Interactions Among Flower-Size QTL of *Mimulus guttatus* Are Abundant but Highly Variable in Nature

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ABSTRACT The frequency and character of interactions among genes influencing complex traits remain unknown. Our ignorance is most acute for segregating variation within natural populations, the epistasis most relevant for quantitative trait evolution. Here, we report a comprehensive survey of interactions among a defined set of flower-size QTL: loci polymorphic within a single natural population of yellow monkeyflower (*Mimulus guttatus*). We find that epistasis is typical. Observed phenotypes routinely differ from those predicted on the basis of direct allelic effects in the isogenic background, although the direction of deviations is highly variable. Across QTL pairs, there are significantly positive and negative interactions for every trait. Across traits, specific locus pairs routinely exhibit both positive and negative interactions. There was a tendency for negative epistasis to accompany positive direct effects and vice versa for the trait of corolla width, which may be due, at least in part, to the fact that QTL were identified from their direct effects on this trait.

EPISTASIS contributes significantly to intrapopulation variation in floral morphology, development time, and male fitness components of *Mimulus guttatus* (Kelly 2005). The aggregate effect of interactions among QTL substantially alters the resemblance of relatives and phenotypic response to inbreeding. However, previous experiments did not identify the specific character of interactions between QTL. For example, it is not clear whether interactions change the rank order of QTL genotypes. If the direction of allelic effect changes with genetic background, so-called “sign epistasis” (Weinreich *et al.* 2005), the same selection pressure may favor different alleles in different genomic contexts, *e.g.*, different subpopulations of a species (De Brito *et al.* 2005). When the trait is fitness, these kinds of interactions naturally generate peaks and valleys in genotypic fitness landscapes (Wright 1932; Burch and Chao 2000).

Sign epistasis can involve a reversal of allelic effect at one or both loci of an interacting pair. Poelwijk *et al.* (2007) define the double reversal as “reciprocal sign epistasis” and con-

trast sign epistasis generally to “magnitude epistasis” where the magnitude but not the direction of allelic effects changes with genetic background. Magnitude epistasis includes synergistic interactions (alleles have greater effect in combination than individually) and less-than-additive or diminishing returns interactions (alleles have lesser effect in combination) (see Crow and Kimura 1970). Alternatively, one can classify interactions as positive or negative (Phillips *et al.* 2000)—positive if the observed phenotype of an allelic combination exceeds that predicted from direct effects at each locus, and negative if the phenotype of the combination is less than the additive prediction. Unfortunately, there is no simple logical mapping from the magnitude/sign epistasis classification to the positive/negative classification, nor to epistasis in the classical sense (Bateson 1909), wherein one locus masks the effect of another. The taxonomy of epistasis is further complicated by dominance (Routman and Cheverud 1997), higher-order interactions (Templeton 2000), and environmental dependencies (Brock *et al.* 2010).

Molecular genetic studies provide clear examples of sign epistasis. Here, the interacting polymorphisms are often within the same gene. For example, the stability of RNA secondary structures requires matching of nucleotides at different positions. Whether a particular nucleotide change increases or reduces stability depends entirely on the identity

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of the nucleotide at its paired site (Chen *et al.* 1999). With “compensatory evolution” (Moore *et al.* 2000), a mutation that is neutral or detrimental in the original genetic background becomes advantageous by compensating for some other mutation that has recently fixed or at least become prevalent within the population. Mutations conferring antibiotic resistance often have deleterious side effects that reduce bacterial fitness in the absence of the drug. These side effects are attenuated by secondary mutations that are often detrimental in the original genotype (Levin *et al.* 1997; Schrag *et al.* 1997).

Despite the progress in research on microbes (Weinreich *et al.* 2005; Elena *et al.* 2010), we currently know little about the prevalence or nature of epistasis for quantitative traits (Carlborg and Haley 2004) and particularly its impact on standing (segregating) variation within natural populations. Eshed and Zamir (1996) found extensive epistasis among QTL in *Lycopersicon* (tomato), but interactions primarily influenced the magnitude of single-locus effects and not their direction. In contrast, Kroymann and Mitchell-Olds (2005) documented a case of QTL effect reversal in *Arabidopsis thaliana*. The high allele for biomass accumulation in the Ler-0 accession becomes the low allele when introgressed into another line (the Col-0 accession). Patterns of gene sequence variation suggest that this polymorphism is maintained by balancing selection. In *Avena barbata*, two loci with negligible average effects exhibit sign epistasis for fitness in a cross between mesic and xeric genotypes (Latta *et al.* 2010).

Even with these examples, it is difficult to evaluate the quantitative frequency of epistasis or regularities in its nature from the current literature. In part, this is because discovery of QTL×QTL interactions is typically idiosyncratic. In a segregating mapping population such as F₂’s or recombinant inbred lines, there is limited replication of particular multi-locus genotypes. As a consequence, it is difficult to accurately estimate the mean phenotype of any particular multi-locus genotype. Also, there are an enormous number of pairwise tests in a full simultaneous scan, which leads to very stringent significance levels (see box 2 of Carlborg and Haley 2004). Thus, while genomic scans have successfully identified interactions (e.g., Li *et al.* 1997; Cheverud 2000; Montooth *et al.* 2003), it is hard to know if the many nonsignificant interactions are due to absence of effect or absence of power.

Our intention in this study was to conduct a comprehensive survey of interactions among a specific set of monkey-flower QTL. These loci are polymorphic within a single contiguous natural population. We first mapped flower-size QTL within nearly isogenic lines (NILs). Measurements on the NILs isolate the “direct effect” of each QTL, *i.e.*, how the polymorphism affects phenotype in a single uniform genetic background. We then intercrossed the various single-QTL NILs in all combinations to generate the four double homozygotes for each pair of QTL: aabb, AAbb, aaBB, and AABB. This design allows high replication of each multi-locus genotype and hence reasonable power to detect even moder-

ate epistasis (e.g., Moyle and Nakazato 2009). Applying this methodology, we find that epistasis is more the rule than the exception, although the form of interaction is highly variable among QTL pairs.

Materials and Methods

Mapping populations

Three panels of nearly isogenic lines (Molly-2, Molly-3, and Molly-4) were synthesized by repeatedly backcrossing a donor genotype into a fixed genetic background (the recipient line). A distinct donor genome was used for each panel. Each donor was an F₁ from a distinct cross of large- and small-flowered parents produced by artificial selection. The initial source population for the selection experiment was a collection of genotypes derived from a single natural population on Iron Mountain in central Oregon (Kelly 2008). As a consequence, our donor genotypes (the F₁’s) were highly heterozygous for flower-size QTL specific to that natural population. The same recipient genome, IM767, was used for each panel. It is one of several hundred randomly extracted lines from Iron Mountain (Willis 1999). It was chosen for this experiment because it has high pollen fertility relative to most inbred lines, yet is intermediate in flower size.

The first generation of backcrossing to IM767 yielded ~200 lineages per panel. After five subsequent generations, Molly-2 retained 198 lines, Molly-3 retained 156 lines, and Molly-4 retained 139 lines. The Mendelian expectation is that each NIL would be (1/2)⁶ donor (1.5% on average) and the remaining genome IM767. We self-fertilized each sixth generation NIL and collected leaf tissue for genotyping (see below). We grew 12 progeny from each selfed family under standard greenhouse conditions (see Arathi and Kelly 2004) and measured the widest width of the corolla on the first two flowers. Each panel was assayed in a distinct grow-up. Including 154 IM767 individuals, a total of 5708 plants were measured across the three panels. There was significant variation among NILs within each panel. A summary of NIL means is given in [supporting information, File S1](#).

QTL identification

We used a selective genotyping method to identify genomic regions affecting corolla width. NILs were ranked by mean corolla width within panels, and we selected NILs from the high and low tails of each distribution for genotyping. Twenty-four NILs were selected from Molly-2, 16 from Molly-3, and 10 from Molly-4. These 50 NILs (the parents of the plants measured for phenotype) were genotyped at 236 length-polymorphic markers. Except for five microsatellite loci, all markers were selected from the MgSTS marker set (<http://www.mimulusevolution.org>). The marker list, with genomic locations on the current Genome build, is reported in [File S2](#). The F₂ map length of *M. guttatus* is ~1500–1700 cM (Fishman *et al.* 2008; Lee 2009), which implies one marker every 6–7 cM. Markers were chosen on the basis

of map position to effectively span the 14 autosomal chromosomes of *M. guttatus*.

As expected, the great majority of NILs were homozygous for the IM767 allele at all markers. Heterozygous loci were largely idiosyncratic to each NIL, given that different portions of the donor genome were randomly retained in each lineage. However, some donor regions, defined by two to five contiguous markers, were over-represented in high or low selected NILs or in both sets. We genotyped the 24 markers in these regions in the selfed progeny of the 50 selected NILs and then estimated genotype–phenotype association, *i.e.*, cosegregation of putative QTL with flower size. This procedure identified seven QTL for subsequent study: x1, x5a, x5b, x8, x9, x10a, and x10b where the number refers to the linkage group according to the convention of Fishman *et al.* (2002). The donor allele at three of these QTL—x1, x5b, and x8—reduced corolla width relative to IM767. The donor allele increased corolla width for QTL x5a, x9, and x10b, while x10a exhibited overdominance in the NIL progeny data set.

The next three generations of the experiment consisted of “cleaning” the background of selected NILs. From the genotype data, we identified a single NIL containing each of the target QTL (File S1). The choice of this NIL among numerous NILs harboring a particular QTL was based on the relative scarcity of donor markers across the genome. We then used a combination of backcrossing to IM767 and selfing to eliminate remaining donor segments from other parts of the genome. The QTL diagnostic markers were MgSTS_198 and MgSTS_757 for x1; MgSTS_40 for x5a; MgSTS_641 and MgSTS_385 for x5b; MgSTS_432, MgSTS_736, and MgSTS_31 for x8; MgSTS_523 for x9; MgSTS_70 and MgSTS_82 for x10a; and MgSTS_308 for x10b. The resulting single-QTL NILs were then intercrossed in all possible combinations to produce 21 doubly heterozygous genotypes. These double heterozygotes were the grandparents of the plants measured for phenotype and used to estimate epistasis.

Each double heterozygote was self-fertilized. We then grew 100–200 progeny from each progeny set and genotyped these plants at the two segregating QTL (the loci heterozygous in that particular parent). Within each progeny set, we selected multiple individuals of each double-homozygote category—AABB, AAbb, aaBB, and aabb—for subsequent self-fertilization and intercrossing of identical genotypes. These four genotypes are true breeding and one of them (aabb) reiterates the recipient line IM767. Their progeny can be assigned to genotype without directly genotyping individuals. The double homozygote, AABB, is unique to each set, but there is considerable redundancy of the other genotypes across sets. A complete array from seven QTL would involve 29 distinct two-locus homozygous genotypes. However, due to failures in crossing or seed germination, the current data set lacks four of the double-donor homozygotes: x1 with x5a, x1 with x8, x5a with x5b, and x5a with x8.

Estimating genotypic effects

Plants were grown in the University of Kansas greenhouses in seven distinct cohorts over a period of 9 months in 2009–2010. The genotypes measured varied across cohorts, but there was extensive overlap. The effects of cohort and genotype are easily distinguished. Each plant was measured for corolla width on the first two flowers, days to first flower, pistil length, anther length, and corolla length of the first flower. We collected pollen from the first two flowers. Total pollen per flower and pollen viability were measured using an updated version of our grain-size method (Kelly *et al.* 2002): Pollen viability was estimated as the proportion of grains in the larger size class divided by 0.9.

During the course of measurements, we noted a flower color polymorphism within the genotype set for x5b with x8. This is likely caused by a heterozygous locus in the double-heterozygote x5b/x8 grandparent, although we do not know if it represents a *de novo* mutation or residual variation from the original donor/recipient cross. Regardless, we recorded the “pale corolla” on all affected plants, and it is included as an additional factor in the statistical analysis. In each cohort, we genotyped a subset of plants to confirm QTL identity. A small number of contaminants were recognized and excluded from the data set.

The phenotypic data were analyzed using general linear models with cohort as a random factor and genotypes as fixed effects. Calculations were conducted using the REML option in JMP v8. We applied two variants of the same statistical model. In the first variant, QTL are characterized in a factorial way with a direct effect of each locus and an interaction term for each pair of loci (Table S2 of File S3). Epistasis is implied by a significantly non-zero coefficient on the cross-product for a pair of loci. In the second variant model, the 25 distinct two-locus genotypes are treated as different levels of the same factor (Table S3 in File S3). This parameterization does not provide tests for epistasis but does yield a standard error for the mean phenotype of each genotype (Figure 1; Table 1). While the coefficients estimated from each variant of the model are different, they yield the same predicted phenotypic values for each genotype.

For each locus pair and each trait, the statistical model yields an observed phenotype for AABB and a predicted value in the absence of epistasis (from the estimated direct effect of alleles in the isogenic background). We regressed observed AABB phenotypes onto predicted values using least squares to estimate the slope and test whether it was different from 1 (Figure 2). For this, we applied a bias correction to account for estimation error in the predicted phenotype (Fuller 1987). The estimation error variance for predicted values of loci A and B was calculated as $s_A^2 + s_B^2$, where s_A and s_B are the standard errors for estimated direct effects at loci A and B, respectively (Table 1). The overall error variance, v_e , was taken as the average of this quantity across locus pairs. The bias corrected slope, b' , was then

Figure 1 QTL interactions for pistil length (y-axis in mm): For each QTL pair, the deviation of AABB (yellow), AAbb (blue), and aaBB (red) from the mean phenotype of IM767 (aabb) is reported. The locus A QTL defines rows while the locus B QTL defines columns. A double asterisk (**) implies that the interaction remains significant after Holm–Bonferroni correction. A single asterisk (*) denotes interactions that are significant in marginal tests ($P < 0.05$) but not after multiple test correction. Error bars are ± 1 standard error (taken from model fits in Table S3 in File S3). The vertical scale is different for the panel with locus A = x5b.

calculated as $b' = b \left(\frac{v_o}{v_o - v_e} \right)$, where v_o is the observed variance in predicted values (the x-axis values). The standard error of b' is calculated as $\left(\frac{v_o}{v_o - v_e} \right)$ times the uncorrected standard error. These calculations treat $\left(\frac{v_o}{v_o - v_e} \right)$ as known (Fuller 1987, section 1.1) and assume that correlations between estimation errors of direct effects are negligible.

Results

Across cohorts, 6523 plants were measured and included in the analysis, although we could not measure all traits on all plants. The mean, standard deviation, standard error, and sample size for each trait are reported in Table S1, File S3. The model fits to each trait including the interaction term are reported in Table S2 in File S3. Table 1 reports mean values for the 25 distinct two-locus genotypes for three dif-

ferent traits (the full reporting of estimates for this variant of the model are given in Table S3 in File S3). The first row of Table 1 reports the mean phenotype for IM767, and subsequent rows are deviations from this value for genotypes that have the donor allele at one or two loci. The coefficients for each individual QTL (rows 2–8 of Table 1) are the estimated effect of each QTL in an isogenic background. Pale corolla is the effect of the visible polymorphism. The remaining rows detail deviations of double-donor homozygotes (AABB) from the recipient genome (aabb). Unlike other rows, the test for each locus pair is not for whether the coefficient is different from zero. Instead, the P -value refers to the test for a significant interaction among loci (see Table S2 in File S3).

All QTL had highly significant effects on corolla width except x1. At the other QTL, the direction of effects was

Table 1 Genotypic effect estimates reported for corolla width, days to flower, and pistil length

	Corolla width (mm)			Days to flower			Pistil length (mm)		
	Coefficient	SE	P-value	Coefficient	SE	P-value	Coefficient	SE	P-value
IM767 (aabb)	17.56	0.42		27.60	1.12		13.77	0.21	
x10a	0.31	0.10	0.0022	−0.25	0.16	0.1203	−0.13	0.05	0.0120
x9	0.60	0.13	0.0000	0.92	0.20	0.0000	0.53	0.07	0.0000
x1	−0.01	0.12	0.9406	0.38	0.20	0.0535	−0.05	0.07	0.4876
x5a	0.70	0.16	0.0000	−1.53	0.25	0.0000	−0.13	0.08	0.1120
x5b	−1.08	0.12	0.0000	0.39	0.20	0.0474	0.09	0.07	0.1887
x10b	0.95	0.10	0.0000	0.53	0.16	0.0009	0.61	0.05	0.0000
x8	−0.64	0.12	0.0000	0.16	0.19	0.3963	−0.28	0.06	0.0000
Pale corolla	−0.34	0.15	0.0213	−1.76	0.24	0.0000	0.00	0.08	0.9928
x10a/x9	0.02	0.42	0.0432	−1.37	0.68	0.0039	−0.21	0.22	0.0082
x10a/x1	−0.38	0.21	0.0073	−0.87	0.34	0.0127	−0.09	0.11	0.4812
x10a/x5a	0.40	0.16	0.0073	−2.70	0.25	0.0103	−0.62	0.08	0.0035
x10a/x5b	0.62	0.30	0.0000	−1.48	0.48	0.0020	0.63	0.16	0.0001
x10a/x10b	1.54	0.17	0.1601	1.04	0.26	0.0173	0.03	0.09	0.0000
x10a/x8	−0.55	0.13	0.2541	−0.13	0.22	0.9006	−0.83	0.08	0.0000
x9/x1	0.86	0.18	0.2502	1.47	0.28	0.6381	0.88	0.09	0.0015
x9/x5a	0.74	0.17	0.0235	−0.27	0.27	0.3810	0.11	0.10	0.0363
x9/x5b	−0.45	0.17	0.9209	0.36	0.26	0.0071	0.62	0.09	1.0000
x9/x10b	1.12	0.25	0.1241	1.19	0.41	0.5771	0.82	0.14	0.0409
x9/x8	0.57	0.23	0.0242	−0.30	0.37	0.0014	0.76	0.12	0.0004
x1/x5b	−0.61	0.18	0.0445	2.24	0.29	0.0001	0.02	0.10	0.8837
x1/x10b	0.67	0.16	0.2011	−0.56	0.26	0.0000	0.39	0.09	0.1323
x5a/x10b	0.53	0.21	0.0000	−0.23	0.34	0.0754	−0.28	0.11	0.0000
x5b/x10b	1.03	0.32	0.0010	4.28	0.50	0.0000	1.55	0.17	0.0000
x5b/x8	−0.52	0.19	0.0000	−0.67	0.29	0.0006	0.15	0.10	0.0059
x10b/x8	−0.35	0.15	0.0011	0.59	0.25	0.7557	−0.69	0.08	0.0000

P-value for locus pairs refers to the test for a significant interaction among loci (from Table S2 in File S3). SE is standard error for the coefficient, and P-values reported as 0 are <0.00005 (boldface type indicates an interaction test with $P < 0.05$).

always as expected from phenotypes measured in the initial NIL mapping. The average magnitude of direct effects was 0.31 environmental standard deviations. Pleiotropy was extensive (Table S3 in File S3). For example, x1 had significant direct effects on days to flower, stigma–anther separation, anther length, pollen viability, and pollen number per flower, while x10a had significant direct effects on anther length, pistil length, corolla length, and pollen viability. Epistasis is evident for the majority of gene combinations (Tables 1 and 2; Tables S2 and S3 in File S3). For corolla width, 11 of 17 locus pairs exhibit a significant interaction (Table 1). Epistasis was equally frequent for other traits: 11 significant interactions for days to flower, 7 for anther length, 13 for pistil length, 8 for corolla length, 8 for stigma–anther separation, 6 for log[pollen viability], and 8 for log[total pollen per flower]. The number of significant interactions per trait was directly related to the within-experiment “heritability,” *i.e.*, the magnitude of the genotypic variance relative to the environmental variance of a trait (Table S1 in File S3).

The significant interaction counts given above are based on a 0.05 critical value per test. If one applies a Holm–Bonferroni (Holm 1979) adjustment to “correct” for the fact that there are 17 simultaneous interaction tests (critical value for smallest P is 0.05/17; critical value for next most significant P is 0.05/16, etc.), then fewer interactions are significant: 5 for corolla width, 7 for days to flower, 5 for

anther length, 10 for pistil length, 4 for corolla length, 6 for stigma–anther separation, 1 for log[pollen viability], and 3 for log[total pollen per flower]. All QTL combinations produced at least two significant interactions across traits. For certain QTL pairs, interactions were observed for multiple traits even when neither QTL had significant direct effects. Table 2 reports epistatic deviations—the observed AABB phenotype minus the value expected without epistasis—for each two-locus combination.

Figure 1 illustrates the spectrum of gene interactions for pistil length. Given that all QTL combinations share the aabb genotype (mean pistil length = 13.77 mm), the remaining genotypes are expressed as deviations from this common value (as in Muir and Moyle 2009). The combination of x8 with x10b exhibits sign epistasis: The donor allele at x10b increases pistil length in the isogenic background (AAbb > aabb) but decreases this trait in the x8 donor background (AABB < aaBB). Locus pairs x10a/x5b, x5a/x10b, and x9/x8 are other possible cases of sign epistasis in this trait (Figure 1). Classical (masking) epistasis (Bateson 1909) is apparent for x9/x1: The x1 donor allele changes from neutral in the isogenic background (aabb ≈ aaBB) to positive in the x9 donor homozygote (AAbb < AABB). The “less-than-additive” interaction is illustrated by x9/x10b where the direct effect of each donor allele is to increase pistil length. The pistil length of the double-donor homozygote is also elevated, but less than expected

Figure 2 The relationship between the observed phenotype of double-donor homozygotes (AABB) and that predicted from the direct effects of each QTL is given for (A) corolla width, (B) days to flower, (C) pistil length, and (D) log[pollen per flower]. The IM767 phenotype (aabb) is the origin (0,0) in these graphs, and significance of the interaction is denoted by symbol. The solid line is the 1:1 relationship (no epistasis), and the broken line is the bias-corrected linear regression of observed onto predicted.

given the direct effect of each QTL. Synergistic interactions, where alleles from different QTL have magnified effects when combined, are illustrated by x5a/x10a and x5b/x10b. The estimates for other traits also reveal high variability of interactions (Table 1 and Table S3 in File S3).

An informative summary of interactions for each trait is the plot of observed AABB phenotypes against the values predicted in the absence of epistasis (Figure 2). Without epistasis, the relationship should be isometric and locus pairs with nonsignificant interactions are usually close to the 1:1 line. Positive/negative epistasis is estimated by the residual from the isometric line (these are the values reported in Table 2). Statistical tendencies in epistatic interactions are revealed by the regression of observed onto predicted phenotypic values (dashed lines in Figure 2; Table 3). The data from most traits are consistent with a slope of 1, although the high variability of interactions produces broad

confidence bands. The regression slope is significantly <1 for corolla width and pollen per flower (Figure 2). For both traits, epistasis is always positive if the predicted direct effect is negative. Epistasis is negative if the direct effect is positive.

Table 4 summarizes correlations among traits. In the full data set (aggregating measurements across cohorts and genotypes), we find strong positive correlations of flower-size dimensions (corolla length and width, pistil length, anther length) and moderate positive correlation of days to flower with flower-size dimensions. Pollen viability and pollen per flower exhibit weak but positive correlations with flower-size measurements and moderate but negative correlation with days to flower. Statistical significance is much less frequent for correlations on the basis of genotypic effects across traits (lower diagonal of Table 4) due to smaller sample sizes (genotypic effect correlations are based on 25 values).

Table 2 Estimated epistatic deviation reported for each QTL pair for each trait

	Days to flower	Corolla width	Anther length	Pistil length	Corolla length	Stigma–anther separation	Log(pollen viability)	Log(pollen per flower)	Log(viable pollen per flower)
x10a/x9	–0.884	–2.040	–0.595	–0.606	–1.370	–0.006	0.004	0.033	0.036
x10a/x1	–0.680	–1.006	0.237	0.095	–0.310	–0.139	0.081	0.185	0.267
x10a/x5a	–0.607	–0.923	–0.196	–0.353	–0.625	–0.125	–0.043	–0.062	–0.105
x10a/x5b	1.391	–1.626	0.060	0.679	0.954	0.616	0.041	0.120	0.163
x10a/x10b	0.281	0.757	–0.021	–0.445	0.233	–0.419	–0.050	–0.034	–0.084
x10a/x8	–0.215	–0.038	–0.272	–0.425	–0.295	–0.146	0.022	–0.017	0.005
x9/x1	0.268	0.172	0.117	0.395	0.219	0.286	0.018	0.129	0.147
x9/x5a	–0.559	0.341	0.155	–0.282	0.011	–0.411	–0.022	–0.058	–0.080
x9/x5b	0.022	–0.952	0.212	0.000	0.356	–0.214	0.000	0.017	0.018
x9/x10b	–0.436	–0.254	0.139	–0.319	0.019	–0.452	0.020	–0.022	–0.002
x9/x8	0.615	–1.375	0.576	0.510	0.764	–0.041	0.043	–0.009	0.035
x1/x5b	0.479	1.470	0.052	–0.019	–0.426	–0.093	–0.003	0.015	0.014
x1/x10b	–0.272	–1.472	0.196	–0.180	0.250	–0.348	0.052	0.083	0.135
x5a/x10b	–1.115	0.765	–0.020	–0.758	–1.002	–0.707	–0.005	–0.080	–0.086
x5b/x10b	1.151	3.354	0.667	0.850	0.976	0.192	0.084	0.119	0.206
x5b/x8	1.201	–1.219	0.197	0.336	0.915	0.197	0.018	0.083	0.088
x10b/x8	–0.658	–0.100	–0.904	–1.029	–0.961	–0.047	–0.050	–0.027	–0.077

The epistatic deviation is the difference between the observed AABB phenotype and that predicted without epistasis. Boldface type indicates a significant interaction test.

However, there is generally excellent agreement between corresponding numerical values in the upper and lower diagonal of Table 4. All of the raw data (genotypes and phenotypes) are presented in [File S4](#).

Discussion

Epistasis clearly contributes to the genetic differences among species and among populations within a species (e.g., Hard *et al.* 1992; Palopoli and Wu 1994; Burton *et al.* 1999; Fenster and Galloway 2000; Demuth and Wade 2007), and such interactions have been mapped to particular QTL in many systems (Carlborg and Haley 2004; Elnaccash and Tonsor 2010). In the great majority of cases, however, it is not clear that interacting loci were ever simultaneously polymorphic within the same evolving population. The present study of yellow monkeyflower considers genetic variability in a single natural population at Iron Mountain in Oregon. The estimates thus speak directly to the consequences of epistasis for quantitative trait evolution.

Table 3 Summary of estimates for the bias-corrected slope and SE obtained from the linear regression of observed AABB phenotype onto that predicted without epistasis

Trait	Slope	SE	Lower bound	Upper bound
Days to flower	1.12	0.44	0.19	2.04
Corolla width	0.45	0.15	0.14	0.75
Anther length	1.21	0.27	0.65	1.78
Pistil length	0.96	0.35	0.23	1.68
Corolla length	0.97	0.28	0.37	1.56
Stigma–anther separation	0.87	0.33	0.18	1.56
Log(pollen viability)	0.65	0.35	–0.08	1.39
Log(pollen per flower)	0.23	0.24	–0.27	0.74
Log(viable pollen per flower)	0.33	0.25	–0.20	0.86

Boldface type indicates that the slope is significantly <1.

A principal finding is that QTL for flower size routinely interact, and these interactions are highly variable in form. Variable interactions among novel or visible mutations have been demonstrated in several systems (Clark and Wang 1997; de Visser *et al.* 1997; Elena and Lenski 1997). The present data extend this generalization to intrapopulation variation in quantitative characters. This is a nontrivial extension, given that standing variation has been sieved by selection and is likely to have different properties from new mutations. Notably, we find sign epistasis, *i.e.*, the reversal of allelic effect with changes in genetic background, for segregating variation. However, other kinds of interactions (less-than-additive, synergism, effect masking) are also routinely observed.

Variability of interactions is multifaceted. Across QTL pairs, there are significantly positive and negative interactions for every trait (Table 2). Across traits, specific locus pairs routinely exhibit both positive and negative interactions. With a couple of notable exceptions (e.g., Figure 2A), the frequency of positive relative to negative interactions is largely unrelated to the direct effect of a locus in the isogenic background. There was some consistency of effects by a locus pair across genetically correlated traits. For example, x10a and x9 interact in a consistently negative way for flower-size dimensions, as does x10a with x5a and x10b with x8 (Table 2). x5b and x10b interact in a consistently positive way for the same collection of measurements. The finding of variation in epistatic effects was clearly anticipated by previous quantitative genetic experiments (Kelly 2005). Kelly (2005) found that the statistical signal of epistasis could not be removed by any scale transformation. Scale transformation can eliminate epistasis if it is simple and directional, but not if interactions are variable in nature.

Directional epistasis is routinely invoked in evolutionary models. For example, synergism between deleterious

Table 4 Raw phenotypic correlations and correlations of genotypic effects (deviations from IM767)

	Corolla width	Days to flower	Anther length	Pistil length	Corolla length	Stigma–anther separation	Log(pollen viability)	Log(pollen per flower)	Log(viable pollen per flower)
Corolla width		0.26	0.63	0.64	0.77	0.08	0.14	0.09	0.13
Days to flower	0.19		0.27	0.23	0.21	–0.02	– 0.19	– 0.30	– 0.30
Anther length	0.50	0.56		0.69	0.71	– 0.30	0.17	0.12	0.16
Pistil length	0.49	0.55	0.81		0.73	0.49	0.17	0.11	0.16
Corolla length	0.65	0.49	0.91	0.81		0.11	0.11	0.05	0.09
Stigma–anther separation	0.05	0.08	–0.15	0.45	–0.01		0.01	–0.01	0.00
Log(pollen viability)	0.34	–0.15	0.36	0.30	0.23	–0.05		0.42	0.77
Log(pollen per flower)	0.26	–0.40	0.08	0.14	0.09	0.11	0.71		0.90
Log(viable pollen per flower)	0.32	–0.32	0.20	0.22	0.15	0.05	0.89	0.95	

Raw phenotypic correlations are reported above the diagonal, and correlations of genotypic effects (deviations from IM767) are reported below the diagonal. Boldface type indicates a correlation significantly different from zero ($P < 0.05$).

mutations is a key component of the mutational deterministic model for the maintenance of sex (Kondrashov 1988). However, a number of novel evolutionary consequences emerge from variation in epistatic effects (Phillips *et al.* 2000). With recurrent deleterious mutation, Otto and Feldman (1997) show that epistatic variation reduces the likelihood that selection will favor sexual reproduction. Variable epistasis also alters the progression of Muller's ratchet (Butcher 1995) and the rate that adaptive evolution within populations generates reproductive isolation among them (Gavrilets and Gravner 1997; Wade 2000).

Epistasis and the genetic variance in flower size

Flower size and shape have diversified extensively in the genus *Mimulus* associated with changes in mating system and pollination syndrome (Vickery 1978; Bradshaw and Schemske 2003). In the Iron Mountain population of *M. guttatus*, flower-size variation is highly polygenic and under strong natural selection (Willis 1996; Mojica and Kelly 2010). Narrow sense heritabilities for floral dimensions are in the range of 0.3–0.5 (Kelly and Arathi 2003; Kelly 2008; Scoville *et al.* 2009), and >40 intrapopulation flower-size QTL have been mapped (Lee 2009). The additive genetic variance in flower size depends on allelic effects (including dominance) at QTL, on the interactions among QTL, and on the frequency of alternative alleles in the natural population.

At present, we have no direct estimates for allele frequency for any of these loci. However, we have estimated the additive genetic variance contribution (within Iron Mountain) of nine flower-size QTL, including x5a, x5b, x9, and x10a of the present study (Scoville *et al.* 2011). V_Q , the additive genetic variance contributed by a locus (as a percentage of the total phenotypic variance), was estimated to be 1.8, 4.8, 1.8, and 1.9 for x5a, x5b, x9, and x10a, respectively. In the absence of epistasis, the additive genetic variance of a trait is a sum of V_Q across all QTL in the genome [plus an increment for any loci exhibiting linkage disequilibria (Bulmer 1980)]. With epistasis, the additive value of an allele is an average across genetic backgrounds. As a consequence, V_Q for a locus will depend on allele frequencies at other QTL.

Epistasis may explain an intriguing pattern from the V_Q study. We found that QTL with moderate effects on corolla

width, as measured in NILs, had the largest V_Q estimates. The QTL with largest effect in the NILs had the lowest V_Q estimates (see figure 1 of Scoville *et al.* 2011). This could be due to the fact that mutations with large effects on corolla width are detrimental for fitness and thus exist at low frequency. Epistasis provides an alternative explanation in that allelic effects inferred from a particular NIL population might be quite different than the average effect obtained by averaging over a diversity of genetic backgrounds. The V_Q estimates of Scoville *et al.* (2011) were obtained by integrating marker-trait associations over 138 distinct “line-cross families” representing genetic variation within the natural population. Relevant here is that our mapping method preferentially selected QTL with the most pronounced effects on corolla width within a single genetic background. We created NILs by repeatedly backcrossing a donor genotype into a fixed genetic background (the IM767 recipient line) and focused on NILs with the most extreme mean corolla widths (File S1). If this procedure preferentially identified QTL with magnified effects in the IM767 background, then we should expect reduced signal as a QTL is assayed in more diverse backgrounds. All nine QTL with V_Q estimates were initially identified by selective genotyping within NILs (Scoville *et al.* 2011).

The net effect of epistasis on the amount of genetic variation in quantitative characters of *M. guttatus* cannot be evaluated from the current data. However, it is informative to consider the variance of observed AABB phenotypes relative to the variance of predicted values in the absence of epistasis. In other words, we compare the variance along the y-axis of Figure 2 to the variance along the x-axis. For most of the traits, the y-axis variance is greater than the x-axis variance, suggesting that epistasis inflates the genetic variation (ignoring estimation issues). The exceptions are corolla width, log[pollen per flower], and log[viable pollen per flower]. Each of these traits exhibits a slope significantly <1 in the regression of observed AABB phenotypes onto predicted values (Figure 2; Table 3). The isolation of QTL on the basis of corolla width effects might explain why this trait differs from other floral dimensions. The pollen traits exhibit lower genetic variance (relative to environmental effects) than other characters (Table S1 in File S3).

Evolutionary consequences

An important question is how variable epistasis affects response to directional selection on a trait (Hallander and Waldmann 2007; Crow 2010). In a large, randomly mating population, response should be proportional to the additive genetic variance. The principal effect of epistasis is in determining the additive value of an allele. Recombination ensures that an allele is expressed in a variety of genetic backgrounds and that its additive value is an average over backgrounds. With sign epistasis, whether an allele increases or decreases trait values will depend on the relative frequencies of different genetic backgrounds. The same selection pressure imposed on different populations, with different initial allele frequencies, might cause different alleles at the same locus to be favored in different populations.

An interesting property of variable epistasis among QTL is that it may be essentially “invisible” at the phenotypic level in a population undergoing sustained directional selection. As allele frequencies change at QTL for the selected trait, V_Q at these loci will also change. With directional epistasis, for example, when alleles increasing trait values interact synergistically, changes in V_Q may be directionally consistent across QTL. This can produce substantial changes in the overall additive variance (see figure 3 of Hansen 2006), causing substantial accelerations or decelerations in the rate of phenotypic change. With variable epistasis, however, changes in V_Q are unlikely to be directionally consistent across QTL. Positive changes at some QTL cancel negative changes at others, yielding approximate constancy of the overall genetic variance in the short term. In this way, the effects of variable epistasis may not be evident at the phenotypic scale, particularly given other factors influencing the genetic variance such as mutation and genetic drift. However, variable epistasis will definitely complicate prediction of allele frequency change at individual QTL, particularly if allelic effects are estimated in a single uniform genetic background and assumed to be constant.

The epistasis for stigma–anther separation (Figure 3) is interesting to consider in relation to the contrasting views of Ronald Fisher and Sewall Wright on the role of epistasis in evolution (Provine 1971). Fisher argued that recombination effectively averages interactions across backgrounds and selection acts on average allelic effects, while Wright felt that selection of specific gene combinations is essential for adaptation. The recombination essential to Fisher’s view depends on random mating. Self-fertilization reduces the efficacy of recombination and may allow selection on gene combinations. In fact, selfing is a more potent mechanism to preserve gene combinations than the population structure/drift mechanism of Wright’s shifting balance model.

Stigma–anther separation is a determinant of selfing rate in many flowering plants (e.g., Vallejo-Marin and Barrett 2009), and several *M. guttatus* experiments have shown correlation of reduced stigma–anther separation with increased selfing (Carr and Fenster 1994; Fishman and Willis

Figure 3 The estimated stigma–anther separation (y-axis in mm) is reported for each of the homozygous genotypes for three loci: x10a (A/a), x5a (B/b), and x10b (C/c). Genotypes differ from IM767 at one locus (shaded bars), two loci (stippled bars), or three loci (open bars). The error bars are ± 1 standard error for the estimated deviation from aabbcc. No error bars are reported for AABbCC because this genotype was not present in the data set. Its value is predicted from the estimated direct and pairwise interactions (Table S2 in File S3), assuming no higher-order interaction.

2008b). The *M. guttatus* species complex includes a number of highly selfing lineages that have evolved reduced stigma–anther separation (Ritland and Ritland 1989; Fenster and Ritland 1994; Wu *et al.* 2008). Figure 3 illustrates an interesting feature of interactions among QTL for this trait: x5a, x10a, and x10b each have minimal direct effects, but combinations of alleles can produce striking reductions in stigma–anther separation. These QTL are potential mating system modifiers, but only in combination. Our pairwise interaction model (Table S2 in File S3) predicts a 1-mm reduction in stigma–anther separation for the x5a/x10a/x10b donor homozygote. A change of this magnitude could substantially elevate selfing rate in Iron Mountain plants (see Fishman and Willis 2008; figure 4 of Bodbyl Roels and Kelly 2011). The stigma–anther results are intriguing in that the phenotypic effect of epistasis might itself facilitate the persistence of gene combinations across generations.

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GENETICS

Supporting Information

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Interactions Among Flower-Size QTL of *Mimulus guttatus* Are Abundant but Highly Variable in Nature

John K. Kelly and Julius P. Mojica

File S1

Supporting Appendix 1

The analysis of variance for panel Molly2 with NIL as factor and corolla width as response is:

Source	DF	SS	MS	F	P
Line	194	1440.21	7.42	1.69	0.000
Error	2058	9049.66	4.40		
Total	2252	10489.87			

The mean corolla width (in mm), days to flower, and sample size (n) are given for each NIL of the Molly2 panel. xx in the last column indicates that DNA from that NIL was scored at markers across the genome. The last row is data for plants of the recipient line (IM767) grown simultaneously with the NILs.

NIL ID	mean CW	mean days	N1	Full genome scan
129	15.68	29.25	12	xx
301	15.79	31.45	11	xx
239	16.13	28.08	12	xx
123	16.16	30.75	12	xx
222	16.25	28.75	12	xx
52	16.58	27.92	12	xx
190	16.87	28.75	12	xx
265	16.87	28.50	12	xx
138	16.90	25.25	12	xx
258	16.91	27.82	11	xx
220	17.05	27.75	12	xx
248	17.09	28.42	12	
153	17.11	27.50	12	
47	17.15	28.75	8	
107	17.22	28.25	12	
240	17.27	31.54	13	
242	17.31	28.00	12	
74	17.32	29.17	12	
230	17.35	32.64	11	
63	17.39	31.82	11	
266	17.40	29.30	10	
257	17.43	30.42	12	xx
189	17.46	28.45	11	
233	17.46	27.33	12	
263	17.47	27.62	13	
66	17.50	33.36	11	
254	17.52	29.50	12	
151	17.54	28.08	12	
251	17.56	30.83	12	
213	17.58	29.08	12	
232	17.60	28.08	12	
117	17.61	29.09	11	
109	17.62	30.54	13	
264	17.62	29.67	9	

217	17.66	29.50	12
154	17.66	30.91	11
241	17.68	28.92	12
274	17.72	29.33	12
65	17.73	28.92	12
39	17.74	31.92	12
30	17.75	29.00	12
1	17.82	30.25	12
23	17.83	28.42	12
75	17.83	28.50	12
8	17.85	29.00	12
219	17.86	31.42	12
58	17.88	28.50	12
69	17.89	30.00	12
53	17.90	27.83	12
100	17.93	27.25	12
14	17.94	29.33	12
269	17.94	28.29	7
19	17.95	28.25	12
50	17.96	27.00	12
249	17.96	30.00	12
133	17.97	28.83	12
171	17.97	28.25	12
221	17.97	28.92	12
60	17.98	31.64	11
285	18.02	31.58	12
201	18.06	28.08	12
16	18.07	29.08	12
288	18.07	29.00	11
162	18.08	28.67	12
238	18.09	28.42	12
145	18.12	28.70	10
180	18.12	33.60	10
294	18.13	28.27	11
218	18.14	30.42	12
80	18.15	28.17	12
206	18.15	29.08	12
7	18.16	29.42	12
136	18.16	28.67	12
237	18.16	28.75	12
276	18.16	29.30	10
291	18.16	27.58	12
157	18.17	29.17	12
231	18.17	28.67	12
106	18.18	30.00	12
11	18.19	27.50	12
3	18.21	27.75	12
5	18.21	28.17	12
235	18.22	28.91	11
95	18.22	28.40	10

277	18.22	30.70	10
168	18.23	28.00	7
150	18.24	27.82	11
273	18.24	30.09	11
116	18.25	27.83	12
6	18.26	28.42	12
174	18.27	28.00	12
244	18.27	28.25	12
128	18.29	28.82	11
214	18.30	28.40	10
85	18.32	30.00	9
78	18.32	31.09	11
292	18.34	27.88	8
229	18.34	28.75	12
208	18.35	29.18	11
17	18.35	29.00	12
161	18.35	28.17	12
175	18.36	28.83	12
68	18.38	28.69	13
165	18.38	28.17	12
51	18.39	31.17	6
227	18.39	27.75	12
125	18.40	30.92	12
24	18.42	27.08	12
43	18.43	26.67	12
127	18.43	31.86	7
163	18.44	29.75	12
211	18.44	28.36	11
124	18.45	29.73	11
25	18.46	27.58	12
56	18.46	27.91	11
94	18.50	31.67	9
290	18.51	29.57	7
118	18.51	28.55	11
283	18.51	29.92	12
284	18.51	29.00	12
271	18.52	30.45	11
31	18.52	26.75	12
147	18.52	28.83	12
280	18.52	29.25	12
252	18.53	30.00	11
243	18.53	28.83	12
158	18.61	27.42	12
212	18.66	30.82	11
179	18.67	28.33	12
18	18.68	27.58	12
20	18.68	29.75	12
207	18.68	29.83	12
49	18.72	27.64	11
36	18.73	29.89	9

192	18.74	29.92	12
28	18.75	28.33	12
83	18.75	27.50	12
272	18.75	30.17	12
12	18.77	27.42	12
261	18.80	27.33	12
113	18.85	26.42	12
130	18.85	29.00	12
101	18.88	28.42	12
29	18.89	30.92	12
178	18.90	26.42	12
256	18.92	29.58	12
155	18.95	29.50	12
32	18.96	28.36	11
152	18.98	27.82	11
71	18.99	27.42	12
122	18.99	27.50	6
96	19.00	27.92	12
142	19.08	28.90	10
279	19.08	30.50	12
86	19.10	28.25	12
27	19.11	30.67	12
134	19.15	31.10	10
13	19.16	27.92	12
92	19.16	28.58	12
143	19.17	28.67	12
119	19.18	28.08	13
88	19.19	28.08	13
286	19.22	28.10	10
210	19.22	30.33	6
10	19.24	28.75	12
156	19.25	34.86	7
46	19.27	28.13	8
126	19.28	28.67	12
187	19.28	27.58	12
203	19.28	28.33	12
204	19.28	29.08	12
76	19.29	30.80	10
26	19.29	28.00	12
79	19.30	30.40	10
160	19.32	30.00	7
226	19.35	28.25	8
70	19.36	29.64	11
67	19.38	29.58	12
121	19.38	29.25	12
120	19.39	28.50	12
102	19.45	27.58	12 xx
139	19.56	27.42	12 xx
191	19.58	27.67	12 xx
255	19.59	30.50	12 xx

73	19.63	27.18	11	xx
105	19.64	29.17	12	xx
15	19.66	30.75	12	xx
200	19.66	28.67	6	
22	19.80	27.33	12	xx
197	19.80	29.50	12	xx
236	19.83	27.92	12	xx
90	19.98	29.50	12	xx
89	20.35	28.67	12	xx
198	20.57	27.00	2	

767	17.89	27.33	64	
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The NILs from which we isolated x10a, x9, x1, and x10b were sampled from Molly-2.

The analysis of variance for panel Molly3 with NIL as factor and corolla width as response is:

Source	DF	SS	MS	F	P
Line	158	1998.23	12.65	3.36	0.000
Error	1662	6258.46	3.77		
Total	1820	8256.68			

The mean corolla width (in mm), days to flower, and sample size (n) are given for each NIL of the Molly3 panel.

NIL ID	mean CW	mean Day	n	Genome scan
82	13.71	30.69	13	xx
284	14.22	34.00	1	
156	15.43	28.75	12	xx
104	15.53	31.25	12	xx
87	15.92	29.42	12	xx
99	15.92	28.38	13	xx
283	16.07	29.40	11	xx
34	16.08	28.58	12	xx
150	16.46	27.42	12	xx
209	16.49	28.92	12	xx
145	16.52	27.77	13	xx
151	16.55	29.29	7	
86	16.68	28.33	12	
47	16.72	26.58	12	
113	16.74	29.58	12	
123	16.80	26.58	12	
286	16.86	28.36	11	
126	16.86	27.92	12	
245	16.88	27.58	12	
79	16.90	29.27	11	
278	16.93	29.36	11	
152	17.04	27.33	12	
84	17.05	27.33	12	
81	17.05	29.58	12	
41	17.09	29.25	12	
136	17.15	29.08	12	
12	17.27	27.38	13	
200	17.28	28.67	12	
77	17.31	26.83	12	
124	17.32	27.17	12	
204	17.45	27.08	12	
213	17.46	27.69	13	
6	17.49	27.25	12	
179	17.50	29.00	11	
9	17.50	29.45	11	
32	17.50	28.25	12	
289	17.52	29.00	12	
148	17.53	27.75	12	
96	17.55	28.00	12	
252	17.56	29.92	12	

59	17.58	28.33	12
158	17.58	29.69	13
172	17.59	29.67	12
72	17.62	25.92	12
64	17.62	26.50	12
185	17.66	27.67	12
159	17.66	27.75	12
135	17.71	29.00	12
203	17.71	29.58	12
141	17.72	28.50	12
279	17.72	28.55	11
89	17.74	28.83	12
230	17.75	28.33	12
120	17.77	28.92	12
8	17.81	27.75	12
90	17.84	28.58	12
7	17.86	29.67	12
161	17.91	27.67	12
221	17.93	28.83	12
3	17.95	26.25	12
75	17.95	27.17	12
201	17.95	28.17	12
232	17.96	27.64	11
182	18.01	27.60	10
5	18.02	29.20	10
50	18.02	28.45	11
267	18.03	26.00	1
24	18.03	27.92	12
30	18.03	28.00	1
188	18.04	26.75	12
144	18.05	28.73	11
273	18.08	26.70	10
223	18.13	28.46	13
268	18.18	28.27	11
78	18.19	28.08	12
299	18.19	28.50	12
261	18.20	29.50	10
162	18.20	28.08	12
280	18.20	28.22	9
131	18.22	29.00	11
180	18.26	30.25	12
117	18.27	28.83	12
160	18.28	28.45	11
69	18.30	26.67	12
73	18.30	29.33	12
97	18.32	29.67	12
269	18.32	30.50	12
147	18.32	28.45	11
52	18.35	29.17	12
239	18.36	28.58	12

153	18.37	28.38	8
4	18.44	28.75	12
225	18.45	28.00	12
186	18.45	30.18	11
300	18.46	27.83	12
292	18.47	28.75	12
16	18.48	29.50	12
164	18.50	28.64	11
42	18.50	27.92	12
205	18.51	28.25	12
105	18.56	27.25	12
115	18.56	28.08	12
254	18.56	30.33	12
211	18.58	28.08	12
61	18.61	29.75	12
106	18.63	28.33	12
140	18.63	31.00	12
33	18.68	28.58	12
197	18.70	26.42	12
241	18.70	27.83	12
174	18.71	28.92	12
54	18.73	27.83	12
243	18.74	29.64	11
173	18.80	32.40	10
168	18.83	29.00	12
176	18.85	29.00	12
266	18.88	29.60	10
157	18.89	30.45	11
146	18.92	28.08	12
2	18.92	28.60	10
101	18.92	28.82	11
85	18.96	28.50	10
29	18.97	28.42	12
193	18.99	29.75	12
116	18.99	28.77	13
270	19.02	28.00	12
14	19.02	31.33	12
214	19.08	29.45	11
265	19.10	28.36	11
248	19.10	27.90	10
260	19.11	28.33	12
208	19.12	28.33	12
287	19.13	29.33	12
53	19.15	28.55	11
181	19.18	30.25	12
166	19.24	30.42	12
212	19.25	30.42	12
253	19.26	28.82	11
220	19.28	28.75	12
217	19.28	29.92	12

229	19.30	29.50	12	
210	19.30	30.36	11	
127	19.35	29.62	13	
1	19.38	27.83	12	xx
178	19.38	29.90	10	
263	19.39	28.50	12	
207	19.47	29.08	12	
250	19.49	29.67	9	
46	19.49	27.25	12	
226	19.51	28.00	11	
169	19.56	29.00	1	
142	19.65	30.18	11	
187	19.85	29.18	11	
98	19.85	28.67	12	xx
76	19.88	30.08	12	xx
216	19.93	29.92	12	xx
235	20.40	32.13	8	xx
36	20.92	31.45	12	xx
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767	19.27	29.04	26	

The analysis of variance for panel Molly4 with NIL as factor and corolla width as response is:

Source	DF	SS	MS	F	P
Line	134	1110.43	8.29	3.92	0.000
Error	1499	3167.93	2.11		
Total	1633	4278.37			

The mean corolla width (in mm), days to flower, and sample size (n) are given for each NIL of the Molly4 panel.

NIL ID	mean cw	mean day	n	Genome scan
78	14.77	32.67	12	xx
122	15.19	31.50	12	xx
139	15.26	30.73	11	xx
41	15.44	30.92	12	xx
206	15.65	27.75	12	xx
197	15.75	28.17	12	
165	15.86	30.75	12	
137	15.93	28.50	12	
179	15.94	32.00	8	
18	16.21	32.33	12	
29	16.22	32.42	12	
192	16.43	31.00	12	
11	16.46	31.83	12	
79	16.46	30.83	12	
164	16.47	29.92	12	
40	16.48	31.92	12	
113	16.51	30.67	12	
6	16.53	31.83	12	
46	16.54	31.67	12	
121	16.58	30.42	12	
34	16.61	29.83	12	
163	16.61	29.77	13	
39	16.63	33.92	12	
169	16.64	31.17	12	
30	16.65	29.17	12	
47	16.65	31.55	12	
72	16.68	31.42	12	
173	16.69	30.50	12	
175	16.71	30.25	12	
171	16.72	29.09	11	
186	16.76	29.17	12	
37	16.82	28.42	12	
185	16.85	28.08	12	
195	16.86	30.17	12	
17	16.88	32.58	12	
55	16.89	29.42	12	
180	16.89	30.45	11	
146	16.90	28.33	12	
161	16.92	31.58	12	
2	16.93	31.75	12	

56	16.93	29.17	12
205	16.97	30.75	12
178	16.98	30.09	12
212	17.00	28.00	12
149	17.01	35.00	12
210	17.03	28.33	12
75	17.06	30.00	12
111	17.06	31.58	12
162	17.06	30.83	12
80	17.08	31.00	12
152	17.08	31.00	12
59	17.09	32.27	11
199	17.09	29.92	12
128	17.13	30.92	13
90	17.13	30.83	12
85	17.15	33.42	12
31	17.16	31.50	12
100	17.16	32.73	11
57	17.17	31.92	12
148	17.19	28.33	12
27	17.20	31.89	9
58	17.20	32.91	11
208	17.22	30.92	12
112	17.23	30.00	12
147	17.24	29.17	12
68	17.29	32.11	9
220	17.29	29.67	12
60	17.30	31.25	12
7	17.32	35.50	12
35	17.34	28.42	12
83	17.34	31.83	12
142	17.34	29.75	12
196	17.34	27.92	12
52	17.36	31.42	12
154	17.41	29.42	12
62	17.43	31.00	12
86	17.44	31.33	3
124	17.44	31.08	12
42	17.48	31.67	6
183	17.49	30.42	12
19	17.50	33.50	12
190	17.50	31.00	6
134	17.57	30.50	12
95	17.59	30.75	12
96	17.59	32.67	12
105	17.61	31.17	12
168	17.61	31.33	12
70	17.64	31.58	12
65	17.68	31.83	12
54	17.70	31.67	12

77	17.70	31.67	12
209	17.71	30.17	12
73	17.75	31.92	12
74	17.75	32.25	12
97	17.80	32.67	12
184	17.80	31.17	12
64	17.83	30.67	12
3	17.84	31.92	12
93	17.84	30.50	12
117	17.85	31.82	11
44	17.90	34.42	12
49	17.90	30.42	12
136	17.92	30.58	12
92	17.98	34.42	12
43	18.00	31.17	12
71	18.01	31.00	10
151	18.01	31.83	12
51	18.04	31.83	12
53	18.04	32.75	12
9	18.09	31.50	12
50	18.09	31.83	12
63	18.09	34.33	12
157	18.09	33.42	12
172	18.09	30.58	12
106	18.13	32.67	12
107	18.13	29.67	12
69	18.14	30.42	12
198	18.14	27.58	12
98	18.17	33.17	12
76	18.18	31.83	12
108	18.19	32.83	12
4	18.20	29.83	12
67	18.36	32.42	12
12	18.40	32.42	12
99	18.46	31.25	12
170	18.53	31.58	12
28	18.55	32.17	12
48	18.55	34.08	12
103	18.60	29.82	11
66	18.76	33.67	12 xx
25	19.15	33.77	13 xx
16	19.39	32.42	12 xx
88	19.57	30.92	12 xx
182	19.91	35.83	12 xx
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767	17.08	30.89	64

The NILs from which we isolated x5a, x8, and x5b were sampled from Molly-4.

File S2

Supporting Appendix 2

Each of the gene based (MgSTS) markers used in the full genome scan is listed with its location on the 8x *Mimulus guttatus* genome build. The ten markers at the bottom of the table are located to scaffold in the 7x genome build because they have not been placed on the current assembly of scaffolds into chromosomes.

MgSTS_marker	Linkage_group	start	end
212	1	71113	72869
98	1	450278	453757
827	1	1082172	1086921
543	1	1206994	1208684
387	1	1641891	1644124
436	1	2048958	2051280
287	1	2234960	2236720
460	1	3886355	3887141
534	1	5522348	5490798
805	1	7817080	7813230
757	1	11072871	11065572
831	2	544019	542829
787	2	1221113	1217862
773	2	1293466	1286632
106	2	1517391	1512102
316	2	1770572	1767042
124	2	1957474	1955708
461	2	2233701	2232695
617	2	2505570	2506690
184	2	3093681	3094835
274	2	3093681	3094835
624	2	4444535	4445844
49	2	13202676	13204180
761	2	17630736	17634422
249	2	18963030	18964853
652	2	18971779	18974369
503	3	2996142	2992702
469	3	9060656	9054862
260	3	9153251	9149570
429	3	13347646	13344970
458	3	16872626	16869548
615	3	17065699	17064514
474	3	17526392	17525121
214	3	17748806	17747049
592	3	18400196	18398195
39	3	18450648	18448956
391	3	18496469	18493820
854	3	18527858	18526347
535	3	18697003	18696873
499	4	990959	989654
94	4	2205801	2204331

822	4	3722446	3720250
362	4	4695959	4693484
738	4	5948156	5945022
554	4	5978616	5976044
228	4	8824681	8823481
213	4	13441209	13439636
73	4	14324879	14318964
455	4	16895256	16897030
234	4	19243538	19242886
219	4	20370414	20368299
755	4	20582947	20584636
729	4	20637091	20641979
641	5	75069	76057
764	5	75069	76057
385	5	143369	145364
255	5	626850	628971
762	5	1040473	1041744
784	5	2509466	2515199
497	5	4788656	4792496
698	5	16437498	16438350
40	5	17194166	17198153
72	6	957637	953365
25	6	1395489	1393178
370	6	2151587	2147554
545	6	2498690	2496978
801	6	2780087	2775104
459	6	2945508	2942725
320	6	3105392	3104047
105	6	3522100	3518079
51	6	3776776	3772846
423	6	4650184	4646946
229	6	4882766	4878329
480	6	5599933	5598942
555	6	6284383	6286776
430	6	17939368	17937996
746	7	538281	540000
376	7	1864371	1861237
717	7	2514240	2513908
34	7	4122729	4127957
441	7	4122729	4127957
251	7	6326263	6331634
46	7	7157262	7164874
331	7	8480344	8484090
707	7	11624545	11625220
23	7	15206795	15208188
537	8	440670	438981
227	8	543327	537249
650	8	548512	544871
772	8	548512	544871
299	8	1844602	1843735

675	8	2044482	2042183
265	8	2485662	2483883
59	8	2636431	2631997
84	8	2943772	2941781
278	8	3605725	3601475
563	8	3695076	3693246
69	8	3717111	3715041
590	8	5022921	5025567
31	8	16604323	16608031
710	8	16708304	16710482
538	8	16802110	16796677
712	8	17292328	17284960
736	8	17441857	17436313
697	8	18025212	18022342
656	8	19203400	19201928
103	8	19244523	19239837
718	8	19378596	19375264
76	8	22971814	22974995
294	8	22974620	22974811
829	8	24766403	24767822
745	9	176712	174622
849	9	2504517	2496496
243	9	7038478	7037385
481	9	8149927	8147576
523	9	12172785	12168872
371	9	12463936	12463251
681	9	12798027	12795899
753	9	13430253	13427676
612	9	14246085	14242062
479	9	14585007	14581155
558	9	14773791	14770925
317	9	14816045	14813999
609	10	125351	121894
58	10	519291	516715
308	10	590467	589749
754	10	1167032	1165241
37	10	1169524	1167479
48	10	1253029	1249871
465	10	2068318	2065439
625	10	3213040	3209970
603	10	4426178	4428610
27	10	5531201	5533569
355	10	6356551	6354732
351	10	13426535	13430244
43	10	14836259	14839779
846	10	16507157	16504327
519	10	18372267	18374427
70	10	18514331	18515436
332	10	18598693	18602352
605	11	150304	153210

20	11	767637	770685
91	11	767637	770685
644	11	778805	782033
767	11	778805	782033
19	11	1953259	1955398
780	11	2382329	2384983
525	11	2590410	2587242
553	11	3039388	3037311
52	11	3133840	3131494
360	11	3423423	3418410
380	11	5433625	5428825
87	11	14010968	14015094
63	11	15539191	15534746
318	11	15800712	15797632
273	11	19380068	19383854
561	11	23069396	23070573
392	11	24027513	24030415
358	11	24179553	24181645
283	11	24202242	24206291
727	12	1074399	1072469
289	12	1431650	1430215
437	12	4165758	4171005
702	12	21127181	21125123
113	12	21250853	21249309
595	12	23057114	23056385
386	12	23543014	23542911
367	12	23544839	23538720
785	12	23646594	23645217
102	12	24021241	24014773
501	12	24239340	24237083
327	12	24458017	24454710
720	12	26733014	26735360
438	12	26827009	26829465
490	13	30716	35644
577	13	210581	215065
45	13	293205	297903
246	13	1729497	1721597
104	13	6120786	6119692
599	13	7651454	7648428
305	13	9020073	9015901
55	13	17691446	17690224
835	13	18571998	18569764
329	13	18582121	18578787
547	13	18802027	18800876
657	13	19547193	19546311
345	13	19818717	19812364
275	13	19840724	19838158
310	13	20445262	20442019
419	13	20858778	20857001
601	13	21049625	21047027

726	14	580360	583521
247	14	1253945	1256340
847	14	1275357	1277728
375	14	1400518	1406086
562	14	1415383	1420809
494	14	3401423	3405528
77	14	4461472	4465451
516	14	4710582	4713123
527	14	4813502	4815255
395	14	7323126	7327203
491	14	7408820	7411520
634	14	7846608	7844603
252	14	7975235	7971241
418	14	12134214	12137358
500	14	13105148	13107626
578	14	14801333	14803321
632	14	15568765	15564809
544	14	21269431	21272141
631	14	21649564	21652751
735	14	22584446	22585784
248	14	22785991	22787561
583	14	24207833	24214473
475	14	24314857	24318245
783	14	25163885	25164881
719	14	26093808	26088857
536	17	250794	252493

MgSTS_marker	Scaffold of 7x build		start	end
75	87		695063	695989
226	79		1122080	1123689
266	64		1181809	1182597
341	102		643398	644993
381	12		2802462	2804515
477	159		21927	23577
560	70		1021599	434469
571	46		1280418	1281481
611	7		3311584	3313523
622	4		830106	830941

File S3

Supporting Appendix 3

Table S1 With data from all cohorts, we report the mean, standard deviation (SD), standard error of the mean (SE), and sample size (n) for each trait.

Trait	Mean	SD	GSD	ESD	SE	n
Days to flower	28.00	4.56	1.36	3.19	0.06	6482
Corolla width	17.88	2.37	0.76	1.96	0.03	6220
Anther length	11.31	1.18	0.47	0.89	0.01	5718
Pistil length	13.96	1.27	0.51	1.03	0.02	5835
Corolla length	20.32	2.39	0.75	2.03	0.03	5694
Stigma-anther separation	2.65	0.96	0.30	0.88	0.01	5699
Pollen viability	0.701	0.199	0.048	0.181	0.003	5380
Pollen per flower	12012	6872	1310	6036	92	5380
Log(Pollen viability)	-0.180	0.171	0.033	0.160	0.002	5380
Log(Pollen per flower)	4.011	0.264	0.050	0.234	0.004	5380
Log(Viable pollen per flower)	3.831	0.372	0.076	0.336	0.005	5380

GSD and ESD are the genotypic and environmental SD, respectively, with cohort the remaining source of variation.

Table S2 Estimates are reported for the statistical model with an interaction term. Genotype data was coded numerically: 1 if the locus was homozygous for the donor allele and 0 if homozygous for the IM767 allele. The interaction is specified by the inter-locus cross product $(x - \bar{x})(y - \bar{y})$ for each pair of loci (x and y). Here, $\bar{x}$ and $\bar{y}$ are the mean values for genotypic scores (of x and y, respectively) in the entire experiment. The p-values are for t-tests of whether the coefficients differ from zero. Non-zero coefficients for the inter-locus cross products indicated epistasis and these p-values are reported in Table 1 of the main text.

Corolla width	coefficient	SE	t	p-value
Intercept	17.55	0.41	42.34	0.0000
x10a	0.23	0.10	2.25	0.0245
x9	0.40	0.13	2.99	0.0028
x1	-0.11	0.08	-1.29	0.1964
x5a	0.23	0.09	2.55	0.0109
x5b	-0.23	0.11	-2.04	0.0414
x10b	0.85	0.09	9.16	0.0000
x8	-0.55	0.07	-7.47	0.0000
Pale Corolla	-0.34	0.15	-2.30	0.0213
(x10a-0.22685)*(x9-0.15852)	-0.88	0.44	-2.02	0.0432
(x10a-0.22685)*(x1-0.14196)	-0.68	0.25	-2.68	0.0073
(x10a-0.22685)*(x5a-0.10386)	-0.61	0.23	-2.68	0.0073
(x10a-0.22685)*(x5b-0.14936)	1.39	0.33	4.21	0.0000
(x10a-0.22685)*(x10b-0.21222)	0.28	0.20	1.40	0.1601
(x10a-0.22685)*(x8-0.17894)	-0.21	0.19	-1.14	0.2541
(x9-0.15852)*(x1-0.14196)	0.27	0.23	1.15	0.2502
(x9-0.15852)*(x5a-0.10386)	-0.56	0.25	-2.27	0.0235
(x9-0.15852)*(x5b-0.14936)	0.02	0.22	0.10	0.9209
(x9-0.15852)*(x10b-0.21222)	-0.44	0.28	-1.54	0.1241
(x9-0.15852)*(x8-0.17894)	0.61	0.27	2.25	0.0242
(x1-0.14196)*(x5b-0.14936)	0.48	0.24	2.01	0.0445
(x1-0.14196)*(x10b-0.21222)	-0.27	0.21	-1.28	0.2011
(x5a-0.10386)*(x10b-0.21222)	-1.12	0.27	-4.15	0.0000
(x5b-0.14936)*(x10b-0.21222)	1.15	0.35	3.29	0.0010
(x5b-0.14936)*(x8-0.17894)	1.20	0.23	5.31	0.0000
(x10b-0.21222)*(x8-0.17894)	-0.66	0.20	-3.25	0.0011
days to flower				
Intercept	27.73	1.12	24.79	0.0000
x10a	-0.91	0.17	-5.37	0.0000
x9	0.07	0.22	0.34	0.7306
x1	0.09	0.13	0.70	0.4815
x5a	-1.52	0.15	-10.41	0.0000
x5b	0.58	0.18	3.24	0.0012
x10b	1.02	0.15	6.92	0.0000
x8	-0.28	0.12	-2.37	0.0180
Pale Corolla	-1.76	0.24	-7.46	0.0000
(x10a-0.224)*(x9-0.16214)	-2.04	0.71	-2.89	0.0039
(x10a-0.224)*(x1-0.14101)	-1.01	0.40	-2.49	0.0127
(x10a-0.224)*(x5a-0.10321)	-0.92	0.36	-2.57	0.0103
(x10a-0.224)*(x5b-0.15134)	-1.63	0.52	-3.10	0.0020

(x10a-0.224)*(x10b-0.21259)	0.76	0.32	2.38	0.0173
(x10a-0.224)*(x8-0.17957)	-0.04	0.30	-0.12	0.9006
(x9-0.16214)*(x1-0.14101)	0.17	0.37	0.47	0.6381
(x9-0.16214)*(x5a-0.10321)	0.34	0.39	0.88	0.3810
(x9-0.16214)*(x5b-0.15134)	-0.95	0.35	-2.69	0.0071
(x9-0.16214)*(x10b-0.21259)	-0.25	0.46	-0.56	0.5771
(x9-0.16214)*(x8-0.17957)	-1.37	0.43	-3.21	0.0014
(x1-0.14101)*(x5b-0.15134)	1.47	0.38	3.86	0.0001
(x1-0.14101)*(x10b-0.21259)	-1.47	0.34	-4.31	0.0000
(x5a-0.10321)*(x10b-0.21259)	0.76	0.43	1.78	0.0754
(x5b-0.15134)*(x10b-0.21259)	3.35	0.55	6.11	0.0000
(x5b-0.15134)*(x8-0.17957)	-1.22	0.36	-3.43	0.0006
(x10b-0.21259)*(x8-0.17957)	-0.10	0.32	-0.31	0.7557

Anther

Intercept	11.30	0.23	49.10	0.0000
x10a	-0.27	0.05	-5.59	0.0000
x9	0.04	0.06	0.70	0.4867
x1	-0.22	0.04	-5.51	0.0000
x5a	-0.25	0.04	-5.76	0.0000
x5b	0.40	0.05	7.63	0.0000
x10b	0.51	0.04	11.73	0.0000
x8	-0.57	0.03	-16.26	0.0000
Pale Corolla	-0.19	0.07	-2.57	0.0102
(x10a-0.22648)*(x9-0.1602)	-0.60	0.20	-2.99	0.0028
(x10a-0.22648)*(x1-0.13956)	0.24	0.12	2.02	0.0431
(x10a-0.22648)*(x5a-0.10196)	-0.20	0.11	-1.83	0.0673
(x10a-0.22648)*(x5b-0.14743)	0.06	0.16	0.38	0.7012
(x10a-0.22648)*(x10b-0.21074)	-0.02	0.09	-0.23	0.8197
(x10a-0.22648)*(x8-0.17664)	-0.27	0.09	-3.01	0.0026
(x9-0.1602)*(x1-0.13956)	0.12	0.11	1.08	0.2803
(x9-0.1602)*(x5a-0.10196)	0.15	0.12	1.31	0.1891
(x9-0.1602)*(x5b-0.14743)	0.21	0.11	1.98	0.0482
(x9-0.1602)*(x10b-0.21074)	0.14	0.14	1.02	0.3061
(x9-0.1602)*(x8-0.17664)	0.58	0.13	4.61	0.0000
(x1-0.13956)*(x5b-0.14743)	0.05	0.11	0.46	0.6457
(x1-0.13956)*(x10b-0.21074)	0.20	0.11	1.87	0.0620
(x5a-0.10196)*(x10b-0.21074)	-0.02	0.13	-0.16	0.8729
(x5b-0.14743)*(x10b-0.21074)	0.67	0.16	4.12	0.0000
(x5b-0.14743)*(x8-0.17664)	0.20	0.11	1.82	0.0691
(x10b-0.21074)*(x8-0.17664)	-0.90	0.09	-9.54	0.0000

pistil

Intercept	13.83	0.21	65.42	0.0000
x10a	-0.32	0.05	-5.87	0.0000
x9	0.44	0.07	6.16	0.0000
x1	0.00	0.04	-0.08	0.9332
x5a	-0.42	0.05	-8.45	0.0000

x5b	0.48	0.06	7.99	0.0000
x10b	0.30	0.05	6.18	0.0000
x8	-0.46	0.04	-11.56	0.0000
Pale Corolla	0.00	0.08	-0.01	0.9928
(x10a-0.22656)*(x9-0.15733)	-0.61	0.23	-2.64	0.0082
(x10a-0.22656)*(x1-0.13796)	0.09	0.13	0.70	0.4812
(x10a-0.22656)*(x5a-0.10266)	-0.35	0.12	-2.92	0.0035
(x10a-0.22656)*(x5b-0.14824)	0.68	0.18	3.84	0.0001
(x10a-0.22656)*(x10b-0.21148)	-0.45	0.11	-4.18	0.0000
(x10a-0.22656)*(x8-0.17755)	-0.42	0.10	-4.12	0.0000
(x9-0.15733)*(x1-0.13796)	0.40	0.12	3.18	0.0015
(x9-0.15733)*(x5a-0.10266)	-0.28	0.13	-2.09	0.0363
(x9-0.15733)*(x5b-0.14824)	0.00	0.12	0.00	1.0000
(x9-0.15733)*(x10b-0.21148)	-0.32	0.16	-2.05	0.0409
(x9-0.15733)*(x8-0.17755)	0.51	0.14	3.55	0.0004
(x1-0.13796)*(x5b-0.14824)	-0.02	0.13	-0.15	0.8837
(x1-0.13796)*(x10b-0.21148)	-0.18	0.12	-1.51	0.1323
(x5a-0.10266)*(x10b-0.21148)	-0.76	0.15	-5.22	0.0000
(x5b-0.14824)*(x10b-0.21148)	0.85	0.18	4.62	0.0000
(x5b-0.14824)*(x8-0.17755)	0.34	0.12	2.75	0.0059
(x10b-0.21148)*(x8-0.17755)	-1.03	0.11	-9.59	0.0000

Corolla length				
Intercept	20.34	0.39	52.39	0.0000
x10a	-0.58	0.11	-5.27	0.0000
x9	0.00	0.14	-0.02	0.9852
x1	-0.08	0.09	-0.93	0.3545
x5a	-0.38	0.10	-3.88	0.0001
x5b	0.39	0.12	3.23	0.0012
x10b	0.90	0.10	9.08	0.0000
x8	-0.90	0.08	-11.22	0.0000
Pale Corolla	-0.19	0.17	-1.16	0.2459
(x10a-0.22568)*(x9-0.16105)	-1.37	0.45	-3.02	0.0025
(x10a-0.22568)*(x1-0.1398)	-0.31	0.27	-1.16	0.2478
(x10a-0.22568)*(x5a-0.10151)	-0.62	0.24	-2.56	0.0105
(x10a-0.22568)*(x5b-0.14735)	0.95	0.36	2.67	0.0077
(x10a-0.22568)*(x10b-0.21022)	0.23	0.21	1.09	0.2761
(x10a-0.22568)*(x8-0.1758)	-0.29	0.21	-1.43	0.1526
(x9-0.16105)*(x1-0.1398)	0.22	0.25	0.89	0.3727
(x9-0.16105)*(x5a-0.10151)	0.01	0.27	0.04	0.9676
(x9-0.16105)*(x5b-0.14735)	0.36	0.24	1.46	0.1444
(x9-0.16105)*(x10b-0.21022)	0.02	0.31	0.06	0.9521
(x9-0.16105)*(x8-0.1758)	0.76	0.28	2.69	0.0072
(x1-0.1398)*(x5b-0.14735)	-0.43	0.26	-1.65	0.0990
(x1-0.1398)*(x10b-0.21022)	0.25	0.24	1.05	0.2956
(x5a-0.10151)*(x10b-0.21022)	-1.00	0.29	-3.43	0.0006
(x5b-0.14735)*(x10b-0.21022)	0.98	0.37	2.65	0.0081
(x5b-0.14735)*(x8-0.1758)	0.91	0.25	3.69	0.0002
(x10b-0.21022)*(x8-0.1758)	-0.96	0.22	-4.44	0.0000

SA_sep				
Intercept	2.53	0.09	26.75	0.0000
x10a	-0.04	0.05	-0.89	0.3722
x9	0.41	0.06	6.67	0.0000
x1	0.21	0.04	5.50	0.0000
x5a	-0.17	0.04	-4.02	0.0001
x5b	0.10	0.05	1.84	0.0665
x10b	-0.19	0.04	-4.48	0.0000
x8	0.12	0.03	3.47	0.0005
Pale Corolla	0.16	0.07	2.26	0.0239
(x10a-0.22636)*(x9-0.16003)	-0.01	0.20	-0.03	0.9756
(x10a-0.22636)*(x1-0.1395)	-0.14	0.12	-1.20	0.2300
(x10a-0.22636)*(x5a-0.10177)	-0.12	0.11	-1.18	0.2396
(x10a-0.22636)*(x5b-0.14687)	0.62	0.16	3.97	0.0001
(x10a-0.22636)*(x10b-0.21127)	-0.42	0.09	-4.52	0.0000
(x10a-0.22636)*(x8-0.17652)	-0.15	0.09	-1.64	0.1021
(x9-0.16003)*(x1-0.1395)	0.29	0.11	2.68	0.0075
(x9-0.16003)*(x5a-0.10177)	-0.41	0.12	-3.53	0.0004
(x9-0.16003)*(x5b-0.14687)	-0.21	0.11	-2.02	0.0431
(x9-0.16003)*(x10b-0.21127)	-0.45	0.13	-3.37	0.0008
(x9-0.16003)*(x8-0.17652)	-0.04	0.12	-0.33	0.7420
(x1-0.1395)*(x5b-0.14687)	-0.09	0.11	-0.83	0.4057
(x1-0.1395)*(x10b-0.21127)	-0.35	0.10	-3.35	0.0008
(x5a-0.10177)*(x10b-0.21127)	-0.71	0.13	-5.64	0.0000
(x5b-0.14687)*(x10b-0.21127)	0.19	0.16	1.20	0.2306
(x5b-0.14687)*(x8-0.17652)	0.20	0.11	1.83	0.0666
(x10b-0.21127)*(x8-0.17652)	-0.05	0.09	-0.50	0.6161

Log(PV)				
Intercept	-0.17	0.02	-8.41	0.0001
x10a	0.03	0.01	2.98	0.0029
x9	0.01	0.01	0.43	0.6674
x1	-0.03	0.01	-4.53	0.0000
x5a	-0.01	0.01	-1.34	0.1797
x5b	0.00	0.01	0.22	0.8228
x10b	0.00	0.01	0.42	0.6709
x8	-0.03	0.01	-4.41	0.0000
Pale Corolla	0.01	0.01	0.81	0.4193
(x10a-0.23086)*(x9-0.1671)	0.00	0.04	0.10	0.9230
(x10a-0.23086)*(x1-0.1448)	0.08	0.02	3.63	0.0003
(x10a-0.23086)*(x5a-0.10725)	-0.04	0.02	-2.18	0.0293
(x10a-0.23086)*(x5b-0.15)	0.04	0.03	1.44	0.1500
(x10a-0.23086)*(x10b-0.20074)	-0.05	0.02	-2.75	0.0060
(x10a-0.23086)*(x8-0.17937)	0.02	0.02	1.34	0.1787
(x9-0.1671)*(x1-0.1448)	0.02	0.02	0.90	0.3661
(x9-0.1671)*(x5a-0.10725)	-0.02	0.02	-1.03	0.3045
(x9-0.1671)*(x5b-0.15)	0.00	0.02	-0.01	0.9941

$(x_9 - 0.1671) * (x_{10b} - 0.20074)$	0.02	0.02	0.84	0.3989
$(x_9 - 0.1671) * (x_8 - 0.17937)$	0.04	0.02	1.83	0.0666
$(x_1 - 0.1448) * (x_{5b} - 0.15)$	0.00	0.02	-0.13	0.8998
$(x_1 - 0.1448) * (x_{10b} - 0.20074)$	0.05	0.02	2.79	0.0053
$(x_{5a} - 0.10725) * (x_{10b} - 0.20074)$	-0.01	0.02	-0.22	0.8236
$(x_{5b} - 0.15) * (x_{10b} - 0.20074)$	0.08	0.03	2.43	0.0150
$(x_{5b} - 0.15) * (x_8 - 0.17937)$	0.02	0.02	0.90	0.3656
$(x_{10b} - 0.20074) * (x_8 - 0.17937)$	-0.05	0.02	-2.73	0.0064

Log(Total Pollen)

Intercept	4.03	0.04	95.46	0.0000
x10a	0.04	0.01	3.19	0.0014
x9	0.01	0.02	0.36	0.7152
x1	-0.01	0.01	-1.34	0.1788
x5a	0.05	0.01	4.12	0.0000
x5b	0.02	0.01	1.67	0.0946
x10b	0.00	0.01	-0.26	0.7922
x8	-0.03	0.01	-3.55	0.0004
Pale Corolla	0.08	0.02	4.19	0.0000
$(x_{10a} - 0.23086) * (x_9 - 0.1671)$	0.03	0.06	0.57	0.5664
$(x_{10a} - 0.23086) * (x_1 - 0.1448)$	0.18	0.03	5.64	0.0000
$(x_{10a} - 0.23086) * (x_{5a} - 0.10725)$	-0.06	0.03	-2.16	0.0310
$(x_{10a} - 0.23086) * (x_{5b} - 0.15)$	0.12	0.04	2.91	0.0036
$(x_{10a} - 0.23086) * (x_{10b} - 0.20074)$	-0.03	0.03	-1.25	0.2099
$(x_{10a} - 0.23086) * (x_8 - 0.17937)$	-0.02	0.02	-0.74	0.4592
$(x_9 - 0.1671) * (x_1 - 0.1448)$	0.13	0.03	4.40	0.0000
$(x_9 - 0.1671) * (x_{5a} - 0.10725)$	-0.06	0.03	-1.88	0.0596
$(x_9 - 0.1671) * (x_{5b} - 0.15)$	0.02	0.03	0.60	0.5506
$(x_9 - 0.1671) * (x_{10b} - 0.20074)$	-0.02	0.04	-0.63	0.5283
$(x_9 - 0.1671) * (x_8 - 0.17937)$	-0.01	0.03	-0.26	0.7954
$(x_1 - 0.1448) * (x_{5b} - 0.15)$	0.01	0.03	0.50	0.6199
$(x_1 - 0.1448) * (x_{10b} - 0.20074)$	0.08	0.03	3.05	0.0023
$(x_{5a} - 0.10725) * (x_{10b} - 0.20074)$	-0.08	0.03	-2.32	0.0206
$(x_{5b} - 0.15) * (x_{10b} - 0.20074)$	0.12	0.05	2.37	0.0176
$(x_{5b} - 0.15) * (x_8 - 0.17937)$	0.08	0.03	2.80	0.0051
$(x_{10b} - 0.20074) * (x_8 - 0.17937)$	-0.03	0.03	-1.00	0.3150

Log(Viable grains)

Intercept	3.86	0.05	72.01	0.0000
x10a	0.07	0.02	3.69	0.0002
x9	0.01	0.02	0.46	0.6430
x1	-0.05	0.02	-3.08	0.0021
x5a	0.04	0.02	2.24	0.0249
x5b	0.02	0.02	1.17	0.2423
x10b	0.00	0.02	0.05	0.9640
x8	-0.06	0.01	-4.73	0.0000
Pale Corolla	0.10	0.03	3.46	0.0006
$(x_{10a} - 0.23086) * (x_9 - 0.1671)$	0.04	0.08	0.44	0.6595

$(x_{10a}-0.23086)*(x_1-0.1448)$	0.27	0.05	5.67	0.0000
$(x_{10a}-0.23086)*(x_{5a}-0.10725)$	-0.10	0.04	-2.55	0.0109
$(x_{10a}-0.23086)*(x_{5b}-0.15)$	0.16	0.06	2.75	0.0059
$(x_{10a}-0.23086)*(x_{10b}-0.20074)$	-0.08	0.04	-2.18	0.0290
$(x_{10a}-0.23086)*(x_8-0.17937)$	0.00	0.03	0.14	0.8902
$(x_9-0.1671)*(x_1-0.1448)$	0.15	0.04	3.50	0.0005
$(x_9-0.1671)*(x_{5a}-0.10725)$	-0.08	0.04	-1.81	0.0702
$(x_9-0.1671)*(x_{5b}-0.15)$	0.02	0.04	0.45	0.6547
$(x_9-0.1671)*(x_{10b}-0.20074)$	0.00	0.05	-0.04	0.9719
$(x_9-0.1671)*(x_8-0.17937)$	0.03	0.05	0.71	0.4800
$(x_1-0.1448)*(x_{5b}-0.15)$	0.01	0.04	0.32	0.7505
$(x_1-0.1448)*(x_{10b}-0.20074)$	0.13	0.04	3.46	0.0006
$(x_{5a}-0.10725)*(x_{10b}-0.20074)$	-0.09	0.05	-1.73	0.0830
$(x_{5b}-0.15)*(x_{10b}-0.20074)$	0.21	0.07	2.86	0.0043
$(x_{5b}-0.15)*(x_8-0.17937)$	0.09	0.04	2.08	0.0379
$(x_{10b}-0.20074)*(x_8-0.17937)$	-0.08	0.04	-1.98	0.0477

Table S3 Estimates are reported for deviations of each genotype from the recipient line (IM767 = intercept of model). The t and p-values refer to a test of whether a term is non-zero. For each double donor homozygote, we also report the predicted value in the absence of epistasis and the residual (observed—predicted).

Corolla width	coefficient	SE	t	p-value	Predicted effect (No Epistasis)	Residual (epistatic effect)
Intercept	17.56	0.42	42.30	0.0000		
x10a	0.31	0.10	3.06	0.0022		
x9	0.60	0.13	4.69	0.0000		
x1	-0.01	0.12	-0.07	0.9406		
x5a	0.70	0.16	4.46	0.0000		
x5b	-1.08	0.12	-8.64	0.0000		
x10b	0.95	0.10	9.41	0.0000		
x8	-0.64	0.12	-5.49	0.0000		
Pale Corolla	-0.34	0.15	-2.30	0.0213		
x10a x9	0.02	0.42	0.06	0.9553	0.91	-0.88
x10a x1	-0.38	0.21	-1.79	0.0732	0.30	-0.68
x10a x5a	0.40	0.16	2.55	0.0108	1.00	-0.61
x10a x5b	0.62	0.30	2.06	0.0398	-0.77	1.39
x10a x10b	1.54	0.17	9.30	0.0000	1.26	0.28
x10a x8	-0.55	0.13	-4.11	0.0000	-0.34	-0.21
x9 x1	0.86	0.18	4.82	0.0000	0.59	0.27
x9 x5a	0.74	0.17	4.36	0.0000	1.30	-0.56
x9 x5b	-0.45	0.17	-2.74	0.0061	-0.47	0.02
x9 x10b	1.12	0.25	4.45	0.0000	1.55	-0.44
x9 x8	0.57	0.23	2.47	0.0135	-0.04	0.61
x1 x5b	-0.61	0.18	-3.32	0.0009	-1.09	0.48
x1 x10b	0.67	0.16	4.11	0.0000	0.94	-0.27
x5a x10b	0.53	0.21	2.53	0.0115	1.65	-1.12
x5b x10b	1.03	0.32	3.19	0.0014	-0.13	1.15
x5b x8	-0.52	0.19	-2.76	0.0058	-1.72	1.20
x10b x8	-0.35	0.15	-2.26	0.0241	0.31	-0.66
Days to flower						
Intercept	27.60	1.12	24.66	0.0000		
x10a	-0.25	0.16	-1.55	0.1203		
x9	0.92	0.20	4.56	0.0000		
x1	0.38	0.20	1.93	0.0535		
x5a	-1.53	0.25	-6.20	0.0000		
x5b	0.39	0.20	1.98	0.0474		
x10b	0.53	0.16	3.32	0.0009		
x8	0.16	0.19	0.85	0.3963		
Pale Corolla	-1.76	0.24	-7.46	0.0000		
x10a x9	-1.37	0.68	-2.02	0.0439	0.67	-2.04
x10a x1	-0.87	0.34	-2.57	0.0103	0.13	-1.01
x10a x5a	-2.70	0.25	-10.85	0.0000	-1.78	-0.92
x10a x5b	-1.48	0.48	-3.09	0.0020	0.14	-1.63
x10a x10b	1.04	0.26	3.95	0.0001	0.28	0.76

x10a x8	-0.13	0.22	-0.59	0.5530	-0.09	-0.04
x9 x1	1.47	0.28	5.23	0.0000	1.30	0.17
x9 x5a	-0.27	0.27	-1.00	0.3163	-0.61	0.34
x9 x5b	0.36	0.26	1.37	0.1710	1.31	-0.95
x9 x10b	1.19	0.41	2.93	0.0034	1.45	-0.25
x9 x8	-0.30	0.37	-0.82	0.4122	1.07	-1.37
x1 x5b	2.24	0.29	7.69	0.0000	0.77	1.47
x1 x10b	-0.56	0.26	-2.12	0.0338	0.91	-1.47
x5a x10b	-0.23	0.34	-0.68	0.4955	-1.00	0.76
x5b x10b	4.28	0.50	8.49	0.0000	0.93	3.35
x5b x8	-0.67	0.29	-2.27	0.0233	0.55	-1.22
x10b x8	0.59	0.25	2.41	0.0160	0.69	-0.10

Anther length

Intercept	11.30	0.23	49.06	0.0000		
x10a	-0.15	0.05	-3.09	0.0020		
x9	-0.02	0.06	-0.27	0.7857		
x1	-0.34	0.06	-5.73	0.0000		
x5a	-0.23	0.07	-3.06	0.0022		
x5b	0.17	0.06	2.94	0.0033		
x10b	0.53	0.05	11.06	0.0000		
x8	-0.44	0.06	-7.96	0.0000		
Pale Corolla	-0.19	0.07	-2.57	0.0102		
x10a x9	-0.76	0.19	-3.96	0.0001	-0.16	-0.60
x10a x1	-0.25	0.10	-2.50	0.0124	-0.48	0.24
x10a x5a	-0.57	0.07	-7.70	0.0000	-0.37	-0.20
x10a x5b	0.09	0.14	0.62	0.5348	0.03	0.06
x10a x10b	0.36	0.08	4.65	0.0000	0.38	-0.02
x10a x8	-0.86	0.07	-12.98	0.0000	-0.58	-0.27
x9 x1	-0.24	0.08	-2.87	0.0041	-0.35	0.12
x9 x5a	-0.09	0.08	-1.06	0.2909	-0.24	0.15
x9 x5b	0.37	0.08	4.62	0.0000	0.16	0.21
x9 x10b	0.65	0.12	5.35	0.0000	0.51	0.14
x9 x8	0.12	0.11	1.15	0.2481	-0.45	0.58
x1 x5b	-0.11	0.09	-1.28	0.1991	-0.16	0.05
x1 x10b	0.39	0.08	4.69	0.0000	0.19	0.20
x5a x10b	0.28	0.10	2.83	0.0047	0.30	-0.02
x5b x10b	1.37	0.15	9.23	0.0000	0.70	0.67
x5b x8	-0.07	0.09	-0.74	0.4600	-0.26	0.20
x10b x8	-0.81	0.07	-11.22	0.0000	0.09	-0.90

Pistil length

Intercept	13.77	0.21	65.05	0.0000
x10a	-0.13	0.05	-2.51	0.0120
x9	0.53	0.07	7.71	0.0000
x1	-0.05	0.07	-0.69	0.4876
x5a	-0.13	0.08	-1.59	0.1120
x5b	0.09	0.07	1.31	0.1887

x10b	0.61	0.05	11.33	0.0000		
x8	-0.28	0.06	-4.39	0.0000		
Pale Corolla	0.00	0.08	-0.01	0.9928		
x10a x9	-0.21	0.22	-0.96	0.3366	0.39	-0.61
x10a x1	-0.09	0.11	-0.77	0.4430	-0.18	0.09
x10a x5a	-0.62	0.08	-7.50	0.0000	-0.27	-0.35
x10a x5b	0.63	0.16	3.92	0.0001	-0.05	0.68
x10a x10b	0.03	0.09	0.40	0.6901	0.48	-0.45
x10a x8	-0.83	0.08	-11.06	0.0000	-0.41	-0.42
x9 x1	0.88	0.09	9.26	0.0000	0.48	0.40
x9 x5a	0.11	0.10	1.20	0.2289	0.40	-0.28
x9 x5b	0.62	0.09	6.74	0.0000	0.62	0.00
x9 x10b	0.82	0.14	5.90	0.0000	1.14	-0.32
x9 x8	0.76	0.12	6.28	0.0000	0.25	0.51
x1 x5b	0.02	0.10	0.23	0.8203	0.04	-0.02
x1 x10b	0.39	0.09	4.12	0.0000	0.57	-0.18
x5a x10b	-0.28	0.11	-2.42	0.0156	0.48	-0.76
x5b x10b	1.55	0.17	9.22	0.0000	0.70	0.85
x5b x8	0.15	0.10	1.48	0.1403	-0.19	0.34
x10b x8	-0.69	0.08	-8.43	0.0000	0.34	-1.03
Corolla length						
Intercept	20.33	0.39	52.27	0.0000		
x10a	-0.39	0.11	-3.67	0.0002		
x9	0.08	0.14	0.62	0.5364		
x1	-0.04	0.13	-0.28	0.7782		
x5a	-0.03	0.17	-0.20	0.8388		
x5b	-0.19	0.14	-1.41	0.1590		
x10b	0.93	0.11	8.60	0.0000		
x8	-0.88	0.13	-7.06	0.0000		
Pale Corolla	-0.19	0.17	-1.16	0.2459		
x10a x9	-1.68	0.43	-3.87	0.0001	-0.31	-1.37
x10a x1	-0.74	0.23	-3.29	0.0010	-0.43	-0.31
x10a x5a	-1.05	0.17	-6.25	0.0000	-0.43	-0.62
x10a x5b	0.37	0.33	1.14	0.2562	-0.58	0.95
x10a x10b	0.77	0.18	4.38	0.0000	0.54	0.23
x10a x8	-1.57	0.15	-10.45	0.0000	-1.28	-0.29
x9 x1	0.27	0.19	1.42	0.1553	0.05	0.22
x9 x5a	0.06	0.19	0.32	0.7464	0.05	0.01
x9 x5b	0.25	0.18	1.37	0.1703	-0.11	0.36
x9 x10b	1.04	0.28	3.75	0.0002	1.02	0.02
x9 x8	-0.04	0.24	-0.15	0.8804	-0.80	0.76
x1 x5b	-0.65	0.20	-3.32	0.0009	-0.23	-0.43
x1 x10b	1.15	0.19	6.11	0.0000	0.90	0.25
x5a x10b	-0.10	0.23	-0.45	0.6534	0.90	-1.00
x5b x10b	1.72	0.34	5.10	0.0000	0.74	0.98
x5b x8	-0.16	0.21	-0.77	0.4391	-1.07	0.91
x10b x8	-0.91	0.17	-5.48	0.0000	0.05	-0.96

Stigma-anther
separation

Intercept	2.47	0.09	26.06	0.0000		
x10a	0.01	0.05	0.31	0.7596		
x9	0.55	0.06	9.25	0.0000		
x1	0.29	0.06	4.91	0.0000		
x5a	0.07	0.07	0.97	0.3315		
x5b	-0.07	0.06	-1.22	0.2236		
x10b	0.08	0.05	1.63	0.1042		
x8	0.14	0.05	2.58	0.0099		
Pale Corolla	0.16	0.07	2.26	0.0239		
x10a x9	0.56	0.19	2.95	0.0032	0.56	-0.01
x10a x1	0.16	0.10	1.66	0.0966	0.30	-0.14
x10a x5a	-0.04	0.07	-0.54	0.5880	0.09	-0.12
x10a x5b	0.56	0.14	3.95	0.0001	-0.06	0.62
x10a x10b	-0.33	0.08	-4.29	0.0000	0.09	-0.42
x10a x8	0.01	0.07	0.13	0.8945	0.15	-0.15
x9 x1	1.12	0.08	13.75	0.0000	0.83	0.29
x9 x5a	0.21	0.08	2.52	0.0118	0.62	-0.41
x9 x5b	0.26	0.08	3.30	0.0010	0.48	-0.21
x9 x10b	0.17	0.12	1.43	0.1518	0.62	-0.45
x9 x8	0.65	0.10	6.20	0.0000	0.69	-0.04
x1 x5b	0.12	0.09	1.40	0.1601	0.21	-0.09
x1 x10b	0.01	0.08	0.17	0.8622	0.36	-0.35
x5a x10b	-0.56	0.10	-5.71	0.0000	0.15	-0.71
x5b x10b	0.20	0.15	1.35	0.1782	0.01	0.19
x5b x8	0.27	0.09	2.98	0.0029	0.07	0.20
x10b x8	0.17	0.07	2.37	0.0177	0.22	-0.05

Log(Pollen Viability)

Intercept	-0.163	0.020	-8.07	0.0001		
x10a	0.020	0.009	2.34	0.0194		
x9	-0.008	0.011	-0.71	0.4777		
x1	-0.065	0.011	-5.89	0.0000		
x5a	0.004	0.013	0.29	0.7690		
x5b	-0.027	0.011	-2.43	0.0152		
x10b	0.001	0.009	0.16	0.8729		
x8	-0.034	0.010	-3.25	0.0012		
Pale Corolla	0.011	0.013	0.81	0.4193		
x10a x9	0.016	0.038	0.44	0.6626	0.01	0.00
x10a x1	0.037	0.019	1.97	0.0491	-0.04	0.08
x10a x5a	-0.018	0.014	-1.34	0.1798	0.02	-0.04
x10a x5b	0.034	0.026	1.34	0.1791	-0.01	0.04
x10a x10b	-0.028	0.015	-1.85	0.0640	0.02	-0.05
x10a x8	0.009	0.011	0.76	0.4462	-0.01	0.02
x9 x1	-0.054	0.015	-3.58	0.0004	-0.07	0.02
x9 x5a	-0.026	0.015	-1.76	0.0787	0.00	-0.02
x9 x5b	-0.035	0.014	-2.45	0.0142	-0.03	0.00
x9 x10b	0.014	0.021	0.66	0.5106	-0.01	0.02

x9 x8	0.001	0.020	0.07	0.9462	-0.04	0.04
x1 x5b	-0.094	0.015	-6.07	0.0000	-0.09	0.00
x1 x10b	-0.011	0.014	-0.80	0.4220	-0.06	0.05
x5a x10b	0.000	0.019	0.01	0.9948	0.01	-0.01
x5b x10b	0.058	0.032	1.82	0.0694	-0.03	0.08
x5b x8	-0.042	0.017	-2.51	0.0119	-0.06	0.02
x10b x8	-0.083	0.014	-5.73	0.0000	-0.03	-0.05

Log(Pollen per flower)

Intercept	4.046	0.042	95.67	0.0000		
x10a	0.010	0.013	0.81	0.4183		
x9	-0.010	0.016	-0.63	0.5283		
x1	-0.097	0.016	-6.07	0.0000		
x5a	0.088	0.020	4.48	0.0000		
x5b	-0.047	0.016	-2.90	0.0038		
x10b	-0.008	0.013	-0.63	0.5311		
x8	-0.035	0.015	-2.34	0.0195		
Pale Corolla	0.082	0.019	4.19	0.0000		
x10a x9	0.033	0.055	0.60	0.5493	0.00	0.03
x10a x1	0.098	0.028	3.53	0.0004	-0.09	0.18
x10a x5a	0.036	0.020	1.82	0.0686	0.10	-0.06
x10a x5b	0.084	0.037	2.24	0.0251	-0.04	0.12
x10a x10b	-0.032	0.022	-1.40	0.1609	0.00	-0.03
x10a x8	-0.042	0.016	-2.59	0.0097	-0.03	-0.02
x9 x1	0.021	0.022	0.96	0.3352	-0.11	0.13
x9 x5a	0.019	0.021	0.90	0.3663	0.08	-0.06
x9 x5b	-0.040	0.021	-1.93	0.0537	-0.06	0.02
x9 x10b	-0.041	0.031	-1.31	0.1887	-0.02	-0.02
x9 x8	-0.054	0.029	-1.88	0.0596	-0.05	-0.01
x1 x5b	-0.129	0.023	-5.70	0.0000	-0.14	0.01
x1 x10b	-0.023	0.020	-1.11	0.2664	-0.11	0.08
x5a x10b	-0.001	0.027	-0.02	0.9849	0.08	-0.08
x5b x10b	0.064	0.047	1.37	0.1710	-0.05	0.12
x5b x8	0.001	0.024	0.04	0.9670	-0.08	0.08
x10b x8	-0.071	0.021	-3.36	0.0008	-0.04	-0.03

Log(Viable pollen per flower)

Intercept	3.883	0.054	72.27	0.0000		
x10a	0.031	0.018	1.70	0.0894		
x9	-0.018	0.023	-0.78	0.4334		
x1	-0.162	0.023	-7.04	0.0000		
x5a	0.092	0.028	3.28	0.0011		
x5b	-0.075	0.023	-3.24	0.0012		
x10b	-0.007	0.019	-0.36	0.7160		
x8	-0.070	0.022	-3.20	0.0014		
Pale Corolla	0.097	0.028	3.46	0.0006		
x10a x9	0.049	0.079	0.62	0.5333	0.01	0.04
x10a x1	0.136	0.040	3.42	0.0006	-0.13	0.27

x10a x5a	0.019	0.029	0.65	0.5146	0.12	-0.10
x10a x5b	0.119	0.054	2.22	0.0261	-0.04	0.16
x10a x10b	-0.060	0.032	-1.85	0.0644	0.02	-0.08
x10a x8	-0.034	0.024	-1.43	0.1532	-0.04	0.00
x9 x1	-0.033	0.032	-1.04	0.2999	-0.18	0.15
x9 x5a	-0.006	0.031	-0.21	0.8322	0.07	-0.08
x9 x5b	-0.075	0.030	-2.52	0.0119	-0.09	0.02
x9 x10b	-0.027	0.044	-0.60	0.5454	-0.03	0.00
x9 x8	-0.053	0.041	-1.28	0.1996	-0.09	0.03
x1 x5b	-0.223	0.032	-6.87	0.0000	-0.24	0.01
x1 x10b	-0.034	0.029	-1.16	0.2444	-0.17	0.13
x5a x10b	-0.001	0.039	-0.02	0.9855	0.09	-0.09
x5b x10b	0.124	0.067	1.84	0.0654	-0.08	0.21
x5b x8	-0.056	0.035	-1.60	0.1097	-0.14	0.09
x10b x8	-0.153	0.030	-5.07	0.0000	-0.08	-0.08

File S4

Supporting Data

Supporting data are available for download at <http://www.genetics.org/content/suppl/2011/09/16/genetics.111.132423.DC1> as a compressed folder (.zip).