallinus*	38.588	-119.786	2066	Megan Peterson
SILVERFORK	1	*M. corallinus*	38.664	-120.219	1959	Megan Peterson
35-48	2	*M.decorus*	45.3054667	-121.66688	1280.16	JMC
BM	2	*M.decorus*	44.4985167	-121.98322	1079.2968	JMC
BR	2	*M.decorus*	44.371	-122.104	1189	JMC
DL	2	*M.decorus*	43.1575333	-122.13343	1576.7304	JMC
HACK	1	*M.decorus*	44.40278	-122.07556	1189	JMC
HAM	2	*M.decorus*	47.5651833	-123.03233	574	Carrie Wu
HJA	2	*M.decorus*	44.2332	-122.1762	888	JMC
HWY15	2	*M.decorus*			1189	JMC
HWY26	2	*M.decorus*	45.2921833	-121.73482	1169.2128	JMC
IMP	2	*M.decorus*	44.3934	-122.149	1676.11549	JMC
KINK	1	*M.decorus*	44.3032	-121.99622	1019.8608	JMC
LL	1	*M.decorus*	44.1739333	-122.0542	528.2184	JMC
NS	2	*M.decorus*	44.5237667	-121.99788	966.216	JMC
ODELL	2	*M.decorus*	43.54795	-121.96278	1457.8584	JMC

TOLL	1	*M.decorus*	45.3210833	-121.90702	530.352	JMC
TRILLII	2	*M.decorus*	45.2665833	-121.74163	1102.4616	JMC
WILD	1	*M.decorus*	45.3498667	-121.99295	365.1504	JMC
ZIG	2	*M.decorus*	45.31105	-121.8892	583.692	JMC
BCB	2	*M.grandis*			4.572	D. Lowry/K. Wright
CVG	2	*M.grandis*	38.3723	-123.05518	224.3328	D. Lowry/K. Wright
DUN	1	*M.grandis*	43.893056	-124.13778	0	D. Lowry/K. Wright
HOC	2	*M.grandis*	47.3854	-123.14728	4.572	D. Lowry/K. Wright
OPB	2	*M.grandis*	42.464017	-124.42292	7.62	D. Lowry/K. Wright
ORO	2	*M.grandis*	35.27335	-120.88912	4.572	D. Lowry/K. Wright
OSW	1	*M.grandis*	45.761083	-123.96657	4.572	D. Lowry/K. Wright
TSG	2	*M.grandis*	53.418833	-131.91573	4.572	D. Lowry
DESCHUTES	2	*M.guttatus*	43.848067	-121.7801	1377.0864	JMC
DNC	2	*M.guttatus*	41.812967	-124.08118	32.948	Jessica Sely
ICE-gutt	2	*M.guttatus*	47.54268	-120.707	786.5	Jessica Sely
NF48	2	*M.guttatus*	45.202083	-121.61492	1031.4432	JMC
NTL	2	*M.guttatus*	43.73045	-121.76328	1328.0136	JMC
SOC	2	*M.guttatus*	41.15748	-122.2907	626	Jessica Sely
TIH	2	*M.guttatus*	40.8764	-123.7563	1065.58	Jessica Sely
WEN	2	*M.guttatus*	47.36433	-120.863	866	Jessica Sely
BAG	2	*M.tilingii*	48.872683	-121.68758	1278.636	Carrie Wu

SOP	2	*M.tilingii*	38.266753	-119.61798	3169.92	Carrie Wu
TWN	2	*M.tilingii*	48.950433	-121.63607	1594.104	Carrie Wu

Table S2: Pearson correlation coefficients between pairs of life history traits across a population survey of 5 perennial taxa (lower left triangle) and within F2s (upper right triangle). FN= Node of first flower; CL= corolla length; LW= leaf width; ST= number of stolons; BR= average branches per node; EM= highest node of stolon emergence; PR= proportion of belowground stolons. Significant correlations (p<0.05) are bolded.

	FN	**CL**	**LW**	**ST**	**BR**	**EM**	**PR**
FN		**0.3**	-0.19	**0.76**	**0.66**	**0.73**	**0.51**
CL	0.09094028		0.15	0.13	**0.36**	0.1	**0.27**
LW	**-0.5645925**	0.04683288		-0.18	-0.16	**-0.28**	**-0.31**
ST	**0.50163204**	**0.14562208**	**-0.2818237**		**0.69**	**0.85**	**0.41**
BR	**0.22947167**	0.0896038	**-0.1898139**	**0.2272746**		**0.68**	**0.5**
EM	**0.6473864**	0.10939216	**-0.5060051**	**0.82404971**	**0.23453082**		**0.37**
PR	**0.1553752**	0.0710367	**-0.1635922**	**0.29215793**	0.05555646	**0.20730242**	

Table S3: Trait means for each parent (OPB= *M. guttatus;* IMP= *M. decorus*) and F1s, as well as the difference between parents, dominance coefficient, and broad sense heritability. Significance was determined by an ANOVA for all traits, except presence/absence, for which we performed a Fisher's Exact Test. Significance is denoted by astrisks: <0.1 +>0.05<0.05 *>0.01, <0.01 **>0.001, <0.001 *>0.0001**

Trait	OPB	F1	IMP	Parental Difference	broad sense heritability
Days to Flower	28.875	26.66667	13.13043	29.5	0.75282011
Node at First Flower	5.208333	5.166667	0.5797101	0.625*	0.80906798
Corolla Length	41.60435	47.38208	18.630835	5.42107**	0.89426937
Corolla Width	31.99957	37.06958	15.068094	1.48668	0.83554148
Leaf Length	28.80043	42.65	81.23007	3.03999	0.33804768
Leaf Width	32.06565	37.665	31.5171	8.32773*	0.25899042
Number of Stolons	7.75	9.833333	5.326087	6***	0.71177093
Stolon Length	144.0463	147.2745	1893.584	29.8441	0.74639957
Stolon Width	3.5225	3.10875	0.3589563	-0.430417	0.51592615
Stolon Leaf Length	40.83208	45.26042	106.81473	2.34125	0.54588417
Branchedness (average branches per node)	1.503472	1.898611	0.17697615	1.123611***	0.57380126
Highest Node of Stolon Emergence	2.166667	2.291667	0.3025362	0.875**	0.67590212
Proportion Belowground	0	0.06539352	0.01442334	0.47951389***	0.48057671
Presence/Absence(percentage)	0	41.66667	100	1***	

Table S4: Effect size for each QTL for each trait (significance determined by 1,000 permutations). GG,GD,DD are the trait means for individuals homozygous for the *M. guttatus* allele (GG), *M. decorus* allele (DD), or heterozygous (GD). D(Alt.Homo)= difference in trait means between alternate homozygotes. D(par)=the difference in trait means between 24 replicates of each parental line. %explained (par)= the effect size/parental difference. %explained (F2_range)= the effect size/range of trait values for F2s. %explained (F2.Vg) is the effect size/(square root(proportion of the genetic variation (Vg)). Total is the total % explained under an additive model, and in the case of the proportion of belowground stolons, a model with non-additive effects between two significant QTL. PR= Proportion of belowground stolons, FN= Node of first flower, ST= number of Stolons, CL= corolla length, LL= Leaf Length, BR= average branches per node, EM= highest node of stolon emergence.

Pheno	CHR	POS	LOD	GG	GD	DD	D(Alt.Homo)	D(par)	%explained (par)	%explained (F2.range)	%explained (F2.Vg)
PR	9	60.9	3.52	0.064	0.086	0.176	0.11152	0.480	23.257	19.565	138.618
PR	11	28.8	3.79	0.0315	0.1094	0.1055	0.07399	0.480	15.431	12.981	91.973
								total (additive):	**38.687**	**32.546**	**230.591**
								total (interaction):	**48.454**	**40.762**	**288.801**
FN	4	49	5.43	6.488	5.76	5.756	-0.732	0.625	117.073	14.634	93.370
FN	8	100.482	4.44	6	5.846	6.359	0.359	0.625	57.436	7.180	45.807
FN	13	42.966	3.25	5.609	5.896	6.273	0.664	0.625	106.245	13.281	84.734
								total (additive):	**280.754**	**35.094**	**223.912**
ST	4	52	2.54	12.897	10.681	12.45	-0.447	6	7.457	1.543	14.135
ST	13	43	4.24	10.174	11.113	13.1272	2.9534	6	49.222	10.184	93.296
								total (additive):	**56.68**	**11.727**	**107.43**

CL	6	19	2.94	45.807	45.435	41.127	-4.680	5.421	86.321	14.442	98.243
								total (additive):	**86.321**	**14.442**	**98.243**
LL	4	60	8.59	21.841	34.827	33.931	12.091	3.04	397.721	18.824	189.055
								total (additive):	**397.721**	**18.824**	**189.055**
BR	9	45.7	3.33	1.956	1.728	2.068	0.112	1.124	9.96	3.198	29.690
								total (additive):	**9.96**	**3.198**	**29.69**
EM	4	51	3.56	3.290	2.606	2.842	-0.447	0.875	51.128	8.947	66.72
EM	13	43	4.39	2.478	2.783	3.2	0.722	0.875	82.484	14.43	107.64
								total (additive):	**133.612**	**23.382**	**174.360**