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Genetic Determinants and Epistasis for Life History Trait Differences in the Common Monkeyflower, *Mimulus guttatus*

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Abstract

Understanding the genetic basis of complex quantitative traits is a central problem in evolutionary biology, particularly for traits that may lead to adaptations in natural populations. The annual and perennial ecotypes of *Mimulus guttatus* provide an excellent experimental system for characterizing the genetic components of population divergence. The 2 life history ecotypes coexist throughout the geographic range. Focusing on population differences in life history traits, I examined the strength and direction of pairwise epistatic interactions between 2 target chromosomal regions (DIV1 and DIV2) when singly and cointrogressed into the alternate population's genetic background. I measured a suite of flowering and vegetative traits related to life history divergence in 804 plants from 18 reciprocal near-isogenic lines. I detected pleiotropic main effects for the DIV1 QTL in both genetic backgrounds and weaker main effects of the DIV2 QTL, primarily in the perennial background. Many of the traits showed epistatic interactions between alleles at the DIV1 and DIV2 QTL. Finally, for many traits, the magnitude of effect size was greater in the perennial background. I evaluate these results in the context of their potential role in population divergence in *M. guttatus* and adaptive evolution in natural populations.

Subject areas: *Quantitative genetics and Mendelian inheritance; Genomics and gene mapping*

Key words: *genetic interactions, life history, near-isogenic lines, phenotypic divergence, pleiotropy, QTL*

The nature of genetic variation underlying complex traits in natural populations is at the center of a long-standing deliberation in evolutionary biology. The key elements of this debate are whether phenotypic variation is generated predominantly via the contributions of many loci, each with small additive effect (Fisher 1930); fewer loci, some with alleles of large effects (Bateson 1913; Orr and Coyne 1992); and/or loci whose allelic contributions are strongly dependent on alleles at other loci (Wright 1931). The relative importance of gene interactions (epistasis) versus additive genetic effects in determining quantitative phenotypes remains contentious, although it has been implicated in maintaining natural variation (Barton and Keightley 2002; Kelly and Mojica 2011), in contributing to local adaptation (Caicedo et al. 2004) and in population or species divergence (Doebley and Stec 1991; Kroymann and Mitchell-Olds 2005).

Epistasis can either enhance (synergistic epistasis) or retard (antagonistic epistasis) the rate of adaptive change depending on the direction and nature of the interactions (e.g., Wade 2000). However, identifying and mapping epistatic interactions is challenging due to the large sample sizes required to detect significant interactions across all possible genetic interactions (Carlborg and Haley 2004; Mackay 2014). Classic empirical tests for epistasis involve molecular genetics approaches that utilize loss of function mutants. Epistasis has been studied in a quantitative genetics framework using inbred line crosses and more recently QTL mapping methods to investigate QTL–QTL interactions (e.g., Carlborg 2003; Juenger et al. 2005; Kroymann and Mitchell-Olds 2005; Muir and Moyle 2009; Latta et al. 2010). The literature contains many reports of epistatic interactions between QTL in various traits and species, but at least as many reports of no interaction (see reviews by Mackay 2001; Orr 2001; Barton and Keightley 2002). In general, we still have a poor understanding about allelic interactions within a species and whether these impact processes like local adaptation and population divergence.

Adding to the complexity arising from genetic interactions among loci, multivariate quantitative phenotypes may be governed by pleiotropic loci. The degree of pleiotropy can affect the evolutionary trajectory of traits and the response to selection (Lande 1979) and remains at the crux of the debate on the effect size of genes underlying phenotypic divergence (for a continued discussion of this debate, see Rockman 2012; Lee et al. 2014). The infinitesimal model predicts many independently acting genes of small effect (Fisher 1930; Dobzhansky 1937), while the major genes model predicts the involvement of few pleiotropic genes of large effect (Orr and Coyne 1992). Empirical research have provided support for both theories, with some studies demonstrating few, large, pleiotropic QTL underlying divergence (e.g., Doebley and Stec 1991; Bradshaw et al. 1998; Colosimo et al. 2004), and other studies showing many loci of small independent effect (e.g., Fishman et al. 2002; Laurie et al. 2004).

The presence of extensive phenotypic divergence among populations within a species provides an opportunity to examine the genetic basis underlying complex adaptations. Here, I use the yellow monkeyflower, *Mimulus guttatus* to study the effects of epistasis and pleiotropy on phenotypic divergence between annual and perennial ecotypes. In *M. guttatus*, there is strong phenotypic divergence between life history ecotypes in flowering time, vegetative and floral characters, despite their close proximity and the potential for gene flow. Lowry and Willis (2010) identified a chromosomal inversion on Linkage Group 8 that affects key life history traits that differ between widespread annual and perennial ecotypes. Previous work has shown that several other QTL have pleiotropic effects on traits directly related to fitness differences between life history ecotypes (Hall et al. 2006) and have demonstrated tradeoffs at individual loci that underlie local adaptation including a second large QTL on Linkage Group 8 (Hall et al. 2010). Furthermore, Kelly 2005 and Kelly and Mojica 2011 have elegantly demonstrated epistatic interactions among a set of polymorphic QTL for flower size characteristics within a single natural population. The nature and degree of pleiotropy and epistasis among genomic regions have consequences for the trajectory of adaptive evolution and may have been involved in the process of phenotypic divergence.

In this paper, I examine pleiotropic and epistatic interactions between 2 QTL that have previously been shown to account for life history differences in *M. guttatus* (Hall et al. 2006; 2010; Lowry and Willis 2010). The first QTL is the large chromosomal inversion that differs in orientation between annual and perennial populations (Lowry and Willis 2010). The second region is a QTL that has been previously implicated in flowering time differences in *M. guttatus* as well as in mapping studies involving closely related species (Zuellig et al. 2014). Here, I utilize near-isogenic lines (NILs) with the 2 QTL regions reciprocally introgressed into alternate annual and perennial genetic backgrounds, to systematically compare the phenotypic effects of each chromosomal region singly and in combination in each background. I first evaluate the main effect of the QTL on a suite of morphological traits that are associated with life history differences and investigate pleiotropy. Next, I assess the degree and nature of epistasis between each QTL and the rest of the background genome and between the 2 introgressed QTL. This approach allows us to begin to understand the complicated genetic mechanisms underlying the phenotypic divergence between the annual and perennial ecotypes in *M. guttatus* and the process of local adaptation and population divergence more generally.

Methods

Study System

The common yellow monkeyflower, *M. guttatus* (sect. *Simiolus*, Phrymaceae) is a highly variable, phenotypically diverse species that is broadly distributed throughout western North America (Grant 1924; Pennell 1951). The species comprises fully interfertile annual and perennial populations inhabiting different edaphic environments, which has led to uncertainty in species delimitation, with various authors recognizing additional taxa within *M. guttatus sensu lato* (reviewed in Nesom 2012). Both ecotypes rely on continuously moist soils, so their life histories are strongly shaped by edaphic conditions. The perennial ecotype inhabits constantly moist soils near permanent water, growing vegetatively via stolons from fall to spring, then flowering in summer and fall. In contrast, the diminutive annual ecotypes thrive in soils that dry out completely during the summer, by germinating in late fall to early spring, growing little vegetatively, and rapidly transitioning to flowering. Previous research has identified several QTL affecting divergence in floral, vegetative, and life history characters (Hall et al. 2006) and has identified 2 QTL in particular as having a role in local adaptation (Hall et al. 2010). I focus on the 2 chromosomal regions (DIV1 and DIV2) that have been previously shown to have a large role in phenotypic divergence for life history traits in a perennial population along the Oregon coast (DUN) and an annual population in the Oregon Cascades (IM). However, I use populations that are located much closer geographically (a perennial population along the California coast, SWB: 39°02'09", 123°41'25", and an annual population located ~50 km away inland, LMC: 38°51'50", 123°05'02"). I use NILs to target these 2 chromosomal regions and systematically measure the direction and strength of epistasis and pleiotropy.

Generation of Experimental Genotypes

In this paper, I use NILs created by David B. Lowry. In Lowry and Willis (2010), the homozygous DIV1 NILs are used in a reciprocal field experiment, however Lowry also created introgression lines that involve a second, unlinked, QTL on the other end of LG 8. As described in Lowry and Willis (2010), the introgression lines were created from 3 independent sets of LMC (Annual) and SWB (Perennial) inbred lines. A single individual from each annual line was crossed with a perennial line to create 3 independent sets of F1 progeny. These were then reciprocally backcrossed as the pollen donor to the parental lines from which they were derived. To facilitate introgression into the alternate genetic background, individuals were genotyped at each generation: 2 flanking makers (DIV1:e571, e772; DIV2:e381, e829) were genotyped around each QTL and 1 marker (DIV1: e173; DIV2: e76) was

genotyped in the middle of each QTL. Marker details can be found at www.mimulusevolution.org. Each generation, 32 backcross hybrids of each type were genotyped for the appropriate markers. Hybrids heterozygous for the 3 markers were then backcrossed to each parental line. For 4 backcross generations, only hybrids heterozygous for both QTL were backcrossed. Fourth generation backcrosses were then self-fertilized and the segregating progeny were used in the current experiment. For a full description of creation of NILs, see Lowry (2010). Thus, the current experiment involves 9 possible genotypes for each genetic background (Figure 1).

Seeds were germinated from all 3 LMC and SWB lines. I used an average of 140 individuals from each line (range: 94–191), for a total of 804 individuals. Every individual in the current experiment was genotyped with the same markers as above to confirm their background genotype, as well as their genotype at the introgressed QTL.

Greenhouse Experiment and Trait Measurements

In mid-April 2013, 3 seeds were planted into 4-inch pots filled with moist Fafard 4P potting mix and stratified in the dark at 4 °C for 1 week. Pots were then moved into the glasshouses at Syracuse University. Seedlings were thinned to one plant per pot, retaining the first germinant. Lighting was supplemented with high-efficiency sodium lights set at 16-h days. Temperature was maintained at 21 °C during the day and decreased to 18 °C at night. Plants were bottom-watered every day by automatic flooding, ensuring adequate and even access to water. The position of pots on the bench was randomized every 3–4 days.

I monitored plants for germination date and the date of first flowering. On the day of first flowering, I measured a suite of morphological traits that have previously been shown to differ between annual and perennial ecotypes of *M. guttatus* (Hall et al. 2006; 2010). These included: node of first flower, stem width (halfway between the first and second node), length of the first internode (between cotyledon and first true leaves), length of the second internode, plant height, number of stolons, length of longest stolon, length of first true leaf, corolla width (at the widest point), and corolla length of first flower. All measurements were made to the nearest millimeter, except stem width, which was measured to the nearest half millimeter. In fulfillment of data archiving guidelines (Baker 2013), I have deposited the primary data with Dryad.

Data Analysis

I checked all variables for normality and transformed as necessary. Node of first flower, both internode lengths and number of stolons were log-transformed. Grouping plants by their genetic background, I calculated Pearson correlation coefficients for all the phenotypic traits using SAS software (SAS Institute Inc., Cary, NC; 2011).

Analysis of Main Effect of QTL

I evaluated the main effect of each QTL in the homozygous and heterozygous NILs for each phenotypic trait using restricted maximum-likelihood general linear models. The model included fixed effects for the genotype at each QTL (DIV1 and DIV2) and analyzed each genetic background separately. I included the random effect of line to account for any differences in our starting inbred lines within each genetic background. Each phenotypic trait was analyzed separately. All analyses were conducted using SAS software.

Quantifying Epistasis

I tested for epistasis in several ways. First, using a restricted maximum-likelihood general linear model, I analyzed

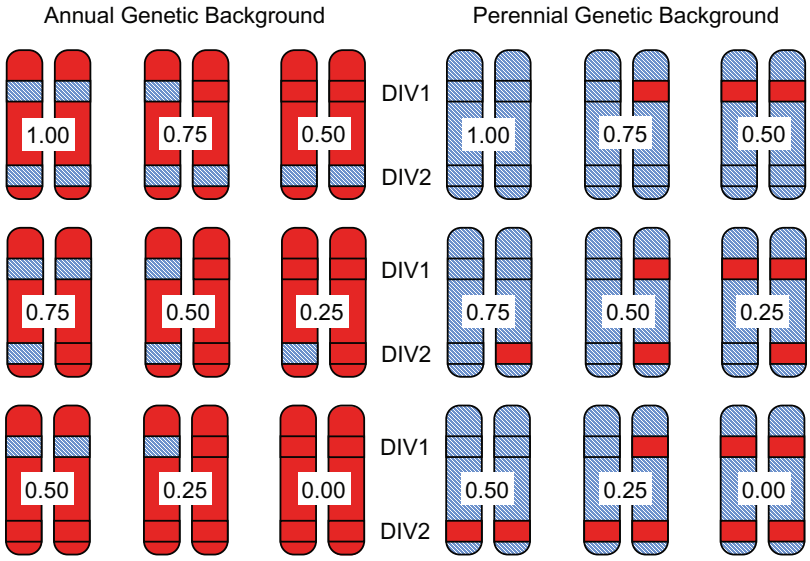

Figure 1. A diagram of the 9 possible genotypes for each genetic background used in the study. For each genotypic class, the proportion of Perennial alleles at the 2 loci is indicated.

whether the main effect of each QTL depended on genetic background. A significant result would indicate epistasis between the known QTL and other genes located elsewhere in the genome. This analysis was performed using a similar model as above, but with both genetic backgrounds analyzed simultaneously and including the main effect of background and background × DIV1 and background × DIV2 interaction terms. For significant interactions, I used contrast statements to determine the source of the variation.

Subsequent tests for epistasis involved an approach similar to the joint scaling test used for line-cross studies (Mather 1949; Hayman 1960; Mather and Jinks 1982). I used the mean phenotypic values for the set of 9 possible 2-locus genotypes to estimate genotypic values (I do not distinguish between the 2 possible double heterozygotes; Figure 1; Table 1). The model included composite genetic effects of additive [*a*], dominance [*d*], and digenic epistatic effects of additive by additive [*aa*], additive by dominance [*ad* and *da*], and dominance by dominance [*dd*]. Least-squares procedures were used to estimate model parameters contained in vector y and their variances from the diagonal of their variance covariance matrix S (Mather and Jinks 1982; Lynch and Walsh 1998). The estimates of y and S are obtained as $\hat{y} = (C^{T}V^{-1}C)^{-1}C^{T}V^{-1}x$ and $\hat{S} = (C^{T}V^{-1}C)^{-1}$, where C is the coefficient matrix (C^{T} its transpose) for the contribution of effects to each genotypic class, V is the diagonal matrix of the error variances of each mean, and x is the vector of observed means. Estimates of parameters are used to predict the means as the algebraic sum of the contribution of each parameter associated with the expected genotype of that class (Mather and Jinks 1982).

The observed means were first tested for fit to a model incorporating only the composite mean (m) and additive parameters (a). Goodness of fit of the observed means to the additive model was tested with the chi-square, calculated as $X^2 = X^{T}V^{-1}X\text{-}X^{T}V^{-1}C\,\hat{y}$ (Hayman 1958). The degrees of freedom is the number of means (in this case, 9) minus the number of parameters estimated in the model (in this case, 3), using a level of significance of $P < 0.05$. Rejection of the additive model indicates that dominance and/or epistasis are contributing to the genetic divergence among classes. The fit of the full 9-parameter model could not be tested because the number of parameters equals the number of observed lines, nonetheless there were no cases in which all parameters explained a significant proportion of the variation ($\alpha = 0.05$). Goodness of fit of each model was tested by X^2 as shown above. F-statistics were used to evaluate the improvement in the goodness of fit for models containing other parameters (Graybill 1961).

Results

Many of the traits showed strong correlations (Table 2). The majority of these correlations were similar in magnitude within the annual genetic background and the perennial genetic background. Notable exceptions include the lack of correlation in flowering time and flower size for perennial plants and no correlation between height and leaf length and internode lengths in perennials. The latter is probably because perennial plants bolt above internode 2, while annuals typically bolt by extending internode 2. There were no instances of significant correlations that showed opposite directions in the 2 backgrounds.

I found highly significant main effects for the DIV1 QTL for most of the traits examined in both the LMC and SWB genetic backgrounds (Table 3). The magnitude of effect size for alleles at each QTL is visible in Figure 2. The DIV2 QTL had fewer significant effects on the traits, and in general, only had strong effects in the perennial background (Table 3). This is further demonstrated in the first test for epistasis by testing the significance of the interaction term between genetic background and QTL in a general linear model. Table 4 shows the results of this test and indicates that there is epistasis between DIV1 and genetic background for 3 vegetative traits: number of stolons, height, and second internode length. There is significant epistasis between DIV2 and genetic background for both floral and vegetative traits, including days to flower, node of first flower, corolla width, number of stolons, and second internode length. Using contrast statements, it was evident that for all 5 traits, the significant interaction arose due to strong genotypic effects of DIV2 in the perennial background, but weak or absent effects of DIV2 in the annual background.

To test for epistasis between DIV1 and DIV2, I used the adjusted means and variances (SE^2) for each trait for the 9 genotypic classes for each genetic background. The regression line resulting from a model with the mean and additive genetic effect is plotted (trait = $m + a$) along with the observed genotypic means in Figure 3. The departure from the regression line and the goodness of fit for the expected genotypic means suggests that in some cases, the additive model is adequate but estimates of other parameters indicates that dominance and epistatic parameters are

Table 1 Mean phenotypes of the 9 genotypic classes for 2 interacting genes (in this study, I do not distinguish between the 2 alternate double heterozygotes). A is the allele from the Annual (LMC) parent, P is the allele from the Perennial (SWB) parent

Genotype at DIV2	Genotype at DIV1		
	X_1X_1 (AA)	X_1X_2 (AP)	X_2X_2 (PP)
Y_1Y_1 (AA)	$a_x + a_y + aa_{xy}$	$d_x + a_y + da_{yx}$	$-a_x + a_y - aa_{xy}$
Y_1Y_2 (AP)	$a_x + d_y + ad_{xy}$	$d_x + d_y + dd_{xy}$	$-a_x + d_y - ad_{xy}$
Y_2Y_2 (PP)	$a_x - a_y - aa_{xy}$	$d_x - a_y - da_{yx}$	$-a_x - a_y + aa_{xy}$

Table 2 Pearson correlation coefficients for 10 phenotypic traits measured on 804 *Mimulus guttatus* plants in the greenhouse. Above the diagonal are the correlations for plants with the annual (LMC) genetic background, below the diagonal are correlations for plants with the perennial (SWB) genetic background

	Days to flower	Node to flower	Corolla width	Corolla length	Number of stolons	Stem width	Leaf length	Height	Internode 1 length	Internode 2 length
Days to flower		0.488***	0.194***	0.065	0.230***	0.141*	−0.023	0.154***	−0.267***	−0.195***
Node to flower	0.654***		0.347***	0.346***	0.403***	0.492***	0.142*	0.560***	−0.289***	−0.135**
Corolla width	0.053	0.060		0.609***	0.172***	0.492***	0.604***	0.426***	−0.066	0.167**
Corolla length	−0.044	−0.121*	0.674***		0.107	0.604***	0.512***	0.686***	0.066	0.421***
Number of stolons	0.319***	0.407***	0.133**	0.017		0.234***	0.070	0.245***	−0.174**	−0.164***
Stem width	0.286***	0.375***	0.355***	0.162***	0.242***		0.590***	0.710***	−0.138*	0.310***
Leaf length	−0.170***	−0.107*	0.281***	0.081	0.019	0.310***		0.560***	0.147**	0.465***
Height	0.283***	0.211***	0.319***	0.352***	0.128*	0.341***	0.079		0.157**	0.548***
Internode 1 length	−0.161**	−0.281***	−0.019	0.072	−0.221***	−0.167***	0.246***	0.010		0.266***
Internode 2 length	−0.567***	−0.763***	0.080	0.253***	−0.354***	−0.348***	0.189***	0.057	0.404***	

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 3 Main effects of the DIV1 and DIV2 QTL in homozygous and heterozygous NILs with either the Annual or Perennial genetic backgrounds

Trait	Annual background		Perennial background	
	QTL - DIV 1	QTL - DIV 2	QTL - DIV 1	QTL - DIV 2
Days to flower	**$F_{2,247} = 10.63$, $P < 0.0001$**	$F_{2,318.5} = 0.70$, $P > 0.5$	**$F_{2,440.6} = 6.91$, $P < 0.01$**	**$F_{2,440.2} = 12.89$, $P < 0.0001$**
Node of first flower	**$F_{2,314.1} = 26.30$, $P < 0.0001$**	**$F_{2,313.2} = 5.82$, $P < 0.01$**	**$F_{2,437.2} = 10.02$, $P < 0.0001$**	**$F_{2,437.1} = 51.30$, $P < 0.0001$**
Corolla length	$F_{2,319} = 1.94$, $P > 0.1$	$F_{2,318.2} = 0.17$, $P > 0.5$	**$F_{2,441} = 3.63$, $P < 0.05$**	**$F_{2,440.3} = 6.60$, $P < 0.01$**
Corolla width	**$F_{2,287.6} = 17.50$, $P < 0.0001$**	$F_{2,318.4} = 1.47$, $P > 0.1$	**$F_{2,441.3} = 16.15$, $P < 0.0001$**	**$F_{2,440.5} = 15.32$, $P < 0.0001$**
Stolon number	**$F_{2,308.6} = 3.37$, $P < 0.05$**	**$F_{2,314.5} = 5.93$, $P < 0.01$**	**$F_{2,437.9} = 12.51$, $P < 0.0001$**	**$F_{2,441.2} = 17.41$, $P < 0.0001$**
Stem width	**$F_{2,318.9} = 8.72$, $P < 0.001$**	$F_{2,318.1} = 1.97$, $P > 0.1$	**$F_{2,440.7} = 28.19$, $P < 0.0001$**	$F_{2,440.2} = 2.45$, $P > 0.05$
Leaf length	**$F_{2,302.1} = 8.59$, $P < 0.001$**	$F_{2,319.2} = 2.94$, $P = 0.05$	**$F_{2,443} = 11.54$, $P < 0.0001$**	$F_{2,441.3} = 1.05$, $P > 0.1$
Height	**$F_{2,318.4} = 5.20$, $P < 0.01$**	**$F_{2,318.1} = 7.50$, $P < 0.001$**	**$F_{2,410.5} = 10.58$, $P < 0.0001$**	$F_{2,441} = 0.70$, $P > 0.5$
Internode 1 length	**$F_{2,296.4} = 6.09$, $P < 0.01$**	$F_{2,295.4} = 0.52$, $P > 0.5$	**$F_{2,440.6} = 4.91$, $P < 0.01$**	$F_{2,440.9} = 0.99$, $P > 0.1$
Internode 2 length	$F_{2,319.4} = 1.27$, $P > 0.1$	$F_{2,318.3} = 0.51$, $P > 0.5$	**$F_{2,440.3} = 36.94$, $P < 0.0001$**	**$F_{2,440.1} = 28.47$, $P < 0.0001$**

Cells in bold indicate significance at $P < 0.05$.

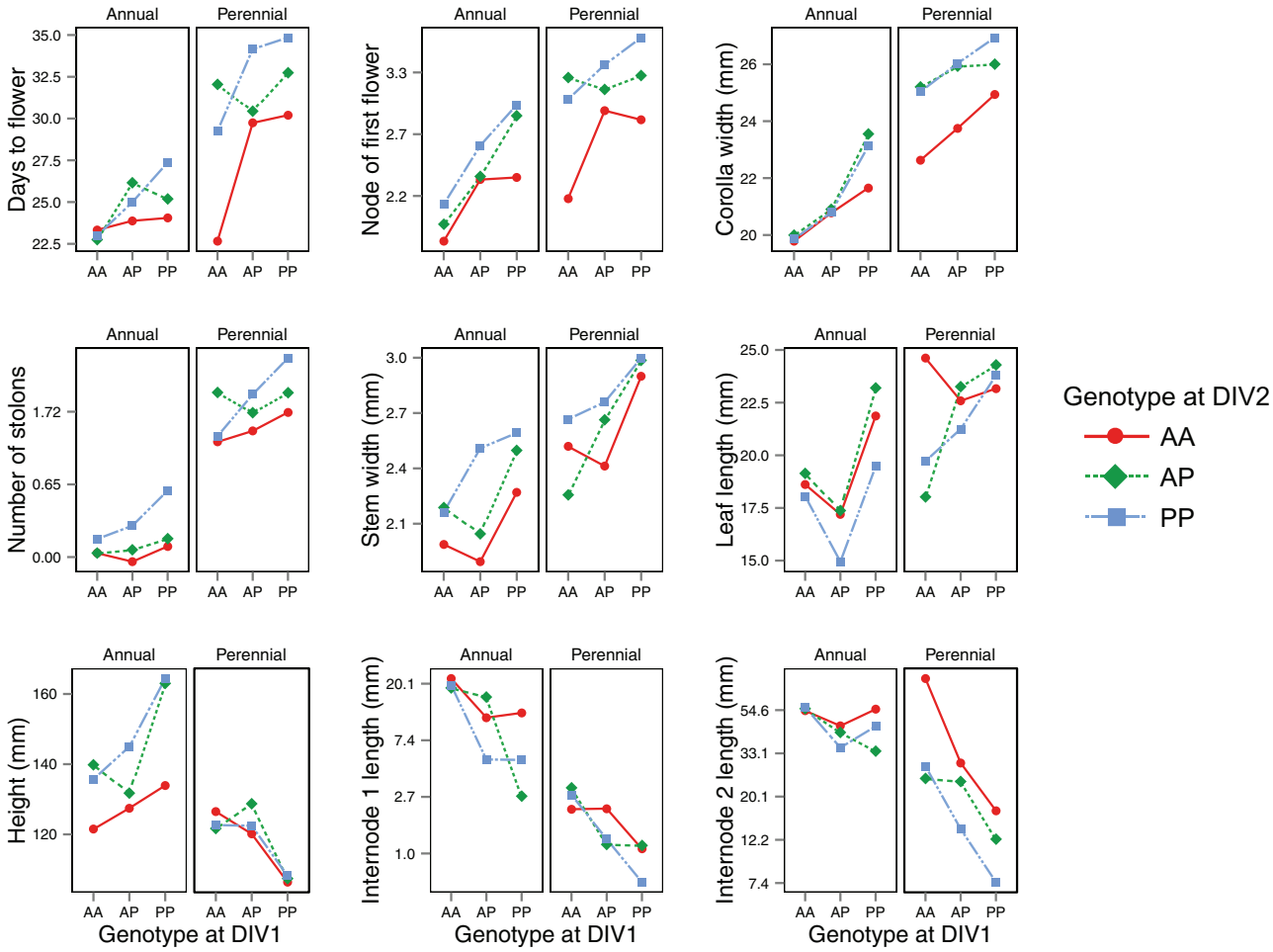

Figure 2. Genotypic effects at 2 biallelic loci (DIV1 and DIV2) for 9 traits. For each trait, the left panel shows the mean genotypic effects in the Annual background, and the right panel shows the mean effect in the Perennial background. The *x*-axis indicates the genotype at DIV1, and the different colored lines represent the genotype at DIV2.

Table 4 Interaction between genetic background and QTL, indicating that the effect of the genotypic class of the QTL depended on genetic background

Trait	QTL - DIV 1	QTL - DIV 2
Days to flower	$F_{2,759.5} = 1.76$, $P > 0.1$	**$F_{2,758.3} = 8.12^{***}$**
Node of first flower	$F_{2,750.7} = 1.51$, $P > 0.1$	**$F_{2,750.1} = 8.15^{***}$**
Corolla length	$F_{2,759.7} = 0.93$, $P > 0.1$	$F_{2,758.3} = 2.41^{*}$
Corolla width	$F_{2,760.7} = 0.04$, $P > 0.5$	**$F_{2,758.2} = 9.81$, $P < 0.0001$**
Stolon number	**$F_{2,761} = 4.78^{*}$**	**$F_{2,759.9} = 5.26^{**}$**
Stem width	$F_{2,759.3} = 1.12$, $P > 0.1$	$F_{2,758.2} = 0.99$, $P > 0.1$
Leaf length	$F_{2,756.9} = 1.10$, $P > 0.1$	$F_{2,760.3} = 0.88$, $P > 0.1$
Height	**$F_{2,758.7} = 11.80$, $P < 0.0001$**	**$F_{2,758.1} = 2.88^{*}$**
Internode 1 length	$F_{2,738.5} = 0.03$, $P > 0.5$	$F_{2,736} = 1.31$, $P > 0.1$
Internode 2 length	**$F_{2,758.7} = 17.80$, $P < 0.0001$**	**$F_{2,758.1} = 15.66$, $P < 0.0001$**

Cells in bold indicate significance at $P < 0.05$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

significantly different from 0 (Tables 5 and 6; Figure 3). Days to flower includes both dominance and epistatic effects, and interestingly the magnitude and sign of these are different in the annual and perennial backgrounds (Table 5), and the magnitude of the additive effects of DIV1 and DIV2 are much greater in the perennial background (Table 5; Figure 2). The second internode distance (a measure of bolting height) shows strong single locus dominance for DIV1 in the annual background and additive by dominance between DIV1 and DIV2; however, in the perennial background, there are only significant single-locus additive effects of both DIV1 and DIV2 (Table 6). Height also shows particularly interesting genotypic effects, the most striking of which is the reversal of the sign of the additive effects in the annual and perennial backgrounds (Table 6; Figure 2—compare across panels).

Finally, by comparing the slopes of the best fit regression lines shown in Figure 3, it is clear that for all traits except height, the combined effect of alleles at DIV1 and DIV2 produce a greater range of phenotypes in the perennial background than in the annual background (i.e., the perennial background has a greater slope).

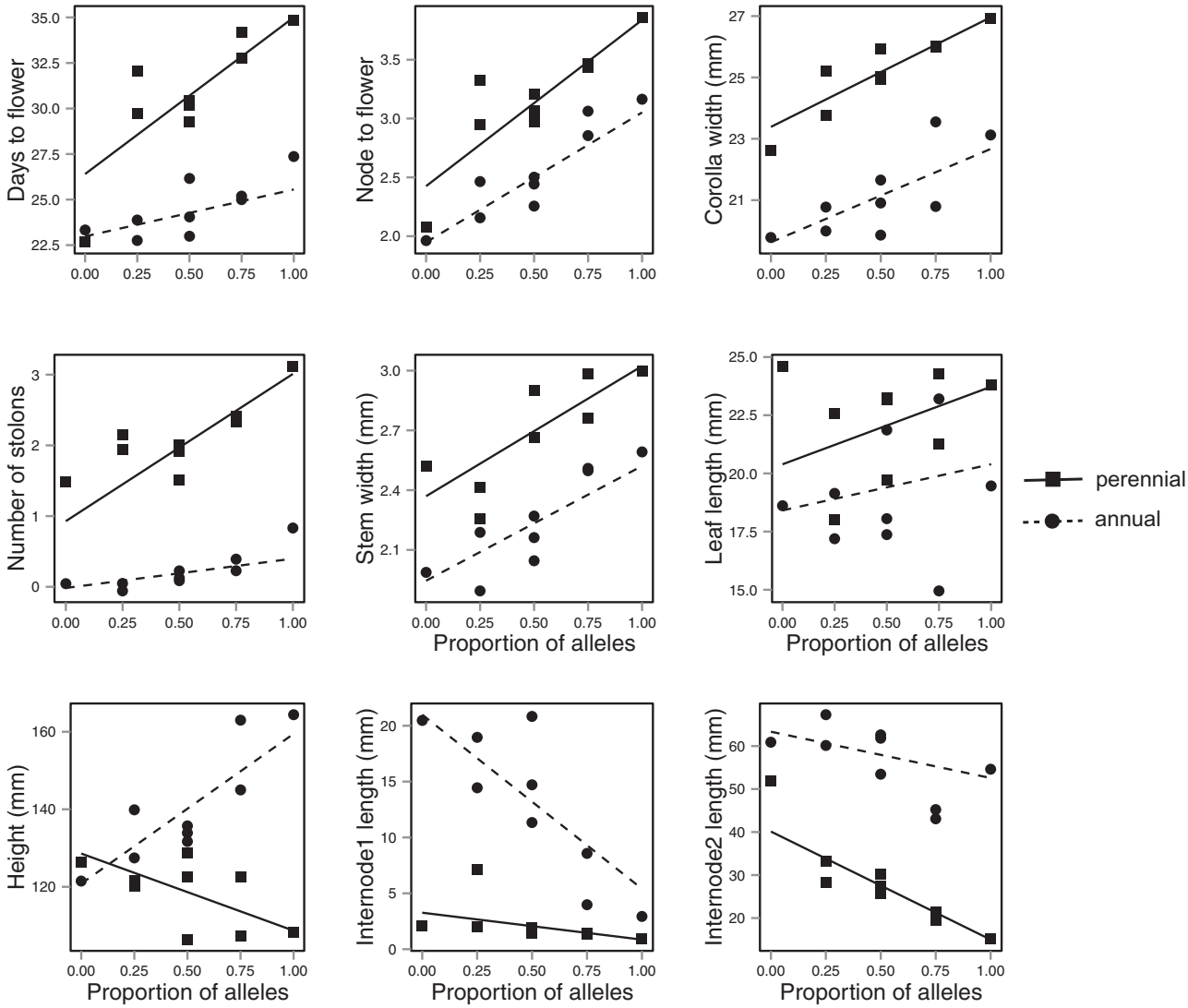

Figure 3. Observed (points) and expected (line) genotype means for selected traits plotted as a function of the proportion of alleles from the Perennial genotype at the 2 QTL loci (i.e., value of 1 is homozygous for both Perennial alleles at both loci; value of 0 is homozygous for both Annual alleles at both loci). The expected lines are for a simple additive genetic model with trait = $m + a$. The squares and solid line are Perennial genetic background NILs, and the circles and dashed line are Annual genetic background NILs.

Discussion

The results of this study indicate that epistatic interactions among loci affecting floral and vegetative life history traits make a substantial contribution to the phenotypic divergence between an annual (LMC) and perennial (SWB) population of *M. guttatus*. There is a substantial amount of pleiotropy in the morphological traits investigated within each genetic background, suggesting that their independent evolution will be constrained. I found evidence for epistatic interactions between the 2 QTL examined and also interactions between the QTL and overall genetic background. The complicated pattern of genetic effects and interactions (encompassing both changes in magnitude and sign) underlying population differences might reflect the evolutionary history that underlies the phenotypic divergence in life history strategies in *M. guttatus*.

The floral, vegetative, and overall life history traits measured here are governed largely by pleiotropic QTL, defined here as a genomic region that affects multiple traits. This finding is similar to previous studies on life history traits (e.g., Westerbergh and Doebley 2004; Li et al. 2006) that showed strong pleiotropy or colocalization of QTL. This could indicate pleiotropic mutations at a single gene or mutations of linked genes that form an adaptive gene complex. Indeed, the DIV1 region is a known inversion that has opposite orientations in the annual and perennial populations studied here (Lowry and Willis 2010). Although we do not know the exact size of the inversion, our current estimate is that

Table 5 Estimates (SE) of parameter values for additive (add) and best-fitting models for 3 flowering traits in the Annual (A) and Perennial (P) genetic backgrounds

	Days to flower				Node of first flower				Corolla width			
	A background		P background		A background		P background		A background		P background	
	Add	Best	Add	Best	Add	Best	Add	Best	Add	Best	Add	Best
m	24.27 (0.36)	24.42 (0.02)	30.70 (0.53)	29.51 (0.31)	2.500 (0.056)		3.131 (0.054)		21.15 (0.19)		25.17 (0.16)	24.93 (0.07)
a_x	−0.99 (0.38)	−1.25 (0.02)	−2.00 (0.57)	−3.00 (0.29)	−0.3547 (0.059)		−0.306 (0.058)		−1.29 (0.21)		−0.81 (0.17)	−0.99 (0.07)
a_y	−0.29 (0.38)	−0.74 (0.02)	−2.29 (0.47)	−2.37 (0.19)	−0.197 (0.059)		−4.00 (0.047)		−0.23 (0.21)		−0.98 (0.14)	−1.04 (0.05)
d_x				2.37 (0.48)								
d_y		−0.44 (0.04)		2.88 (0.60)								0.83 (0.13)
ad_{xy}				2.64 (0.60)								0.72 (0.14)
da_{yx}												
aa_{xy}		0.90 (0.02)										
dd_{xy}		2.17 (0.07)		−4.32 (0.90)								
X^2	4.026	0.026	8.08	0.21	7.007		7.95		4.847		3.088	0.277
df	6	3	6	2	6		6		6		6	4
F-stat	$F_{3,3} = 148.30$, $P < 0.001$		$F_{4,2} = 18.80$, $P = 0.05$								$F_{2,4} = 20.36$, $P < 0.01$	

The following effects are shown: the composite mean (m), additive (a), dominance (d), additive by dominance (ad and da), additive by additive (aa), and dominance by dominance (dd).

Table 6 Estimates (SE) of parameter values for additive (add) and best-fitting models for 6 vegetative traits in the Annual (A) and Perennial (P) genetic backgrounds

	Number of stolons				Stem thickness				Leaf length			
	A background		P background		A background		P background		A background		P background	
	Add	Best	Add	Best	Add	Best	Add	Best	Add	Best	Add	Best
m	0.191 (0.045)		1.969 (0.118)	2.086 (0.077)	2.233 (0.048)	2.229 (0.018)	2.696 (0.040)		19.40 (0.68)	19.79 (0.29)	22.06 (0.47)	
a_x	−0.075 (0.46)		−0.569 (0.125)	−0.419 (0.085)	−0.148 (0.051)	−0.155 (0.016)	−0.25 (0.043)		−1.33 (0.72)	−1.58 (0.325	−1.80 (0.51)	
a_y	−0.132 (0.045)		−0.472 (0.099)	−0.233 (0.089)	−0.140 (0.052)	−0.099 (0.018)	−0.076 (0.035)		0.34 (0.73)	0.52 (0.25)	0.13 (0.41)	
d_x										−3.47 (0.51)		
d_y						0.112 (0.034)				1.21 (0.46)		
ad_{xy}												
da_{yx}						−0.224 (0.04)						
aa_{xy}				0.345 (0.098)								
dd_{xy}						−0.297 (0.059)						
X^2	1.817		11.24	3.237	7.606	0.37	4.799		10.327	0.76	4.47	
df	6		6	5	6	3	6		6	4	6	
F-stat			$F_{1,5} = 12.37$, $P < 0.05$		$F_{3,3} = 19.42$, $P < 0.05$				$F_{3,3} = 38.85$, $P < 0.001$			

	Height				Internode 1 distance				Internode 2 distance			
m	140.14 (2.56)	138.54 (0.84)	118.64 (2.07)	115.26 (0.67)	13.20 (1.13)	12.45 (0.66)	2.06 (0.39)	1.66 (0.04)	57.95 (2.30)	60.22 (1.09)	27.55 (1.08)	
a_x	−9.29 (2.73)	−10.48 (0.78)	10.38 (2.27)	7.76 (0.67)	4.80 (1.21)	5.11 (0.68)	0.85 (0.42)	0.48 (0.05)	3.63 (2.39)		6.75 (1.15)	
a_y	−10.03 (2.83)	−10.82 (0.86)	−0.44 (1.88)		2.99 (1.22)	3.25 (0.68)	0.35 (0.34)	0.27 (0.03)	1.75 (2.37)		5.81 (0.91)	
d_x				6.48 (1.21)						−7.84 (2.87)		
d_y		12.61 (1.49)						2.57 (0.10)				
ad_{xy}								2.39 (0.11)		9.87 (2.33)		
da_{yx}										8.10 (3.26)		
aa_{xy}		3.98 (0.92)										
dd_{xy}		−19.44 (2.53)		7.03 (1.76)		8.37 (2.21)		−2.53 (0.14)				
X^2	7.942	0.199	3.64	0.205	9.64	2.49	1.065	0.004	13.26	2.83	2.61	
df	6	3	6	5	6	5	6	3	6	5	6	
F-stat	$F_{3,3} = 38.85$, $P < 0.001$		$F_{1,5} = 83.56$, $P < 0.001$		$F_{1,5} = 14.36$, $P < 0.05$		$F_{3,3} = 216.65$, $P < 0.001$		$F_{1,5} = 18.39$, $P < 0.01$			

The following effects are shown: the composite mean (m), additive (a), dominance (d), additive by dominance (ad and da), additive by additive (aa), and dominance by dominance (dd).

it is about 6 MB and contains dozens of genes that could be involved in flowering and vegetative traits. Because of suppressed recombination, we are precluded from using fine mapping techniques to locate specific genes that might be involved. However, by utilizing differences in gene expression between the ecotypes in the inverted region, we hope to better understand which genes are consistently differentially expressed and thus identify candidate causative genes. The large effect of the DIV1 QTL in both this study and previous ones (Hall et al. 2006; Lowry and Willis 2010) suggests that it is involved in the divergence of the annual and perennial ecotypes.

The DIV2 QTL region appears to have more complicated genetic effects, including epistasis with genetic background (other unidentified genes) and less pleiotropy. Hall et al. (2010) found a pattern consistent with conditional neutrality at the DIV2 locus in an experiment that included recombinant inbred lines from annual IM population and perennial DUN population. In particular, for plants backcrossed to IM (BC-IM), individuals with a native DUN allele at DIV2 had significantly greater fitness than nonnative IM homozygotes at the Dunes site, but there was no affect of this locus on fitness at the Cascades site. The DIV2 QTL region is also quite large, currently spanning about 1.8 MB. However, there are some obvious candidate genes involved in the flowering time pathway including *GIBBERELLIC ACID REQUIRING 1* (*GA1*), *VERNALIZATION1* (*VRN1*), and 3 copies of *SHORT VEGETATIVE PHASE* (*SVP*). *SVP* has been implicated in *Arabidopsis* as a repressor of flowering and regulates the expression of other floral pathway integrator genes including *FT*, *TSF*, and *SOC1* which all promote flowering (Jang et al. 2009; Gregis et al. 2013).

In general, using inbred line crosses to study evolutionary quantitative genetics poses several limitations. Inbred line and QTL approaches will always sample a small proportion of the total naturally occurring allelic variation. These analyses focus only on the nature of fixed genetic variation between the parental lines in the context of an artificially created experimental population. I attempted to mitigate some of this problem by using 3 separate parental lines for each genetic background. Interestingly, the statistical effect of line nested within population was always significant, suggesting that the different lines were fixed for different alleles throughout their genome.

We do not yet have a clear understanding of the evolutionary relations among the annual and perennial populations of *M. guttatus* and do not know whether the 2 loci investigated here were polymorphic during evolutionary divergence. It is possible that during population divergence, there was sequential evolution at the 2 loci. The finding that the DIV2 QTL has larger effect in the perennial (SWB) background provides fodder for some intriguing scenarios for how evolution may have proceeded. If the annual form were derived from the perennial form, then early variation at DIV2 in the perennial background would have been under strong selection during the initial stages of the transition to the annual form. However, if the transition went in the other direction (perennial to annual), then this locus would only contribute to divergence at the very end of the process when the perennial form was established. As we gain more insight into the population genetic relations among populations, the specific role of epistatic interactions during adaptive divergence should be clarified.

Interestingly, the effect size of alternate alleles is much greater in the perennial background than in the annual background (e.g., the slope of the lines in Figure 3 is always greater for SWB than LMC). This might suggest that the range of phenotypic possibilities for the annual type is more constrained due to other interacting (modifying) loci in the genome. Given that many of the traits are pleiotropic, one could imagine that modifier loci somewhere else in the genome affect this network of traits. The network of genes in the flowering time pathway, and genes involved in life history developmental processes, are functionally well characterized in other plant systems (e.g., reviews by Koornneef et al. 1998; Mouradov et al. 2002; Ingram and Waites 2006; Busov et al. 2008; Krizek 2009) and should facilitate candidate gene identification underlying the traits in *Mimulus*.

There are compelling ecological reasons why the evolutionary history of the annual ecotypes might favor a more narrow range of phenotypes. The annuals grow in environments that deteriorate rapidly with the onset of summer drought, while the perennials grow in habitats with constant access to water. Thus, the harsh environment of the annual habitat might impose stronger selection for rapid transition to flowering and very limited vegetative growth, creating a more constrained phenotypic space. Evidence for this comes from reciprocal transplant field experiments with DIV1 introgression lines by Lowry and Willis (2010). In this study, they showed that there was stronger selection against the perennial alleles in the annual habitat than there was against the annual alleles in the perennial habitat. Thus, the general weaker selection imposed by the habitat of the perennial plants might enable a much larger range of morphological traits. Indeed I have data to suggest that across the entire range of *M. guttatus*, the multivariate phenotypes of perennial populations are more variable than the multivariate phenotypes of annual populations (J.F. and Alex Twyford, unpublished data.).

The maintenance of 2 distinct life history strategies in *M. guttatus* provides an excellent opportunity to study the genetic architecture of adaptively important complex traits differences. Previous research has clearly demonstrated the adaptive significance of flowering time differences, in particular, the benefit of early flowering for annual populations growing on soils that dry out rapidly during summer (Hall and Willis 2006; Lowry 2010). It is still unclear how selection acts on perennial populations and vegetative growth, although one can presume that delayed flowering allows them to accumulate resources during the spring and summer, and become larger, more competitive individuals. Field experiments would be necessary to validate this hypothesis. Overall, our results here suggest that the differentiation between ecotypes could be strongly influenced by the strength and nature of genetic interactions underlying adaptive phenotypic differences, especially because the phenotypic divergence involves

complex mechanisms with both pleiotropic QTL and epistatic interactions.

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