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

Authors: Jenn M. Coughlan[1,2], Maya Wilson Brown[1,3] & J.H. Willis[1]

Affiliations

1. Biology Department, Duke University, 125 Science Dr. Durham, NC, 27708
2. Biology Department, University of North Carolina, Chapel Hill, 250 Bell Tower Dr. Chapel Hill, NC, 27599.
3. Department of Plant Biology, Michigan State University, 612 Wilson Rd, East Lansing, MI, 48824.

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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 in geographic and ecological distributions. Divergent natural selection imposed by ecological differences is a major source of phenotypic differentiation between closely related species [1–3]. Such phenotypic differentiation can promote species coexistence at broader geographic scales [4–7], and contribute to reproductive isolation [8–11]. 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 [12]. 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 [13,14]. Alternatively, if traits are controlled by many loci, only some of which have correlated effects on multiple traits, then evolution may be more flexible [15–17].

One cause of phenotypic divergence among closely related plant species is elevation [18,19]. Plant populations predictably differ in phenotypes along elevational gradients; both within species [20–25], and between close relatives [26–28]. Phenotypes can diverge along elevational gradients in response to water availability, herbivore pressures, climate, growing season, or a combination of factors [21,22,25,29–31]. 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 transitions to reproduction, investment in vegetative biomass, and the timing of senescence [20–22,27,32], as well as unique phenotypes, like shorter stature [33], changes in leaf physiology [34], or production of belowground morphological structures [35,36].

Importantly, investment in traits that confer adaptation to high elevation habitats may also change with ontogeny. This can occur simply because more time has passed for particular morphological features to develop (e.g. investment in vegetative biomass; [37]), and/or because the importance of different selective pressures can change throughout the course of development [38,39]. If closely related species or populations differ in the production and/or timing 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 [40]).

The *Mimulus guttatus* species complex (Section *Simiolus*, DC, Phrymaceae) is a unique system to study trait divergence among closely related species. The complex comprises about a dozen closely related species that exhibit substantial phenotypic variation, and inhabit a broad altitudinal range across the Pacific Northwest [41,42]. A major axis of ecologically associated phenotypic variation is life history, including highly selfing, rapidly reproducing annual species, diminutive, but outcrossing annual species, and several perennial taxa [28,41–45]. A considerable effort has been made to characterize life history in this group, although most studies have focused on variation within individual annual species [21,30,46–49], between closely related annual species [50–53], or between annuals and perennials [14,31,43,54–62]. Very little is known about the variation in ecologically associated trait divergence among perennial taxa in this group, despite the fact that 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 closely related 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 taxonomic designations in the *M. guttatus* species complex are debated [63–65], at least five known genetically distinct perennial taxa are recognized: *M. tilingii*

143 (*sensu lato*)*, M. decorus, M. corallinus,* and two ecotypes of perennial *M. guttatus;*
144 coastal perennials (sometimes referred to as *M. grandis*; [63]) and inland perennials
145 (sometimes referred to as *M. guttatus sensu stricto*; [63]). *Mimulus decorus* and *M.*
146 *tilingii* both comprise multiple genetic lineages [44,63], but as these lineages form
147 monophyletic groups, we include each as a single taxa.
148 Perenniality in the *M. guttatus* species complex is defined by the production of
149 stolons. Stolons are horizontally growing, vegetative stems that develop from axillary
150 meristems at basal nodes of a plant. Although vegetative at first, stolons have the ability
151 to become reproductive. Thus early investment in vegetative biomass can provide later
152 reproductive rewards. In addition to above ground stolons, some perennials produce
153 stolons that grow just below the soil's surface (sometimes referred to as rhizomes;
154 [66,67]). Belowground stolons, like their above ground counterparts, are horizontally
155 growing, vegetative stems that originate above ground. However, once underground,
156 their morphology changes; they become high branched, achlorotic (i.e. white), and
157 repress leaf growth (Fig. S1, Fig. S7). Belowground stolons can eventually re-emerge
158 above ground, wherein, their morphology reverts to that of a typical above ground
159 stolon, including the potential to become reproductive. Belowground stolons thus
160 represent a unique axis of investment in vegetative biomass, and may provide
161 additional benefits in high elevation habitats (discussed below), but little is known about
162 their ecological or phylogenetic distribution or their genetic architecture.
163
164 *Estimating ecological distributions of perennials of the* M. guttatus *species complex*
165
166 To categorize the climate niche, we compiled occurrence records from Global
167 Biodiversity Information Facility (GBIF) for each species within the native range and
168 combined these with collection records from the *Mimulus* community (available:
169 http://mimubase.org/). These ranges largely agree with previously published ranges
170 [66], although we include more northerly occurrence records for *M. decorus* than
171 described in [66]. For records in which elevation was not recorded, we estimated
172 elevation using the USGS National elevation map
173 (https://viewer.nationalmap.gov/theme/elevation/##bottom) or the R package *raster* [68].

We then downloaded BIOCLIM variables for all unique locations for each species at a resolution of 10 arc-degrees using the *raster* package. We performed a PCA on all BIOCLIM variables plus elevation using the R function *prcomp* with the variables scaled to have unit variance. A broken stick model [69] determined that PCs 1 and 2 explained significantly more variation than expected. We assessed if species differed in climate niche by performing an ANOVA using the *aov* function for each significant PC with species identity as the independent variable, and pairwise T-tests with Holm correction for multiple testing to determine which species varied. All statistical analyses were carried out in R [70].

Quantifying life history variation in perennials of the M. guttatus *species complex*

In order to assess trait divergence among perennials of the *M. guttatus* species complex, we performed two common garden experiments. For both, 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 (with an 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 (outlined below). 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 roots and stolons were 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). In addition, 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 (e.g. plants were unpotted to measure below ground traits), 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 perennial members of the *M. guttatus* species complex in a common garden. Many life history traits are correlated (Table S2), and we therefore 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 at which stolons were emerging, the number and proportion of belowground stolons, and the length, width, and leaf length of above ground stolons. PC1 explained 49.82% of the variance and was the only significant PC according to a broken stick model. We then performed linear mixed models using the *lme4* and *car* packages in R [71,72] 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 developmental 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 [71,72].

We next determined whether variation in life history among populations was related to a population's source environment. We used 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 also performed submodels with a species by elevation interaction, but this term was not significant at any developmental time point (Vegetative: *p*=0.84 Early reproductive: *p*=0.88; Late reproductive: *p*=0.29), these models had higher AIC scores (Vegetative: 211 vs 170; Early reproductive: 249 vs 210; Late reproductive: 258 vs 214) and models were not significantly improved by the addition of this interaction term (Vegetative: *p*=0.76; Early reproductive: *p*=0.84; Late reproductive: *p*=0.2). Therefore the interaction term was dropped.

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* [14,56,57]. To determine if perennials exhibited an association between timing to reproduction and investment in stolons 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. We additionally performed linear mixed models that included a species by stolon number interaction, but this term was not significant (*p*=0.59), a model which included an interaction term had a higher AIC (205 vs 213) and was not significantly improved (*p*=0.5). We therefore dropped the interaction term.

Determining the genetic basis of life history divergence among perennials

I. F2 grow out and trait measurement

We concurrently grew 429 F2s derived from a single self-fertilized F1 hybrid between *M. decorus* (IMP) and coastal perennial *M. guttatus* (OPB). These parents vary in several perennial traits, including the total number of stolons produced, the production of belowground stolons, and flowering time (Table S3; Fig. S6). We additionally grew 24

267 replicates of each inbred parental line and 24 F1s. Due to an asymmetric seed barrier
268 between *M. decorus* and *M. guttatus* [44], all F1s were created with *M. guttatus* as the
269 maternal donor.
270 Individuals were surveyed at the same developmental time points as above, but,
271 we did not destructively survey plants. Therefore, at the three and six week post
272 stratification time points individuals were assessed only for the number of above and
273 belowground stolons. On the day of first flower, individuals were assessed for flowering
274 time (date and node of first flower), corolla length and width, length and width of the first
275 true leaf, and the highest node of stolon emergence, and above ground stolons were
276 measured for several traits (length, width, leaf length, number of branches per node,
277 highest node of emergence). A total of 410 F2 individuals were phenotyped, as well as
278 24 of each parental line and F1.
279
280 *II. Library construction and sequencing of F2s*
281
282 We extracted DNA from F2 individuals using a modified CTAB protocol from fresh leaf
283 and bud tissue ([73]; dx.doi.org/10.17504/protocols.io.bgv6jw9e). We created libraries
284 for 384 F2s and each parental line using a modified Multiplexed Shotgun Genotyping
285 approach with the enzyme Csp6I ([74]; dx.doi.org/10.17504/protocols.io.bbjbikin),
286 wherein each individual sample contained a unique barcode-adapter combination. All
287 eight libraries were split across 2 lanes of Illumina 4000 High Seq at the Duke
288 Sequencing Facility, for an average of 97.4 million reads per library (~2 million reads
289 per F2 individual).
290
291 *III. Read processing, linkage map construction, genotype estimation & QTL mapping*
292
293 Reads were demultiplexed using Stacks 2.2 [75,76]. Each .fastq file was aligned to the
294 V2.0 *M.guttatus* reference genome (https://phytozome.jgi.doe.gov/) using the BWA
295 *mem* function [77]. All .bam files were cleaned and sorted using Picard tools
296 (http://broadinstitute.github.io/picard/). We used GATK to call SNPs for each sample
297 individually, then all individuals were jointly genotyped using *GenotypeGVCFs* [78]. We

298 then removed indels and included only sites with a minimum genotype quality of 30
299 using VCFtools [79]. We polarized SNPs in the F2s based on sites that were alternative
300 homozygotes in the parental inbred lines, and determined genotype markers by using
301 the number of counts for each parental allele across 100kb windows. A window was
302 determined to be homozygous if >95% of reads were of one parent, and heterozygous if
303 the percent of reads were between 0.1 and 0.9. We then filtered the dataset to remove
304 individuals with very low genotyping (less than 50 markers across the genome) and
305 markers with genotype information for <10% of individuals.
306 We implemented GOOGA [80] to estimate individual genotyping error rates,
307 construct a linkage map, and estimate genotype posterior probabilities for each
308 individual at each marker. We defined genotypes using a posterior probability >/=0.95.
309 We removed individuals with substantial missing data (>90% missing data) or severely
310 distorted overall genotypic compositions (<20% or >80% heterozygous calls). The
311 remaining dataset comprised 213 individuals with 1,311 markers.
312 We used the *scanone* function in the R/*qtl* [81,82] with Haley-Knott regressions to
313 identify LOD scores, set significance thresholds using 1,000 permutations, and define
314 the 1.5 LOD interval for each significant QTL. For one QTL on LG4 which affected
315 flowering time and the highest node of stolon emergence, we find a modest effect on
316 the number of stolons, although this did not pass the genome-wide permutation
317 threshold. However, we include this QTL in our final map, as single marker analysis
318 using the *fitqtl* function determined there was a significant association between this
319 marker and the number of stolons (F=2.33, p=0.033). For traits in which more than one
320 QTL reached significance, we assessed non-additive interactions among QTLs using
321 the *addint* function in R/qtl. Except in one case, we do not recover any significant
322 interactions among QTLs. We calculated effect sizes of each QTL as the difference
323 between alternate homozygotes as well as the percentage of the parental difference
324 explained. We lastly estimated how much phenotypic variance in the F2 was explained
325 by each QTL in two ways. First, we estimated Vg (proportion of phenotypic variation that
326 could be explained by genetic variation) for each trait by subtracting the phenotypic
327 variance observed in F1s from the phenotypic variance in F2s. As variance is measured
328 in the square of the unit of measurement for each trait, we then calculated the difference

329 between homozygotes divided by the square root of Vg for each QTL to estimate the
330 proportion of heritable genetic variation explained by each QTL. Second, we calculated
331 how much of the range of F2 phenotypes was explained by each QTL as the difference
332 in phenotype between alternate homozygotes as a proportion of the difference in
333 phenotype between the two most extreme F2 individuals.
334
335 **Results:**
336 *Perennial taxa vary in both climate niche and life history*
337
338 Perennial taxa of the *M. guttatus* species complex differ in their geographic and
339 environmental distributions (Fig. 1). Four of five taxa are relatively restricted
340 geographically and ecologically, while inland perennial *M. guttatus* is geographically and
341 ecologically widespread. Of the more restricted taxa, three inhabit almost exclusively
342 mid to high elevation habitats, with *M. tilingii* exhibiting a more broad distribution across
343 the Sierras, Cascades, Rockies, and Pacific Coastal ranges (although it is restricted to
344 high elevation habitats within these mountain ranges; Fig. S2; Fig. S3) In contrast, *M.*
345 *decorus* and *M. corallinus* exhibit relatively restricted ranges, with *M. corallinus* found
346 exclusively at mid to high elevation sites in the Sierras. Coastal perennial *M. guttatus*
347 tends to inhabit coastal cliffs and seeps, and are found largely at low elevation along the
348 Pacific coast. The climate niches of these habitats are summarized by PC1 and PC2,
349 which explain 44.41% and 30.98% of the variance, respectively, and for which species
350 show significant divergence (PC1: F=96.2, df=4, p<0.0001; PC2: F=189.1, df=4,
351 p<0.0001; Fig. 1C; Fig. S3). Higher values of PC1 represent habitats at higher
352 elevation, with more precipitation in the hottest and driest quarters, and higher
353 seasonality, while higher values of PC2 represent habitats with greater precipitation
354 overall and in the wettest month and quarter (Fig. S3). Thus *M. decorus, M. corallinus,*
355 and *M. tilingii* tend to live in more seasonal, high elevation habitats.
356

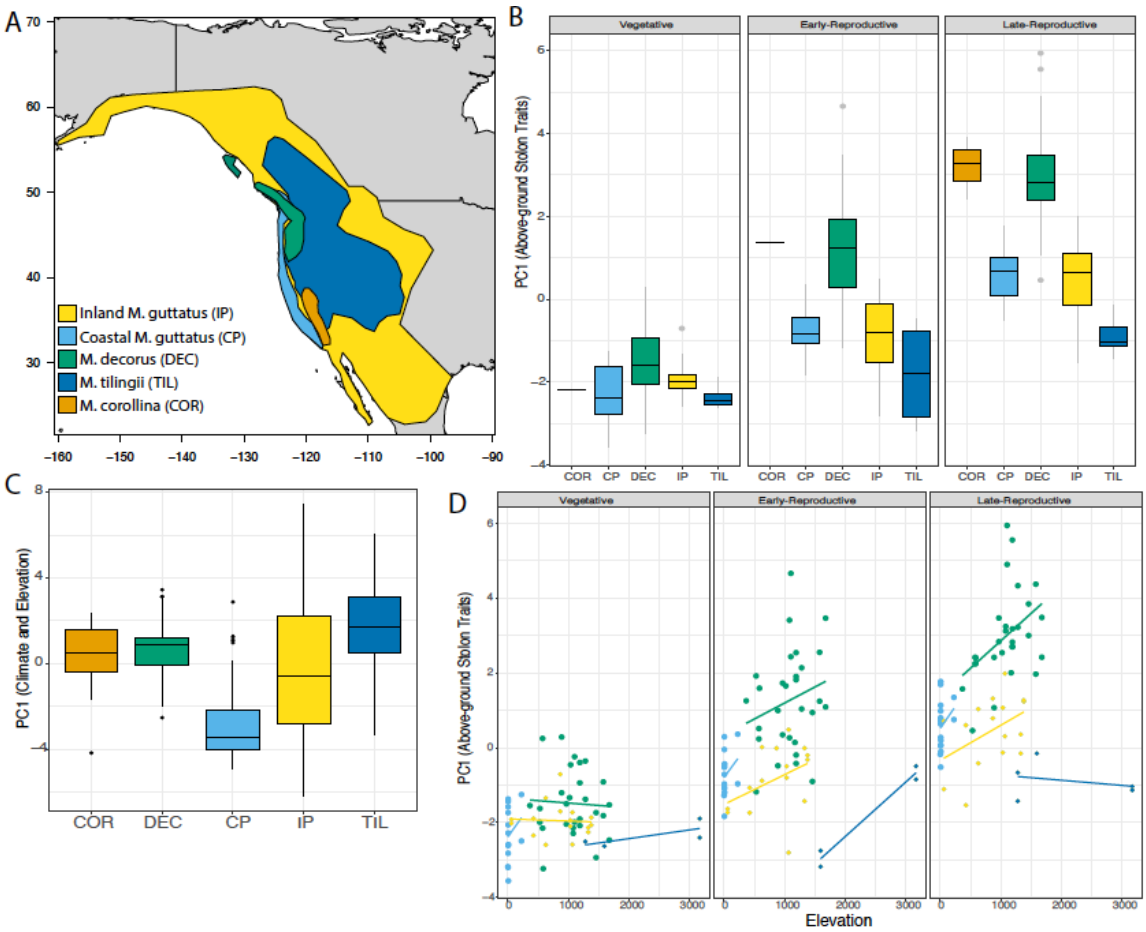

Fig.1: Perennials vary in niche and life history. (A) Geographic ranges of different perennials based on herbarium records. (B) Perennials vary in life history, and these differences extenuate with developmental time (panels). Greater values of PC1 indicate more investment in vegetative biomass. (C) PC1 of climate and elevation across species. Greater values indicate more seasonal environments at higher elevation with greater precipitation in the driest quarter and month. (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, but the extent of differentiation among taxa depends on developmental time. Perennial taxa vary along PC1 (which explained 49.82% of the variance) at all time points, 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 aboveground 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 overall stolons. A PCA of traits measured on the day of first-flower 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).

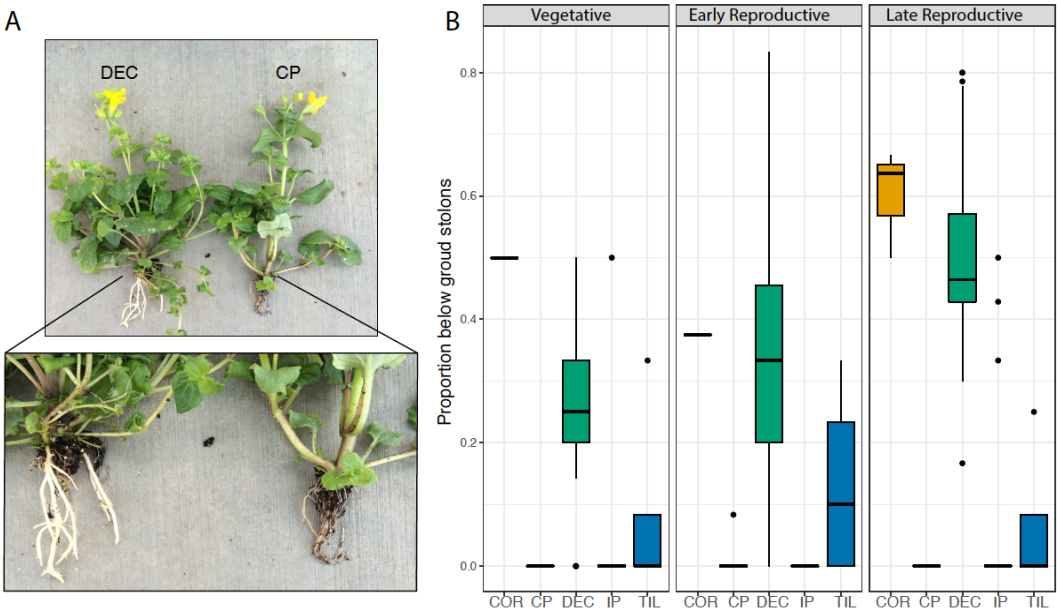

Fig.2: Mid and high elevation perennials make belowground stolons. (A) Representative *M. decorus* and coastal perennial *M. guttatus*. Both plants make above ground stolons, but only *M. decorus* produces belowground stolons. (B) Proportion of belowground stolons across development by species. COR= *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: ^{2}=4.969, *df*=1, *p*=0.026). Populations that exist at higher elevations invest more in vegetative biomass; they produce more stolons, and stolons that emerge at higher nodes and are more branched (Fig. 1D; Fig. S4). For species that produce belowground stolons, higher elevation populations also tend to produce more belowground stolons.

We also scored the presence of a unique life history trait; the production of belowground stolons. Belowground stolons are thinner, shorter, and more branched than their above ground counterparts (Fig. S1). They also lack chlorophyll, are substantially more prone to breakage, and repress the growth of vegetative structures relative to above ground stolons (Fig. S1; personal obs). Three of five perennial taxa produced belowground stolons (Fig. 2), all of which live exclusively in mid to high elevation habitats (Fig. 1C).

The genetic basis of life history variation among perennials is relatively simple

In order to dissect the genetic architecture of life history differences between two perennial members of the *M. guttatus* species complex, we constructed an F2 mapping population between *M. decorus* and coastal perennial *M. guttatus*. These two parents vary in a number of life history traits (Table S3; Fig. S6), including flowering time and size, leaf size, investment in stolons, and the presence of belowground stolons, and this is representative of differences among the two species (Fig. 1). Using reduced representation sequencing, we constructed a linkage map using 1,311 markers across 14 chromosomes. The total map length was 1,232.3cM (range: 52.1-125.8cM per linkage group), with an average marker spacing of 1cM.

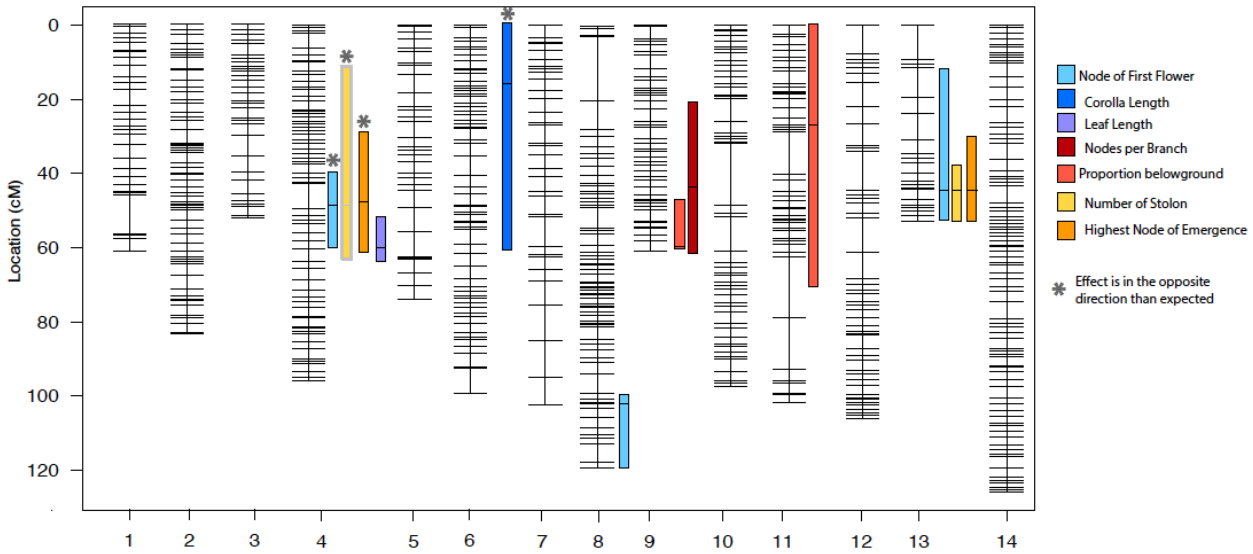

Fig. 3: QTL map of life history traits between coastal perennial *M. guttatus* and *M. decorus*. Each colored box represents the 1.5 LOD interval surrounding significant associations (thick horizontal line). Asterisks indicate that the effect of the QTL is opposite to the expected effect, based on the parental difference. One QTL on LG4 is highlighted in grey, as this QTL did not pass a genome-wide significance threshold, but was found to be significantly associated based on individual marker analysis.

The genetic architecture of several quantitative 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. On average, 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). 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 (as well as some individual QTLs effects) 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; ^{2}=47.4; *df*=1; *p*<0.0001, with a marginally significant species effect: ^{2}=9.11; *df*=4; *p*=0.06). Correlations between time to reproduction and investment in vegetative growth via stolons persisted among F2s between coastal perennial *M. guttatus* and *M. decorus,* suggesting that they are genetic (Fig. 4; r^2=0.54, *p*<0.0001; Table S2). In line with this, we find that these traits have a shared genetic basis; QTLs on LG4 and LG13 affect flowering time, highest node of emergence, and stolon number (Fig. 3; Table S4). The allelic effects of these two QTL are opposite; for LG4 the *M. guttatus* allele is associated with later flowering and more stolons (typically *M. decorus* traits), while the LG13 QTL shows effects in the predicted direction (Fig. 4; Table S4). We also observe some transgressive segregation for both of these traits among F2s (Fig. 4).

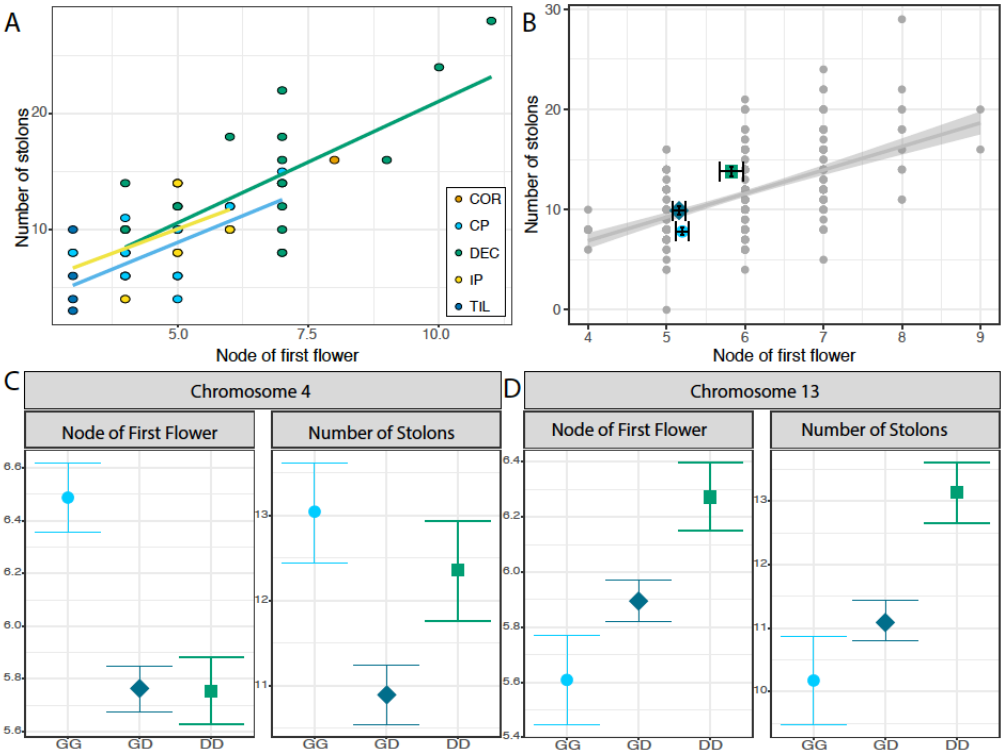

Fig. 4: Life history traits covary among perennial species, and share a genetic basis. (A) Phenotypic correlation between node of first-flower and number of stolons across 40 populations of 5 perennial taxa. COR= *M. corallinus,* DEC= *M. decorus,* TIL= *M. tilingii,* IP= inland perennial *M. guttatus,* CP= coastal perennial *M. guttatus.* Lines represent linear regressions for each species. (B) Genetic

correlations between flowering time and number of stolons in F2s derived between *M. decorus* and coastal perennial *M. guttatus.* Grey circles are individual F2s, blue circle = trait average for replicates of the coastal perennial *M. guttatus* parent (OPB), turquoise diamond = trait average for replicate F1s, and green square = trait average for replicates of the *M. decorus* parent (IMP). (C,D) Phenotypic effects of two QTL on flowering time and the number of stolons. Error bars represent standard errors.

Lastly, we tracked the genetic architecture of investment in belowground stolons through development. We find that the production of belowground stolons changes through development in hybrids and as such, so does the genetic architecture of this trait. Early on, very few F1s or F2s make belowground stolons, and we are unable to detect any significant QTL associated with this trait while plants are vegetative (Fig. 5). At reproduction, a larger number of hybrids produce belowground stolons, but the trait remains largely recessive. At this developmental time point, 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 hybrids (both F1s and F2s) make belowground stolons. At this point, no interactive effects are detected (p=0.89), and a single locus (marker 940 on LG11) 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), we note that these traits do not appear to segregate independently in F2s (personal obs; Fig. S7).

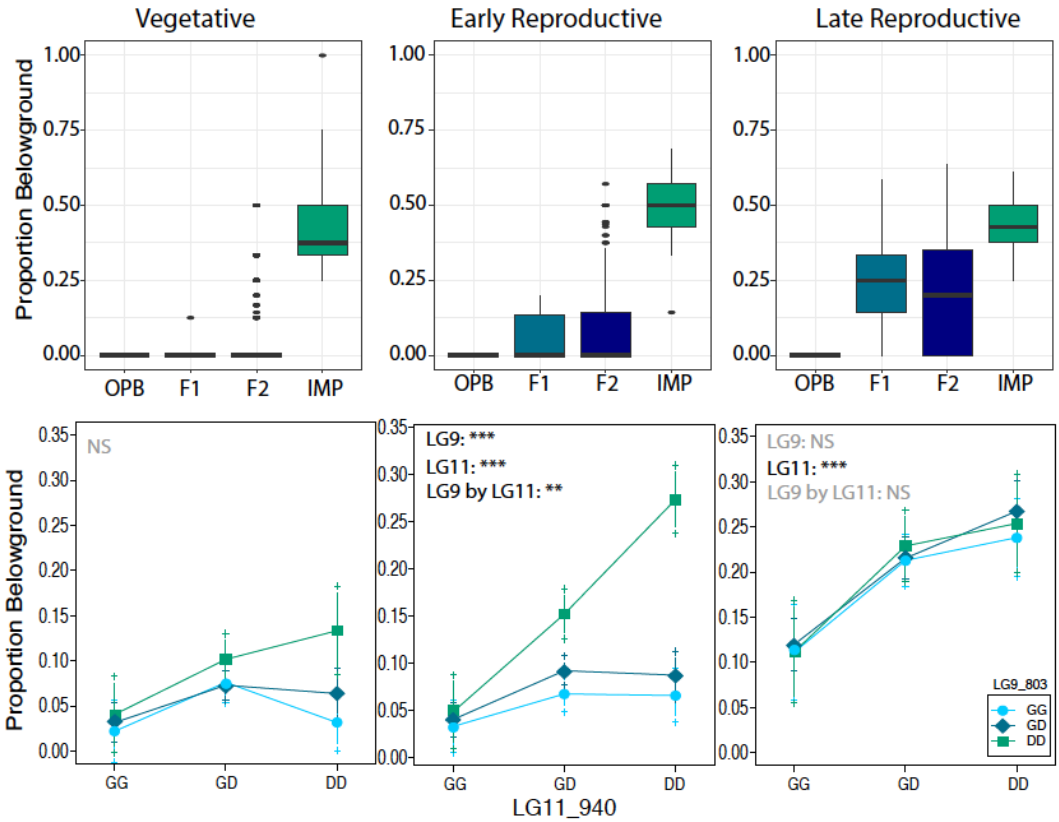

Fig. 5: The genetic architecture of ability to produce belowground stolons changes across development. Top row: trait distributions for each parent (OPB= coastal perennial *M. guttatus* and IMP= *M. decorus*), F1s, and F2s. Bottom row: Effect of two markers on the proportion of belowground stolons, with the significance of each marker and their interaction 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 perennial taxa live in a variety of habitats, though many taxa are restricted to mid to high elevations. Perennials also vary in life history, but the extent of life history 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, elevation of origin is associated with investment in vegetative biomass. Together, this suggests 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 species

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. These clines are particularly apparent in inland perennial *M. guttatus* and *M. decorus*, which live across a broader range of elevations, and for which we were able to survey several populations. For both species, individuals from higher elevation populations produced more stolons, that were more branched, and continued to produce stolons at higher nodes. 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 eventually become reproductive. In line with this, perennials have higher fitness than annuals in a high elevation common garden [28]. Similarly, adaptive clines in investment in growth and delayed reproduction exist along elevational gradients of annual *M. guttatus* [21,30]**,** as well as other perennial taxa [20,22,24,26,32].

Although populations of *M. tilingii* from higher elevations also tend to produce more vegetative biomass than those from lower elevations, the species as a whole invested the least in vegetative biomass in our common garden experiment (as well as a previous common garden experiment [44,83]), despite living at the highest elevation. Phenotypic descriptions of *M. tilingii* from field collected plants suggest that *M. tilingii* invests quite heavily in vegetative biomass in nature [66,67]. Differences between wild plants and plants grown in a common garden may stem from plastic responses to environmental differences, wherein high elevation habitats stimulate more investment in vegetative biomass. Alternatively, they may also arise from differences in age, wherein

536 naturally collected plants tend to be older and therefore have had more time to invest in
537 vegetative biomass.
538 We also find the taxa that are restricted to mid to high elevation habitats exhibit a
539 novel trait relative to most *M. guttatus* populations; the presence of belowground
540 stolons. One hypothesis for the presence of belowground stolons in high elevation
541 plants is that these stolons may afford perennials a greater chance of survival in
542 environments with extreme cold temperatures and sudden temperature drops. While
543 uncommon in our experiment, we find that 4/48 inland perennial *M. guttatus* individuals
544 produced belowground stolons during the course of the population survey. These
545 individuals hailed from 2 mid-elevation sites from the California Coastal Range and
546 Cascade mountains of Washington (1,065m and 866m, respectively). High elevation
547 populations of *M. guttatus* can co-occur with *M. decorus* and/or *M. tilingii,* and this trait
548 may have introgressed between species. Belowground stolons may also have evolved
549 independently in each species or existed as a shared polymorphism in the ancestor of
550 all four taxa (as discussed in [9,84,85]). Alternatively, belowground stolons may
551 represent an ancestral state, wherein *M. guttatus* has lost this character with the
552 colonization of low elevation habitats. Further population genetics studies will be
553 required to test these potential scenarios.
554
555 *The genetic architecture of life history divergence is relatively simple*
556
557 We find that life history divergence between two perennial species has a relatively
558 simple genetic basis. One to three QTLs explain a significant proportion of the parental
559 difference in most life history traits, and the effects of these QTLs are largely additive.
560 This is much simpler than the genetic architecture of life history divergence in other
561 systems [14,48,50,55,86–88]. One explanation for why some genetic architectures are
562 complex versus simple is how natural selection acts on the trait in question [89,90].
563 Strong, divergent natural selection between populations is expected to result in more
564 simple genetic architectures than stabilizing or balancing selection within populations
565 [89–91], and this is true for a number of examples of adaptations to discrete habitats
566 [9,92,93]. In the case of life history divergence between coastal perennial *M. guttatus*

567 and *M. decorus*, we suggest that trait differences may have arisen due to divergent
568 natural selection via elevation. Further reciprocal transplants or manipulative common
569 gardens will be needed to assess the agents, strength, and targets of natural selection
570 in this system.

571 Despite the general simplicity of our mapping results, we uncover some curious
572 features of life history divergence between coastal perennial *M. guttatus* and *M.*
573 *decorus.* First, we find the presence of two QTLs that are associated with three highly
574 correlated traits; stolon number, flowering time, and the highest node of stolon
575 emergence. QTLs that affect both flowering time and stolon number have been
576 described between annual and perennial *Mimulus guttatus* [14,55,57], and may
577 represent a genetic control of early meristem commitment (e.g. production of floral buds
578 versus stolons; [60,94]). Interestingly, the LG4 QTL exhibits opposite allelic effects than
579 predicted by the parental difference. Consequently, a small proportion of F2s that
580 exhibit transgressive phenotypes- delayed flowering and the production of more stolons
581 than either parent. Such extreme phenotypes may have negative fitness consequences
582 in nature, highlighting a potential for reproductive isolation among perennials.

583 Second, we find that the genetic architecture of the proportion of belowground
584 stolons changes with development, and this is due to a change in dominance through
585 development. Early in reproduction, the ability to produce belowground stolons is
586 recessive, and is genetically controlled by two interacting loci. However, later in
587 development, the ability to produce belowground stolons becomes dominant (with the
588 proportion of belowground stolons showing no dominance), and only one of the
589 previously determined QTL continues to affect the proportion of belowground stolons
590 produced. One potential genetic model for the change in genetic architecture through
591 development is if the LG11 QTL largely controls the ability to produce belowground
592 stolons, but timing of expression of this QTL is modulated by the LG9 QTL. Previous
593 work in *Arabidopsis* suggests that the genetic basis of trichome density is largely
594 controlled by a single large effect QTL in both juvenile and adult leaves [40]. Although
595 much less studied, we argue that an understanding of how the genetic architecture of
596 traits may change through development may also provide details of the molecular
597 genetic basis of development in ecologically important traits.

Lastly, although belowground stolons differ from above ground stolons in a number of traits (Fig. S1), these traits do not appear to segregate independently in F2s (Fig. S7, personal obs). This suggests that, although repurposing aboveground stolons to belowground stolons requires changes along many phenotypic axes, the evolution of this trait may be accomplished with few genetic changes. Similarly, complex behavioral shifts in *Drosophila* are largely attributable to a single retrotransposon insertion [95], and in *Penstamon* single QTL influence several floral phenotypes associated with pollinator shifts [16]. Alternatively, this complex trait may be controlled by many closely linked alleles (such as [96]). Further work determining if and how relatively few genetic changes has allowed *Mimulus* to inhabit high elevation habitats can provide insights into the evolution of novel traits.

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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 collect phenotype information and helped with DNA extractions. JW contributed substantially 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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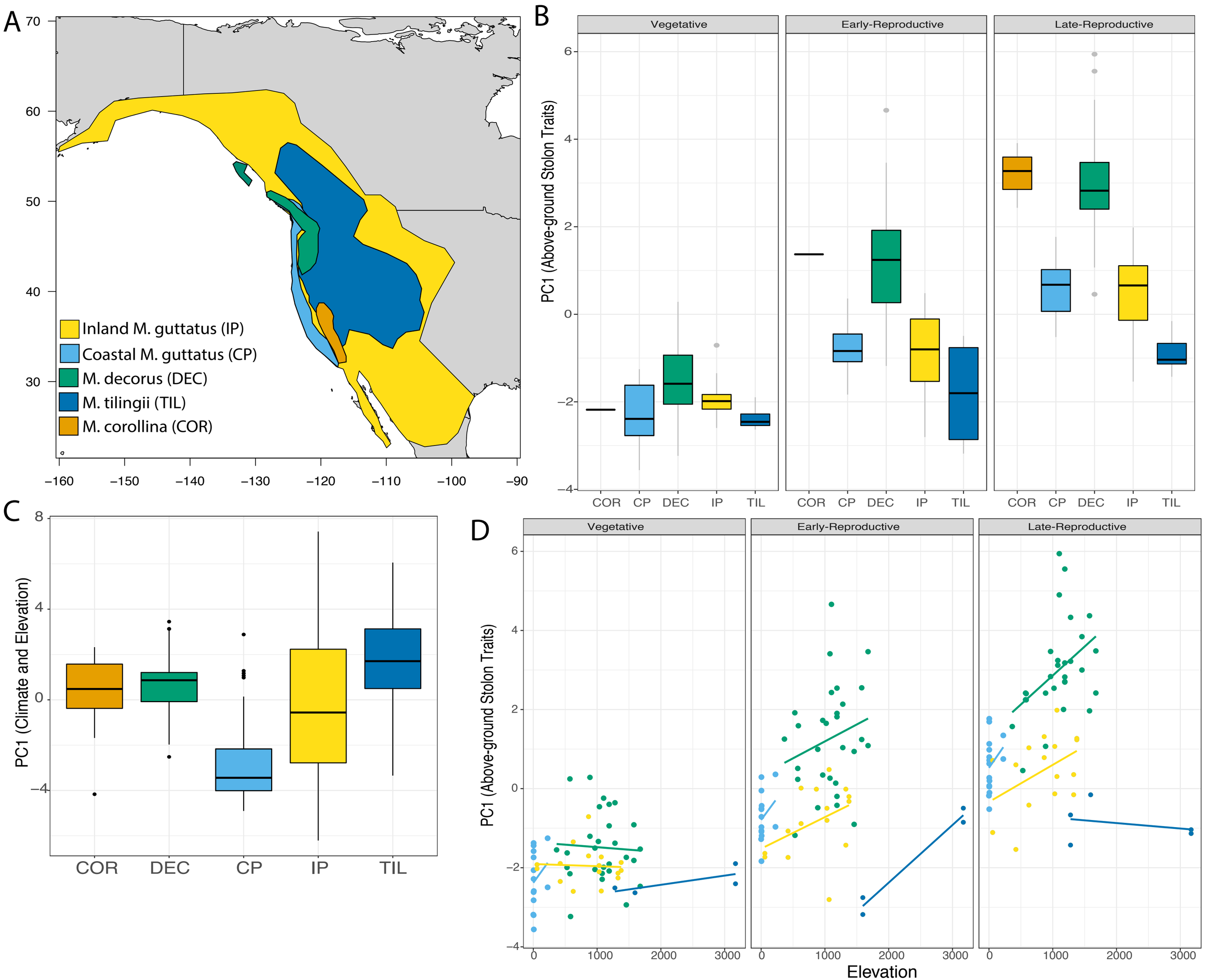
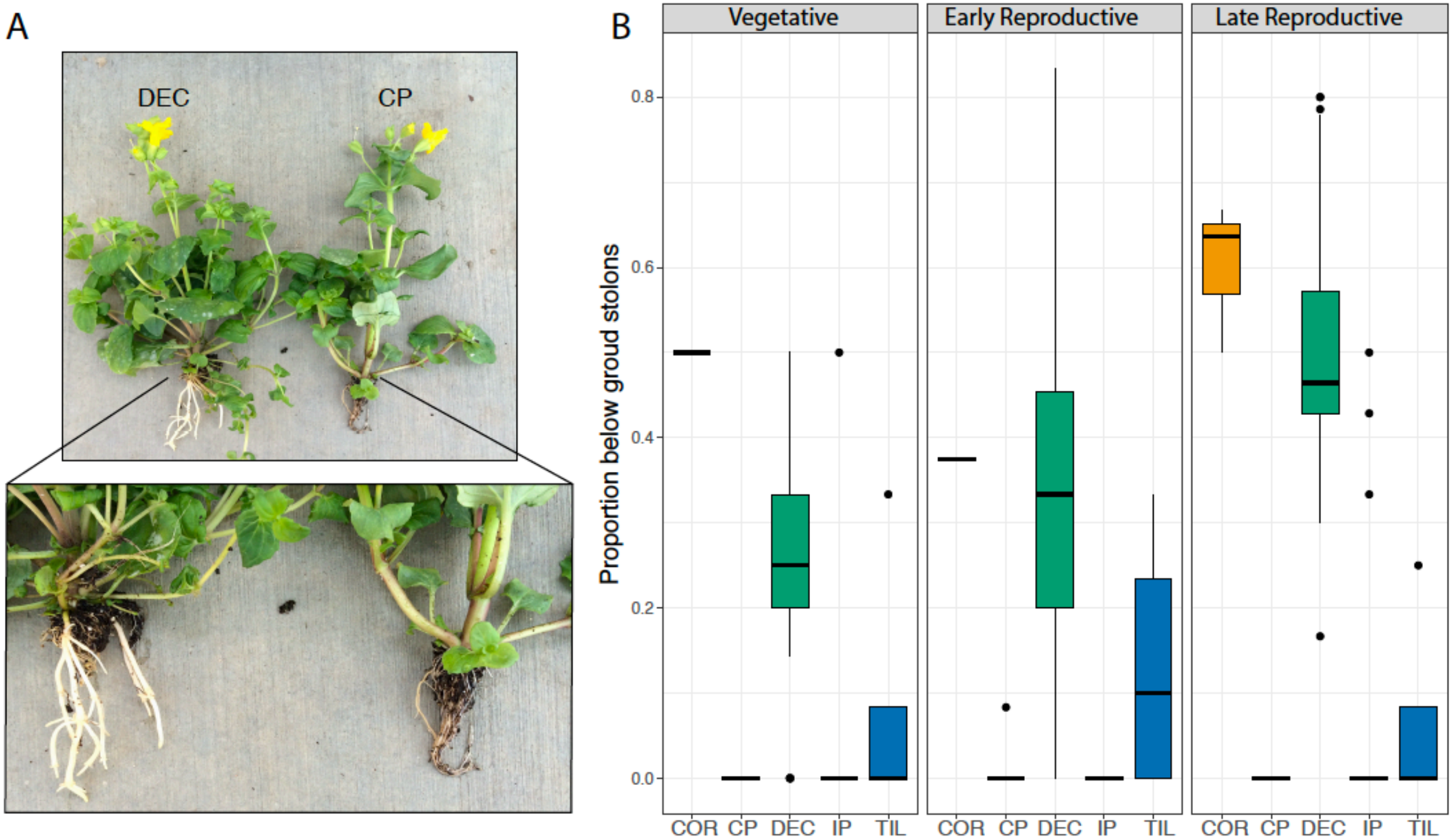
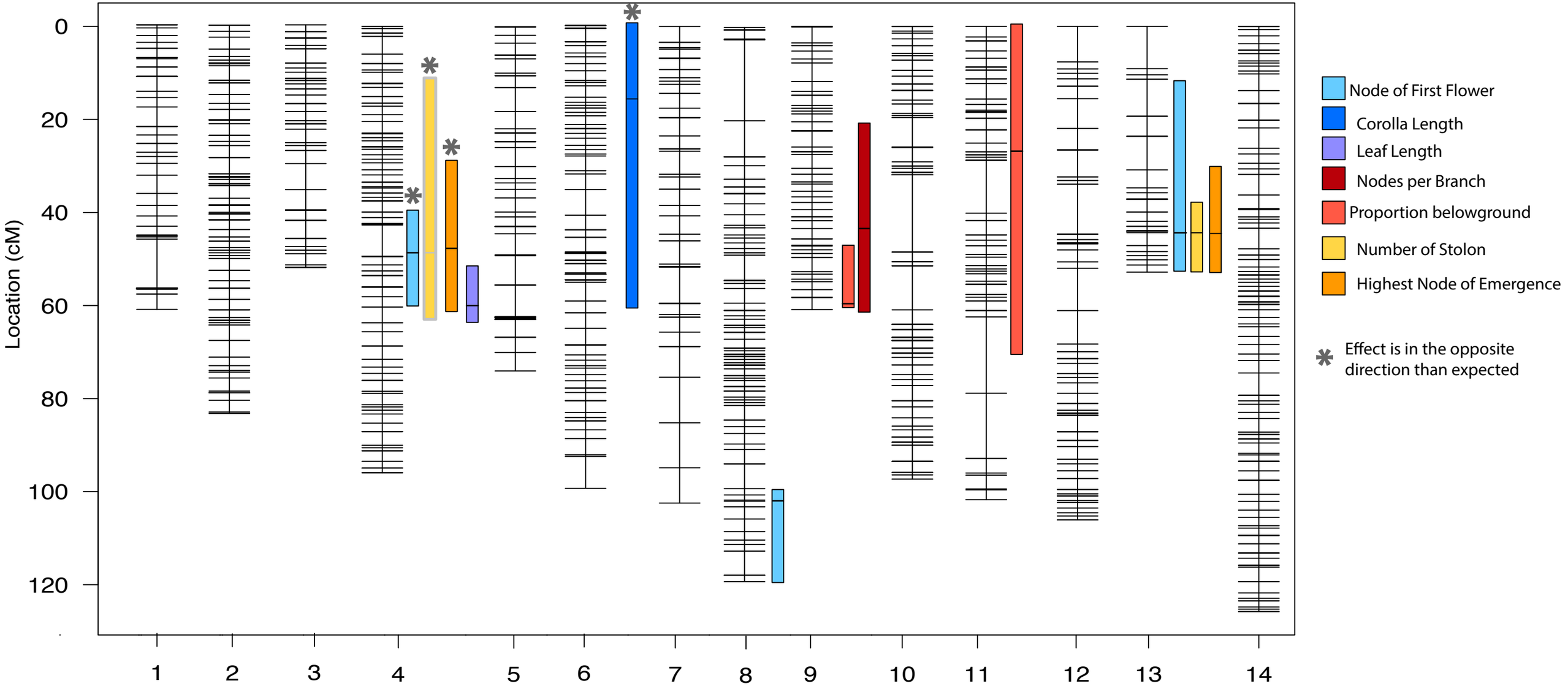
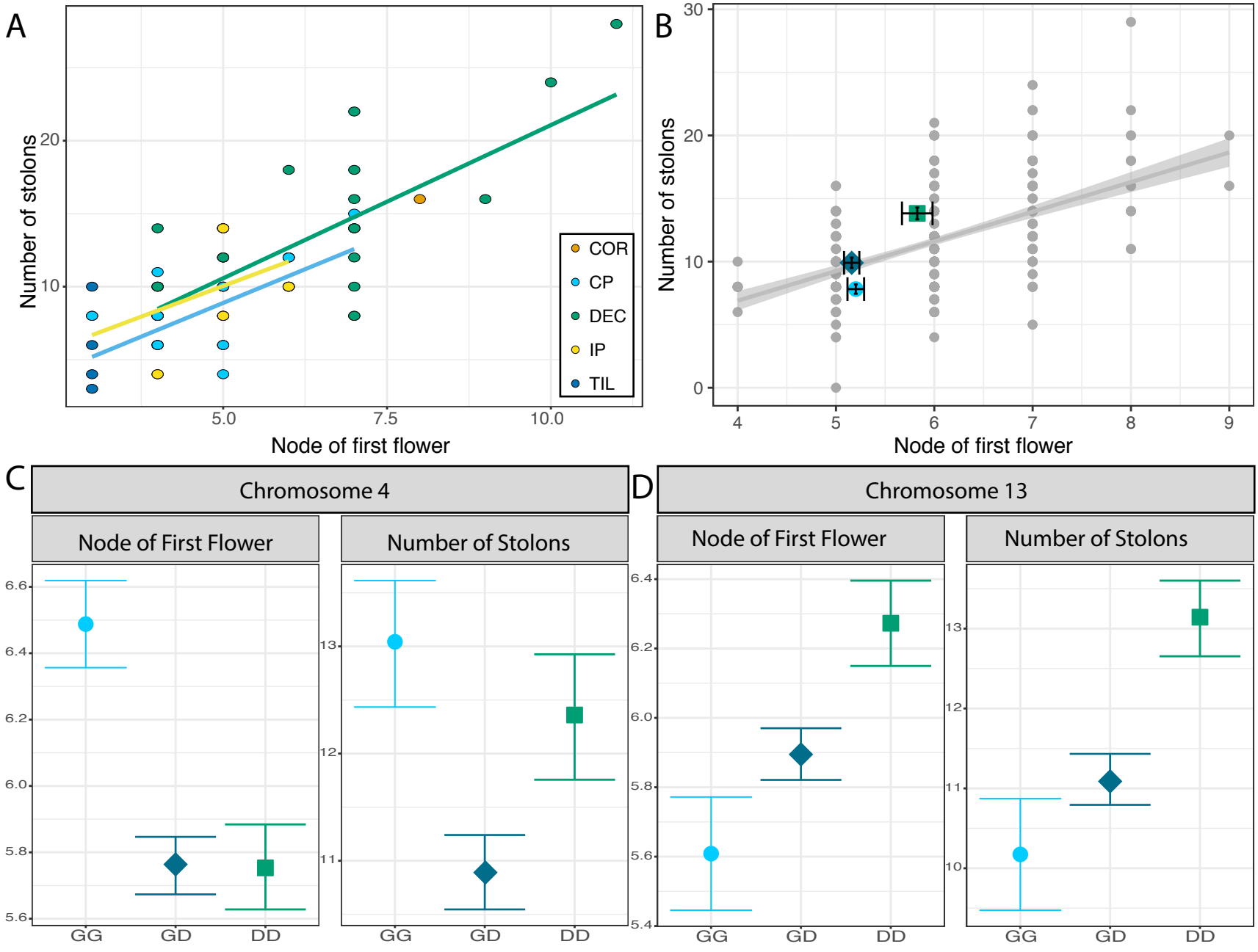
875 **Figure Captions**
876
877 **Fig. 1: Perennials vary in niche and life history. (A) Geographic ranges of different**
878 **perennials based on herbarium records. (B) Perennials vary in life history, and**
879 **these differences extenuate with developmental time (panels). Greater values of**
880 **PC1 indicate more investment in vegetative biomass. (C) PC1 of climate and**
881 **elevation across species. Greater values indicate more seasonal environments at**
882 **higher elevation with greater precipitation in the driest quarter and month. (D) Life**
883 **history in a common garden as a function of elevation of the source population**
884 **for all perennial taxa. Each panel represents a developmental sampling time.**
885 **Lines represent significant regressions.**
886
887 **Fig. 2: Mid and high elevation perennials make belowground stolons. (A)**
888 **Representative *M. decorus* and coastal perennial *M. guttatus*. Both plants make**
889 **above ground stolons, but only *M. decorus* produces belowground stolons. (B)**
890 **Proportion of belowground stolons across development by species. COR= *M.***
891 ***corallinus,* CP= Coastal Perennial *M. guttatus,* DEC= *M. decorus*, IP= Inland**
892 **Perennial *M. guttatus*, TIL= *M. tilingii.***
893
894 **Fig. 3: QTL map of life history traits between coastal perennial *M. guttatus* and *M.***
895 ***decorus*. Each colored box represents the 1.5 LOD interval surrounding**
896 **significant associations (thick horizontal line). Asterisks indicate that the effect of**
897 **the QTL is opposite to the expected effect, based on the parental difference. One**
898 **QTL on LG4 is highlighted in grey, as this QTL did not pass a genome-wide**
899 **significance threshold, but was found to be significantly associated based on**
900 **individual marker analysis.**
901
902 **Fig. 4: Life history traits covary among perennial species, and share a genetic**
903 **basis. (A) Phenotypic correlation between node of first-flower and number of**
904 **stolons across 40 populations of 5 perennial taxa. COR= *M. corallinus,* DEC= *M.***
905 ***decorus,* TIL= *M. tilingii,* IP= inland perennial *M. guttatus,* CP= coastal perennial**
906 ***M. guttatus.* Lines represent linear regressions for each species. (B) Genetic**
907 **correlations between flowering time and number of stolons in F2s derived**
908 **between *M. decorus* and coastal perennial *M. guttatus.* Grey circles are individual**
909 **F2s, blue circle = trait average for replicates of the coastal perennial *M. guttatus***
910 **parent (OPB), turquoise diamond = trait average for replicate F1s, and green**
911 **square = trait average for replicates of the *M. decorus* parent (IMP). (C,D)**
912 **Phenotypic effects of two QTL on flowering time and the number of stolons. Error**
913 **bars represent standard errors.**
914

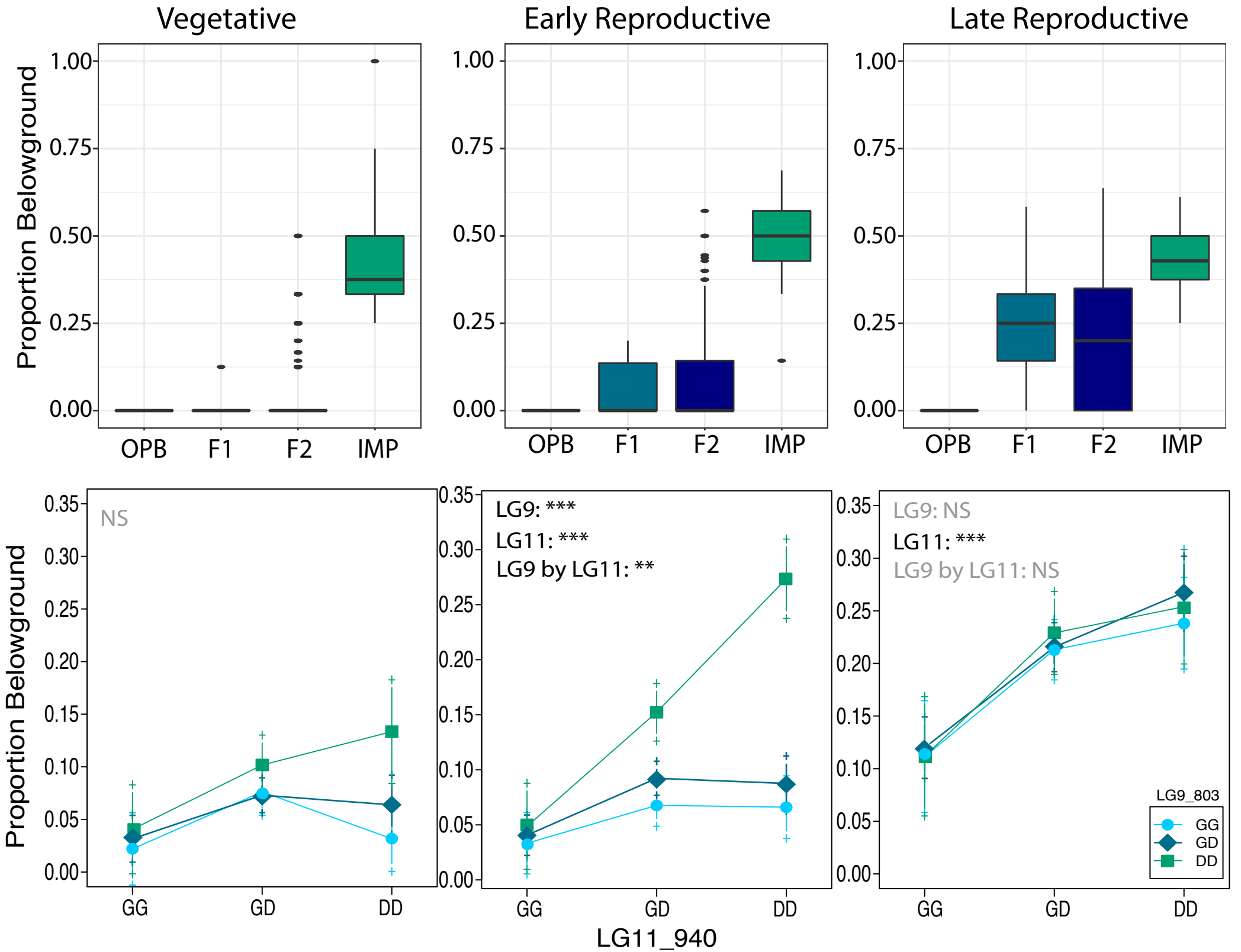
915 **Fig. 5: The genetic architecture of ability to produce belowground stolons**
916 **changes across development. Top row: trait distributions for each parent (OPB=**
917 **coastal perennial *M. guttatus* and IMP= *M. decorus*), F1s, and F2s. Bottom row:**
918 **Effect of two markers on the proportion of belowground stolons, with the**
919 **significance of each marker and their interaction listed in the top left corner. G=**
920 **the coastal perennial *M. guttatus* allele, D= the *M. decorus* allele.**
921


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
60
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
0.20
0.15
0.10
0.05
0.00
GG
GD
DD
LG11_940
LG9_803
GG
GD
DD

Supplemental Figures

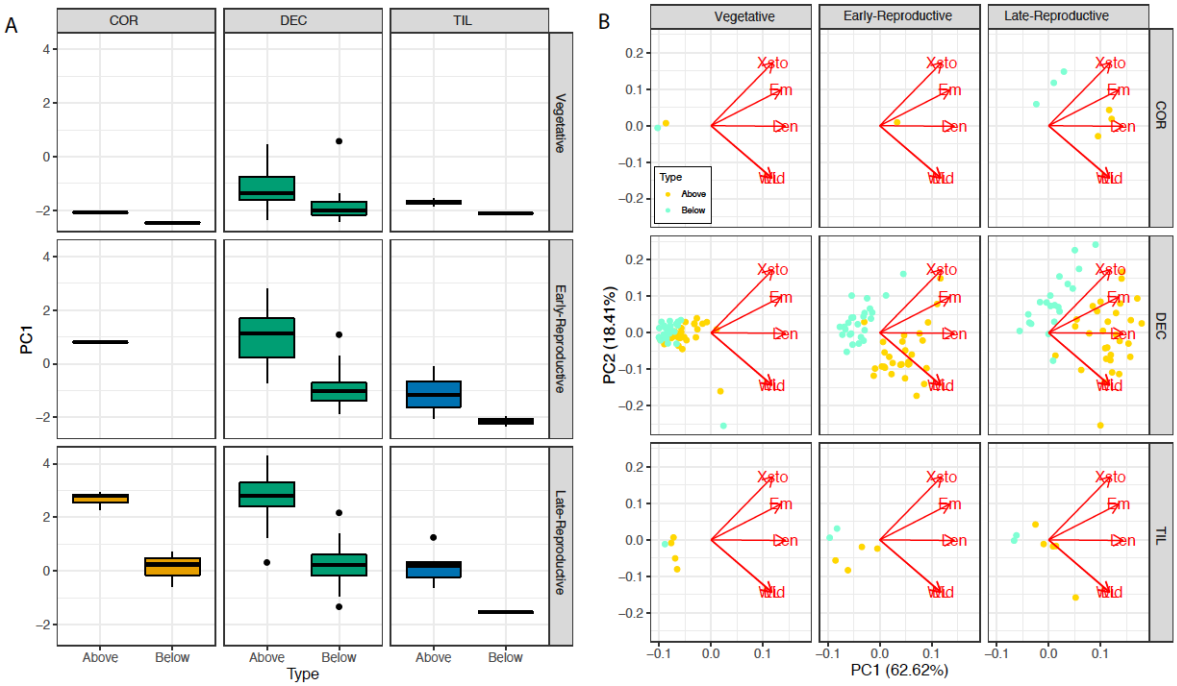


Figure S1: Above and Below ground stolons differ, and these differences grow through development. Analyses were restricted to the three species that made both above and below ground stolons (*M. decorus, M. tilingii,* and *M. corallinus*). We performed a PCA of all stolon traits measured for both above 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.

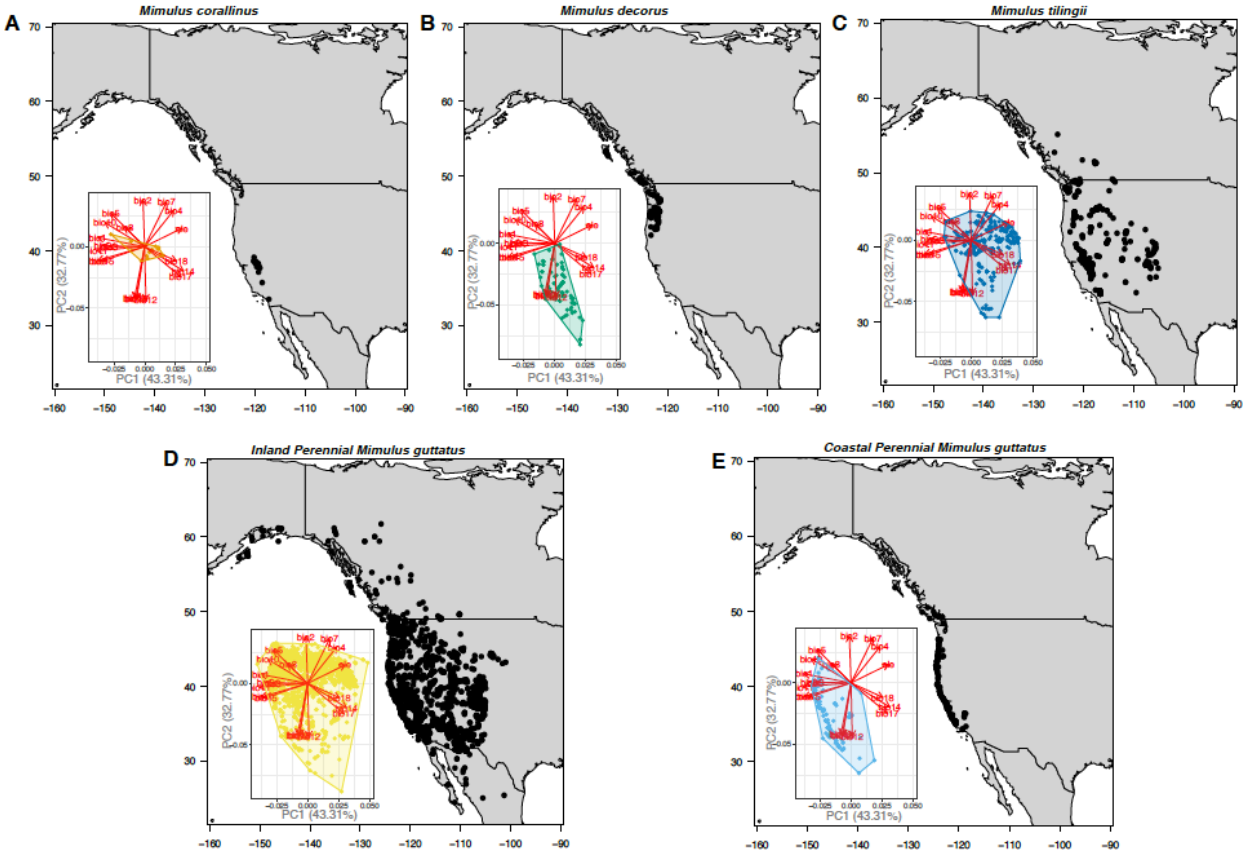


Figure S2: Raw geographic distributions and ecological niche for five perennial *Mimulus* taxa: *M. corallinus, M. decorus,* inland perennial *M. guttatus* (*M. guttatus sensu stricto,* according to the taxonomic revision prevented in Baker *et al.* 2012), coastal perennial *M. guttatus* (*M. grandis,* 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.

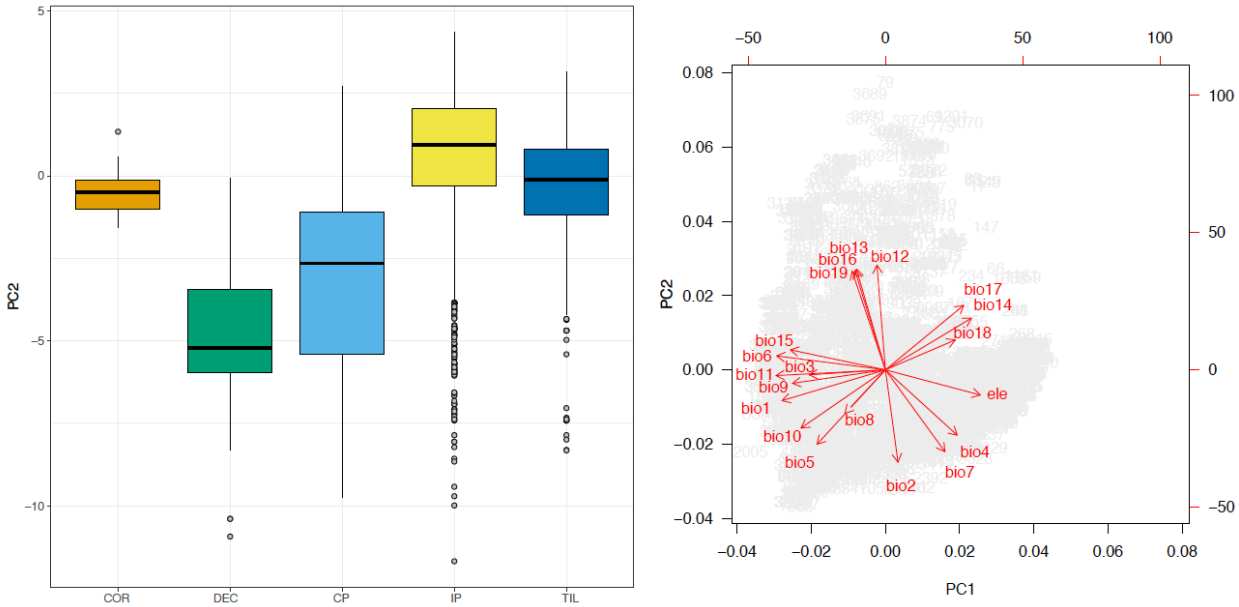


Figure S3: Climate PC2 (30.98% of the variation; Left) as a function of species. Right: Biplot for all 19 BIOCLIM variables and elevation for the climate PC.

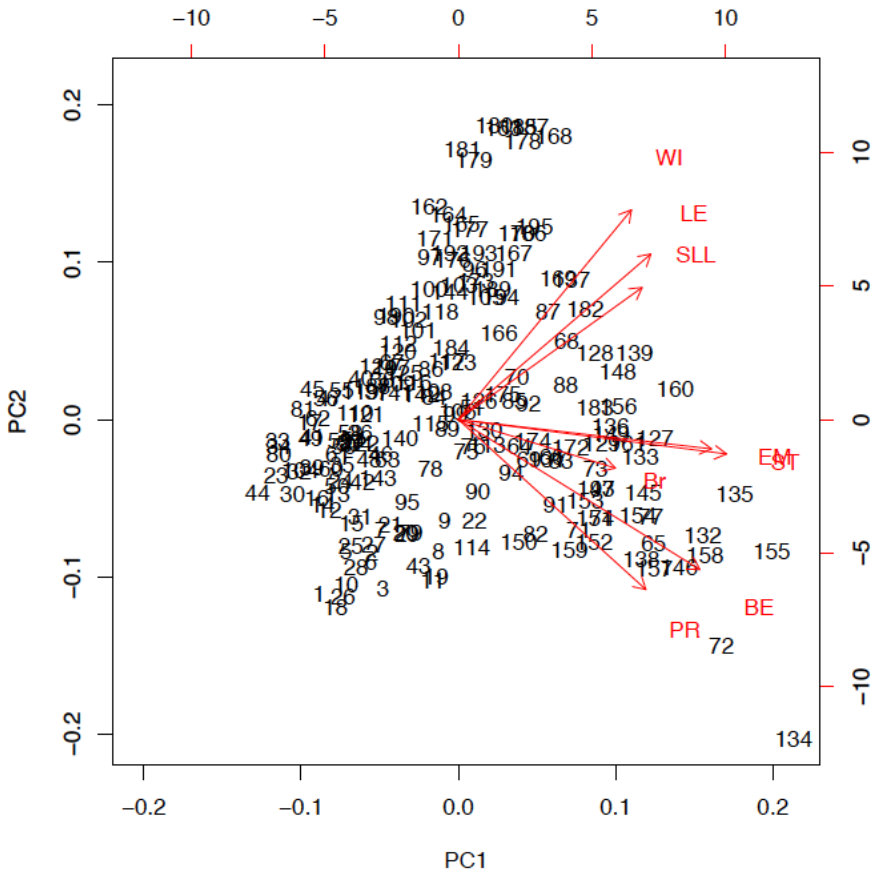


Figure S4: Biplot for a PCA involving life history traits across 40 populations of five perennial taxa across three developmental times. LE= stolon length, WI: stolon width, SLL= stolon leaf length, EM= highest node of emergence, ST= the number of stolons, BE= average number of branches per node, and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

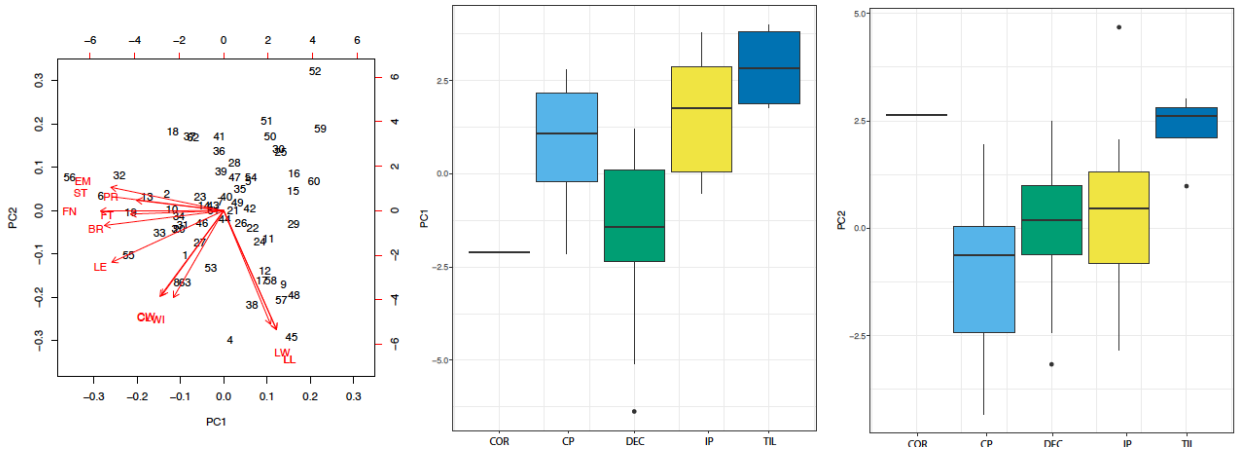


Figure S5: An additional PCA of both stolon traits and reproductive traits at only the day of first flower. Far Left: Biplot of the PCA, LE= stolon length, WI: stolon width, SLL= stolon leaf length, EM= highest node of emergence, ST= the number of stolons, BR= average number of branches per node, and 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).

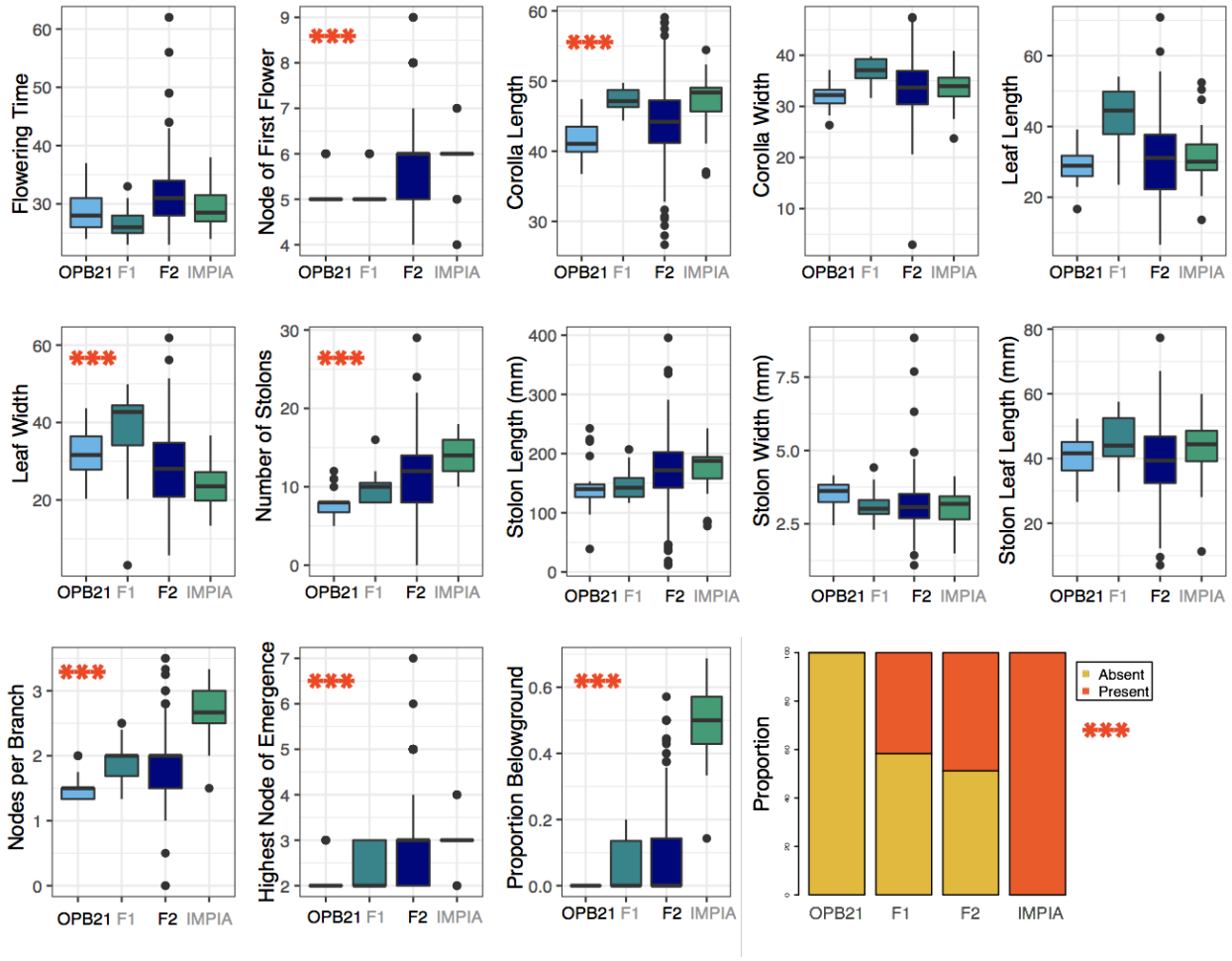
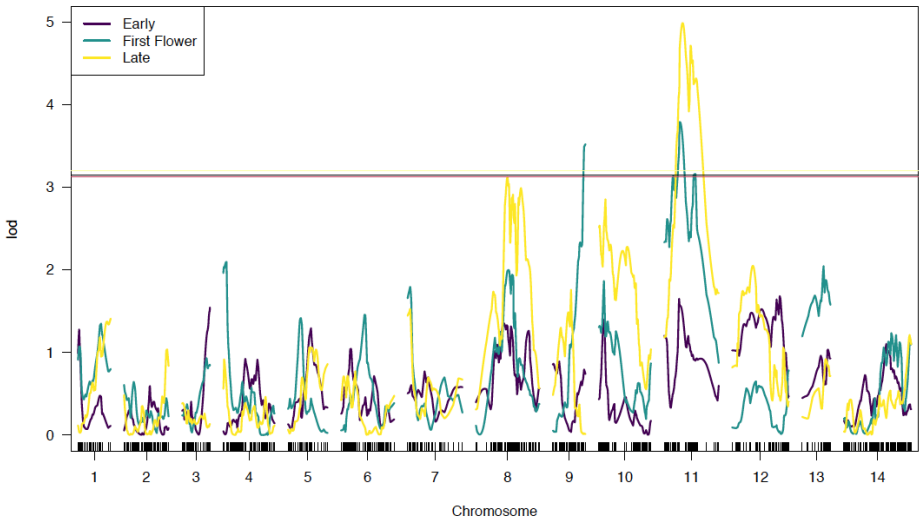
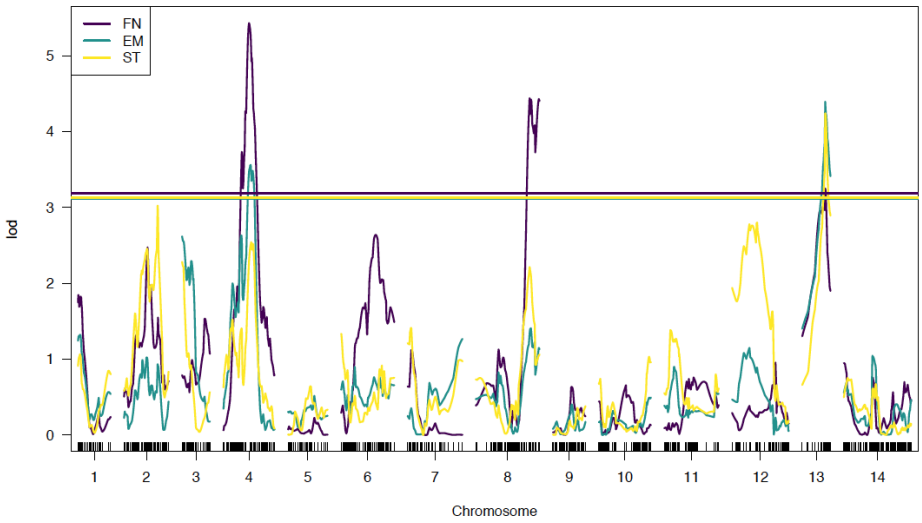


Figure S6: Trait distributions for each parental line and their hybrids. Significant differences between parental lines denoted by three red asterisks.

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

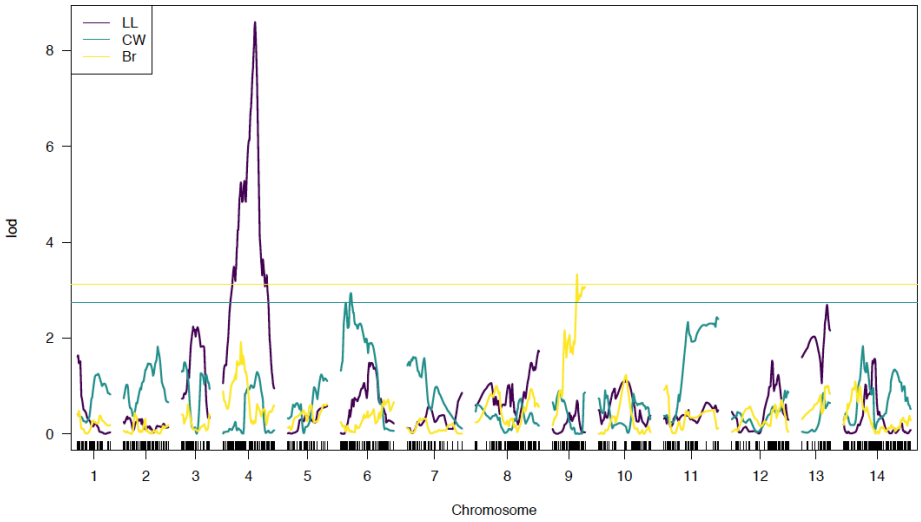

LL
CW
Br
lod
Chromosome

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.

ɜmental Tables

S1: Seed collections used in a common garden experiment.

ion	Number of Maternal Families	Species	Latitude	Longitude	Elevation (m)	Collector
MEADOW	1	*M. corallinus*	38.32	-119.92	2046	Megan Peterson
CREEK	1	*M. corallinus*	38.588	-119.786	2066	Megan Peterson
FORK	1	*M. corallinus*	38.664	-120.219	1959	Megan Peterson
	2	*M.decorus*	45.3054667	-121.66688	1280.16	JMC
	2	*M.decorus*	44.4985167	-121.98322	1079.2968	JMC
	2	*M.decorus*	44.371	-122.104	1189	JMC
	2	*M.decorus*	43.1575333	-122.13343	1576.7304	JMC
	1	*M.decorus*	44.40278	-122.07556	1189	JMC
	2	*M.decorus*	47.5651833	-123.03233	574	Carrie Wu
	2	*M.decorus*	44.2332	-122.1762	888	JMC
	2	*M.decorus*			1189	JMC
	2	*M.decorus*	45.2921833	-121.73482	1169.2128	JMC
	2	*M.decorus*	44.3934	-122.149	1676.11549	JMC

	1	*M.decorus*	44.3032	-121.99622	1019.8608	JMC
	1	*M.decorus*	44.1739333	-122.0542	528.2184	JMC
	2	*M.decorus*	44.5237667	-121.99788	966.216	JMC
	2	*M.decorus*	43.54795	-121.96278	1457.8584	JMC
	1	*M.decorus*	45.3210833	-121.90702	530.352	JMC
	2	*M.decorus*	45.2665833	-121.74163	1102.4616	JMC
	1	*M.decorus*	45.3498667	-121.99295	365.1504	JMC
	2	*M.decorus*	45.31105	-121.8892	583.692	JMC
	2	*M.grandis*			4.572	D. Lowry/K. Wright
	2	*M.grandis*	38.3723	-123.05518	224.3328	D. Lowry/K. Wright
	1	*M.grandis*	43.893056	-124.13778	0	D. Lowry/K. Wright
	2	*M.grandis*	47.3854	-123.14728	4.572	D. Lowry/K. Wright
	2	*M.grandis*	42.464017	-124.42292	7.62	D. Lowry/K. Wright
	2	*M.grandis*	35.27335	-120.88912	4.572	D. Lowry/K. Wright
	1	*M.grandis*	45.761083	-123.96657	4.572	D. Lowry/K. Wright
	2	*M.grandis*	53.418833	-131.91573	4.572	D. Lowry

JTES	2	*M.guttatus*	43.848067	-121.7801	1377.0864	JMC
	2	*M.guttatus*	41.812967	-124.08118	32.948	Jessica Sely
	2	*M.guttatus*	47.54268	-120.707	786.5	Jessica Sely
	2	*M.guttatus*	45.202083	-121.61492	1031.4432	JMC
	2	*M.guttatus*	43.73045	-121.76328	1328.0136	JMC
	2	*M.guttatus*	41.15748	-122.2907	626	Jessica Sely
	2	*M.guttatus*	40.8764	-123.7563	1065.58	Jessica Sely
	2	*M.guttatus*	47.36433	-120.863	866	Jessica Sely
	2	*M.tilingii*	48.872683	-121.68758	1278.636	Carrie Wu
	2	*M.tilingii*	38.266753	-119.61798	3169.92	Carrie Wu
	2	*M.tilingii*	48.950433	-121.63607	1594.104	Carrie Wu

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

	FN	CL	LW	ST	BR	EM	PR
		0.3	-0.19	**0.76**	**0.66**	**0.73**	**0.51**
	0.09094028		0.15	0.13	**0.36**	0.1	**0.27**
	-0.5645925	0.04683288		-0.18	-0.16	**-0.28**	**-0.31**
	0.50163204	**0.14562208**	**-0.2818237**		**0.69**	**0.85**	**0.41**

	0.22947167	0.0896038	**-0.1898139**	**0.2272746**		**0.68**	**0.5**
	0.6473864	0.10939216	**-0.5060051**	**0.82404971**	**0.23453082**		**0.37**
	0.1553752	0.0710367	**-0.1635922**	**0.29215793**	0.05555646	**0.20730242**	

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

	OPB	**F1**	**IMP**	**Parental Difference**	**broad sense heritability**
Flower	28.875	26.66667	13.13043	29.5	0.75282011
First Flower	5.208333	5.166667	0.5797101	0.625*	0.80906798

Length	41.60435	47.38208	18.630835	5.42107**	0.89426937
Width	31.99957	37.06958	15.068094	1.48668	0.83554148
ıgth	28.80043	42.65	81.23007	3.03999	0.33804768
dth	32.06565	37.665	31.5171	8.32773*	0.25899042
of Stolons	7.75	9.833333	5.326087	6***	0.71177093
.ength	144.0463	147.2745	1893.584	29.8441	0.74639957
Vidth	3.5225	3.10875	0.3589563	-0.430417	0.51592615
.eaf Length	40.83208	45.26042	106.81473	2.34125	0.54588417
dness (average branches per node)	1.503472	1.898611	0.17697615	1.123611***	0.57380126
Node of Stolon Emergence	2.166667	2.291667	0.3025362	0.875**	0.67590212
on Belowground	0	0.06539352	0.01442334	0.47951389***	0.48057671
e/Absence(percentage)	0	41.66667	100	1***	

4: Effect size for each QTL for each trait (significance determined by 1,000 permutations). GG,GD,DD are the trait means fo
uals homozygous for the *M. guttatus* allele (GG), *M. decorus* allele (DD), or heterozygous (GD). D(Alt.Homo)= difference in t
between alternate homozygotes. D(par)=the difference in trait means between 24 replicates of each parental line. %explain
he effect size/parental difference. %explained (F2_range)= the effect size/range of trait values for F2s. %explained (F2.Vg) i
ize/(square root(proportion of the genetic variation (Vg)). Total is the total % explained under an additive model, and in the
roportion of belowground stolons, a model with non-additive effects between two significant QTL. PR= Proportion of

round stolons, FN= Node of first flower, ST= number of Stolons, CL= corolla length, LL= Leaf Length, BR= average branch :M= highest node of stolon emergence.

CHR	POS	LOD	GG	GD	DD	D(Alt.Homo)	D(par)	%explained (par)	%explained (F2.range)	%explai (F2.Vg)
9	60.9	3.52	0.064	0.086	0.176	0.11152	0.480	23.257	19.565	138.618
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**
4	49	5.43	6.488	5.76	5.756	-0.732	0.625	117.073	14.634	93.370
8	100.482	4.44	6	5.846	6.359	0.359	0.625	57.436	7.180	45.807
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**
4	52	2.54	12.897	10.681	12.45	-0.447	6	7.457	1.543	14.135
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**
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**
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**
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**
4	51	3.56	3.290	2.606	2.842	-0.447	0.875	51.128	8.947	66.72
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**