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Supplementary Materials for

Selective trade-offs maintain alleles underpinning complex trait variation in plants

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Other supplementary material for this manuscript includes the following:

Tables S1 to S5 (Excel format)

Materials and Methods

The study system

M. guttatus can occur as either an annual or perennial, and numerous selfing forms with diminutive flowers exist within the complex (*35, 36*). The most extensively studied population of *M. guttatus* is near Iron Mountain in the Cascade Mountains of Oregon (44.402217N, 122.153317W) at roughly 1400 m elevation. At this site, *M. guttatus* is annual / winter-annual and its lifespan is typically a brief race against the summer drought. During a window of 6 to 10 weeks (spring snowmelt to summer drought), *M. guttatus* at IM must germinate, flower, and set seed. Plants that flower later have the potential to ensure greater seed set due to larger flowers, but potentially at the cost of death by drought prior to seed set (*18*). Individuals from this population have been shown to be largely outcrossing, though self-compatible (*37*), and the population as a whole demonstrates little internal spatial structure at neutral markers (*38*). The distribution of flowering time is continuous and usually unimodal (*27, 39*). At least two chromosomal inversions segregate within IM and nearby populations (*32, 40*). Within IM, nucleotide diversity is remarkably high (synonymous $\pi = 3.3\%$) and linkage disequilibrium is high at short distances (~ 100 base pairs), but decays quickly. Patterns of long-range linkage disequilibrium vary between chromosomes (*34*).

Line generation and greenhouse phenotyping

We initiated inbred lines from two separate collections of wild plants from IM for sequencing (*17, 41-43*). Lineages were propagated by single seed descent for 5-19 generations to create inbred lines. Lines from the first collection have the prefix "IM," lines from the second have the prefix "Z." We further synthesized F1 lines by randomly pairing distinct inbred lines. Each F1 line was made from the same two inbred parental lines crossed in the same direction. Due to crossing error, two distinct F1 lines share a parental inbred line.

To determine inbred and F1 line averages for phenotypic traits, we grew seed from each line in the Duke University Biology Greenhouse. Five cohorts, comprised of at least ten individuals each from 401 lines (338 inbred and 63 F1) were grown starting from late January through March of 2015. Lines were grown in cohorts of roughly 1000 plants at a time, with overlap between lines planted across cohorts, and a core set of lines planted in a majority of cohorts. An additional cohort was grown in mid-February of 2017 to finalize phenotyping of mainly F1 lines, as well as a handful of previously unsuccessfully grown inbred lines. Plants from all cohorts were grown according to the same schedule. Seed from each line was planted in 2.5 inch square pots on Fafard 4P potting soil, and placed into a cold room for seven days. Following cold treatment, pots were transferred to a flooded bench in the greenhouse. On approximately the third day out of the cold room, germination would begin. As seedlings emerged they were transferred to their own labeled 2.5 inch pot, and randomized into flats. Up to ten germinants were transplanted for each line. Germination date was recorded as the transplant date for each individual. Transplanting of germinants continued for ten days, by which time viable seeds would likely have germinated. Plants that died within the first ten days due to transplant shock

were replaced. Benches remained flooded for the first ten days to increase survival rates of newly transplanted germinants. On the eleventh day, benches were drained, and normal fertilization and water schedules resumed. Flats were reorganized once a week to minimize the effect of location on the bench.

We measured each plant for corolla width, corolla length, floral tube length, floral throat width, pistil length, stamen length (length of the longest filament and anther), node of flower, height at flowering node, and width of the widest leaf on the date of first flower (44). The date of first flower was recorded, and number of days since germination calculated to determine days to flower. Lines with fewer than three individuals across all cohorts were not used for further analysis. In total, 307 inbred lines were successfully phenotyped, with an average of 13 plants per line ($3 \leq n \leq 68$, mean = 12.65), as well as 94 F1 lines with an average of 11 plants per line ($5 \leq n \leq 20$, mean = 11).

DNA extraction, library preparation, and sequencing

We generated sequences of the 187 inbred IM lines in stages. We combined sequence data for 152 previously unpublished lines with data from 26 lines described by (*34*) and nine lines published by (*45*). For 81 of the 152 newly sequenced lines, DNA extraction, library preparation, and sequencing was performed according to the methods outlined in (*34*). For the remaining 71 lines, leaf or flower bud tissue was collected and frozen, and DNA was extracted using the ThermoFisher Scientific GeneJET Plant Genomic DNA Purification Kit. Libraries for Illumina sequencing were prepared using the Illumina Kapa Hyper Prep Kit. Individual barcodes were added during library preparation, and libraries were pooled up to 24 per lane for sequencing. Pooled libraries were run on the Illumina HiSeq 4000, generating 150 bp paired-end reads, which was comparable to previously sequenced lines. To augment data for lines with low read depth, we made new libraries for 24 of the lines. Each was sequenced (150bp, single end) at the University of Kansas Genomics core using the Illumina HiSeq 2500.

Alignment and genotype calling

We processed sequenced data from each line with Scythe (https://github.com/vsbuffalo/scythe) to remove adaptors and with Sickle (*46*) to trim low quality sites. We mapped reads to the *M. guttatus* v2 genome build using BWA (v 0.7.15) on the 14 main chromosomal scaffolds as well as scaffold 17 which is the end of chromosome 9 (*47*). We called SNPs and small Indels using the GATK UnifiedGenotyper (*48*). Once a genome-wide set of variants had been determined, we further filtered such that all sites were required to have a minimum mapping quality of 30, and be called in at least 100 lines. We also culled any polymorphism where the minor allele was not homozygous in at least two lines. We performed a genome-wide pairwise contrast between all lines and eliminated those that were excessively divergent or excessively heterozygous as probable contaminants. This left 187 largely homozygous lines that were clearly IM derived. We excluded some lines that were exceptionally divergent as possible contaminants (*M. guttatus* genotypes but not from IM population). Our final variant set consists of 10,199,239 polymorphisms, with median read depth of 6.5 per site per line. F1 lines genotypes were inferred from sequences of their inbred parents.

Line means and phenotypic principal components

We calculated inbred and F1 line least square means (LSmeans) from greenhouse phenotypic data for subsequent analyses using mixed linear models with cohort, flat, and line as random effects. All models were fit using the "fit model" function in JMP v. *13* (SAS Institute Inc., Cary, NC). Principal components analyses were also performed on the four major flower size traits (corolla width, corolla length, floral tube length, and floral throat width) for both inbred and F1 line individuals. The first and second principal components of flower size were included as additional traits with PC1 adopted as an overall measures of flower size. The Pearson correlation coefficient and correlation probability was determined for each set of inbred or F1 traits using JMP v. *13*.

GWAS and permutation analysis

We performed genome-wide association analyses using a mixed linear model (MLM) incorporating a kinship matrix (K) to account for line relatedness (a second, complementary analysis is described below). Kinship matrices were calculated using all sites from all 187 sequenced inbred lines and 66 sequenced F1 lines independently. We applied model fits only to the 165 sequenced lines and 55 F1s with phenotype data. The vector of observed phenotypes (y) was modeled as $y = X\beta + Zu + e$, where β is the vector of fixed effects (including all tested SNPs), u is the vector of random additive genetic effects, and e is an unobserved vector of residuals. X and Z are the known design matrices, relating y to β, and y to u, respectively. K is incorporated into the MLM as part of the variance of random effects: $Var(u) = 2KV_g$, with K as the n x n kinship matrix and V_g as the genetic variance (*49, 50*). The kinship matrix was calculated using equation 13 of (*51*). We used restricted maximum likelihood for variance component estimation using the P3D compression option to improve algorithm efficiency by clustering lines into related groups (*52*). All association and variance component analyses were carried out using TASSEL *v* 5.0 (*49*).

To synthesize effect estimates across the two datasets and improve statistical power, we used Fisher's combined probability test to combined combine p values from inbred and F1 line data. For each trait, p values were combined on a per site basis into a single test statistic as $-2\log(inbred\ p\ value) - 2\log(F1\ p\ value)$ (*21, 53*). Under standard assumptions, this quantity follows a chi-square distribution with four degrees of freedom if the null hypothesis (no effect of the locus) is true. Here, we do not rely on this feature given that permutation is used to obtain p-values. We developed a pipeline to implement permutation (whole genome sequences against the vector of phenotypes for each line and F1) by splitting both chromosomes and replicates across parallel computing nodes. In each permutation replicate, phenotypic data was randomly shuffled both within the inbred line set and the F1 line set 100 times. Each permuted data set (both F1 and inbred lines) was then analyzed using the TASSEL model described above. For each trait, we extracted the lowest p values from each replicate to determine appropriate adjusted p-value cutoffs to reflect standard 95th and 90th percentile significance thresholds. We determined these thresholds for both the inbred data in isolation as well as for the combined p values.

We first ran the TASSEL MLM only on polymorphisms with a minimum minor allele frequency (MAF) of 0.05 in the lines (following other GWAS studies). After running permutations with this set of variants, we discovered 16 polymorphisms that were significant for at least one trait (loci 1-16 in Table S1). We noticed that several of the most significant tests were for SNPs with an MAF very close to 0.05 (loci 8-13). For this reason, we reran the entire pipeline to include all polymorphisms with the minor allele scored in two or more phenotyped inbred lines. The greater inclusion of data increased thresholds obtained by permutation, but still added a substantial number of associations (loci 17-27 in Table S1).

The model that we fit (TASSEL "K") allows relatedness among lines, but assumes the sampled population is generally well-mixed and unstructured. To accommodate population structure for samples that span numerous natural demes, (*50*) suggest including genomic covariates in the model. While there is no evidence of cryptic population structure *within* IM, we also fit the "Q+K" model of TASSEL to our inbred line data for plant height and flower size PC1. For this, we created a distance matrix among lines for multi-dimensional scaling (Principal Coordinate Analysis) and used the first five axes as covariates (same kinship matrix as used previously). We then compared p-values of common SNPs between the "K" and "Q+K" analyses. For plant height, we find that the most significant polymorphisms in the "K" model usually become more significant in the "Q+K" model. The median p-value reduction is 18% for the top 50 "K" model SNPs – the range goes from 5-fold reduction to 40% increase. The slight increase in power with "Q+K" suggests that the inclusion of covariates removes a small amount of residual noise for individual locus tests. In a simple linear regression with only the five genomic PCs as predictors, there is a slight but significant effect of one genomic PC on plant height. None of the genomic PCs is a significant predictor of flower size PC1. For flower size, the "K" and "Q+K" produce generally similar p-values, neither consistently lower. Given the minimal effect of including genomic covariates, we retained the "K" model to define trait-associated loci (Tables S1 and S3).

Linkage Disequilibrium among polymorphisms and thinning of sites for analysis

The loci in Table S1 fall into two groups based on allele frequency. The first seven (locus IDs in Table S1) have a Minor Allele Frequency (MAF) of greater than 8% and exhibit minimal association with other significant loci (Table S2). Loci 1-7 do sometimes have strong LD with sites within 20kb. LD measured as r^2 is sometimes greater than 0.8 within genes. This level of haplotype structure is typical for the IM population (*34*), and as a consequence, we can map to the gene scale but it is difficult to infer specific nucleotide variants that cause phenotypic differences. The remainder of significant loci (Loci 8-24) have a MAF of < 6% with the minor allele present in nine or fewer lines. These polymorphisms often show a moderate level of association ($0.6 > r^2 > 0.2$) with at least one other locus in this set (Table S2); associations that are likely minimal (or absent) in the natural population. These group 2 SNPs exhibit LD in the inbred line population because their minor alleles happen to co-occur in a few specific inbred lines. In fact, four of the initial 45 significant sites were perfectly associated, the minor alleles present in exactly the same set of lines. We collapsed them to a single locus for Fig. 1 as they are indistinguishable in their effects. Importantly, all strongly associated sites have approximately the same allele frequency. Thus, inferences about allele frequency (e.g. Fig. 1B) are not dependent on identifying the particular causal sites among a correlated set.

Linkage disequilibrium is an important consideration for downstream analyses such as comparison to the population re-sequencing (Poolseq) datasets (e.g. Fig. 2) and the analysis of selection (Fig. 3-4). However, the nature of the problem with respect to hypothesis testing is different for the population re-sequencing and field studies, respectively. Both of these of rely on the 10^{-5} set, which exhibits the same LD patterns as the genome-wide significant set in Table S1. A difficulty is that LD present in a sample of 187 alleles from a population is different from that present in the natural populations. Even if one treats the 187 as a purely random sample of haplotypes from IM, sampling will generate associations between loci in the lines distinct from those in the population. While this effect may be slight on average, it can generate occasional associations that are quite strong in inbred lines even if weak in nature (*54*, *55*).

If the lines (or F1s between the lines) constitute the entire dataset, such as in the field study, then it is only the LD specific to the lines that matters. We can thin closely linked sites to a single data point because they convey exactly the same information (e.g. exactly the same estimated effect on survival). For the analysis of field data, we distilled the 10^{-5} set into a set of 845 SNP/indel loci and 94 structural polymorphisms by collapsing closely linked sites with high r^2 (Fig. 4). This eliminates double counting. It does not create fully independent loci as moderate associations ($0.2 < r^2 < 0.6$) are sometimes observed between loci that are not closely linked when minor alleles happen to co-occur in a few specific inbred lines. For this reason, we cannot treat individual tests (SNPs versus fitness components) as independent. The permutation procedure tests whether averages across SNPs (say the mean effect of minor alleles) is significantly non-zero (Figs. 3-4). Since fitness estimates per line are permuted against whole genome sequences (preserving all inter-locus associations), no assumption of independence among loci is required.

The contrast of greenhouse GWAS results to the population re-sequencing data confronts essentially the opposite problem. Here, the greenhouse study is used simply to classify SNPs as relevant to a trait (e.g. Fig. 2A) or associated with a trait (Fig. 2B). The response variable (where independent realizations are exploited for testing) consists of differences in allele frequencies between population re-sequencing populations. These variables will exhibit much weaker associations. For the High and Low experiments, inter-locus associations will reflect LD within the IM population followed by many generations of independent propagation in the High and Low population. For IM versus Quarry, inter-locus associations should be even weaker since the LD within IM is distinct from the LD in Quarry and each population is sampled independently. A potentially serious difficulty in detecting real effects (avoiding Type II errors) emerges when several closely linked sites have high r^2 in the lines. All will show an association with phenotype in the greenhouse GWAS, even if only one of these polymorphisms is causal. When assayed in the population re-sequencing samples, where associations of allele frequency change estimates between linked sites is much lower, only the causal site is predicted to show the signal. Change at the causal site (driven by a real effect) will be diminished by averaging with neighboring sites (where differences are driven by sampling). The effect should weaken real associations but not inflate Type I error rates.

Identification of putative structural variants

We applied the Lumpy pipeline (*23*) using default parameters in an attempt to identify major insertions, deletions, and inversions relative to the reference genome. Only 181 of the 187 sequenced lines had sequence data suitable for this analysis. The initial inputs were the sam files obtained from read mapping to the v2 reference genome (as described above). These were processed using Samblaster (*56*): options --excludeDups --addMateTags --maxSplitCount 2 --minNonOverlap 20. The insert size distribution was estimated using the Lumpy program pairend_distro.py. For each line, we created a subset bam specifically for discordantly mapped read pairs using samtools view -b -F 1294 [input bam]. A second subset bam was created for split read pairs by piping the output from "extractSplitReads" (a program within Lumpy) to BWA mem. These operations provided necessary inputs (as well as the sample specific mean and standard deviation of insert sizes) to run lumpy with using default settings (-mw 4 -tt 0 -pe, min_non_overlap:75, back_distance:10, weight:1, min_mapping_threshold:20). We obtained vcfs on a per chromosome basis, only considering inversions, deletions, and duplication calls within these vcfs. We called genotypes using svtyper (https://github.com/hall-lab/svtyper).

The median read depth for our data (6.5x on average) is low for optimal genotyping of structural variants. Perhaps as a consequence, there is evidence of under-calling of alternative genotypes to the reference and perhaps of spurious calling of distinct alternative homozygotes as heterozygotes. For this reason, we binned all calls that were not reference homozygotes into a single alternative. This essentially treats the reference genome alternatives as dominant markers. This procedure is conservative with respect to testing because, if multiple alternatives exist, we will not be distinguishing their effects in subsequent model fitting. Genotyping error could diminish real associations, but not inflate Type I error rates. After creating a genotype file for each inbred line, we determined the various F1 genotypes and then ran the TASSEL MLM on the resulting files to estimate genotype-phenotype associations. The minor allele count for the structural polymorphisms are in the range of 2-14 lines, and as a consequence exhibit the sort of associations described above that are typical for low frequency minor alleles (MAF < 6%). All loci yielding $p < 10^{-5}$ for any trait are reported in Table S3.

Meiotic Drive Locus and Chromosome 6 Inversion

Important structural mutations segregate on chromosome 11 (a meiotic drive locus, (*57*) and chromosome 6 (inv6, (*32*)) in IM. The derived allele (orientation) of each is associated with a specific nucleotide sequence over mega-bases of DNA so these features are easily scored in the inbred lines. Letting D represent the drive allele, we obtained genotype counts of DD = 57, Dd = 6, and dd =124 at the drive locus. The frequency of the D allele in the lines (0.32) is close to the value obtained in direct surveys of field collected plants (*40*). Only three of the 187 lines have the derived karyotype for inv6, a frequency substantially lower than in the field (*32*). For the drive locus, we tested for effects on the greenhouse phenotypes for both inbred and F1 lines. Only Days-To-Flower in the F1s exhibited a significant effect ($p<0.05$; Table S4). The Drive locus exhibits more obvious effects in the field experiment, particularly in 2015 (Table S5). Unfortunately, the few lines harboring the inv6 mutation were not phenotyped or employed in the field studies.

Interrogation of population re-sequencing data

We compared the GWAS results to several previously published pooled population samples (Poolseq) by recalling variants from these datasets using a common pipeline. We simultaneously scored variant sites within a collection of pooled samples, including the High, Ancestral, and Low populations from (*25*) and the IM and Quarry sample from (*27*) using Varscan v2.3.6. We piped the output from samtools mpileup (version 1.2) to the varscan functions mpileup2snp (for SNPs) and mpileup2indel (for indels). We obtained the read count (number of alleles) and reference allele frequency at each variant site for each sample. We then performed distinct but parallel contrasts of the GWAS significant loci to the pooled-sample VCF for High/ Low samples from the selection experiment and for the IM/Quarry field data. In both cases, we included a variant position only if each pooled sample (e.g. High and Low) was scored for at least 20 reads and allele frequency within the inbred lines of the GWAS was within 0.1 (+/-) of the "combined IM pooled" sample. The latter is the combined sample of Ancestral population reads from (*25*), which are two greenhouse generations removed from a large sampling from IM, and the IM samples from (*27*) which are DNAs directly from field plants. Ideally, allele frequencies from both the GWAS line and combined IM pooled samples provide unbiased estimates of the true allele frequency in IM. However, either could deviate owing to allele frequency change during line formation and/or genotyping error (more likely for the pooled samples).

After filtering, 1342 polymorphisms were ascertained in the High/Low data. We did not consider contrasts of the Ancestral sample to High or Low owing to its lower median read depths (it is only used for the combined IM pooled allele frequency). At these 1342 polymorphisms, median read depths were 74 (Low) and 79 (High), respectively. We first polarized SNPs with the 'minor allele' defined from its frequency in the inbred lines. We performed a one sample t-test to determine if $\Delta minor$[HL], the difference in the frequency of the minor base High – Low, is non-zero. For loci polymorphic in High/Low, but not in the 10^{-5} set (sites not affecting traits in the GWAS), there is essentially no difference: mean $\Delta minor$[HL] = 0.001. We applied linear regression with the difference in reference allele frequency (High-Low) as the response variable and the effect of the reference allele on traits in the GWAS as the predictor. We used simple summary statistics (mean and SEM) to compare the expected heterozygosity ($\overline{2pq}$) between populations.

After filtering by the same criteria, except with read depths specific Quarry and IM, 1477 of the 10^{-5} set polymorphisms were ascertained in the Quarry/IM data. With SNP alleles defined as minor in the inbred lines, we performed a one sample t-test to determine if $\Delta minor$[Q,IM] (Quarry – IM) is non-zero. To provide a comparison of $\Delta minor$[Q,IM] at 10^{-5} set polymorphisms to the genomic background distribution (as in Fig. 2A), we extracted all polymorphisms in Quarry/IM that that were not near fixation for the same allele (p not less than 0.01 in both populations and p not greater than 0.99 in both populations). Finally, we applied linear regression with the difference in reference allele frequency (Quarry – IM) as the response variable and the effect of the reference allele on traits in the GWAS as the predictor.

Field experiment and analysis

The same F1 lines phenotyped in the greenhouse were also assayed in the field. Fresh F1 seed was generated each year by crossing inbred lines grown in the Duke greenhouse and germinated at the University of Oregon greenhouse in 2014, 2015, and 2016. Each F1 line had two unique parents, and was always created with the same maternal and paternal line. Seeds were cold treated for five days in 2015 and 2016 to synchronize germination. Germination began approximately 5/6/14, 5/2/15, and 4/18/16. Earlier germination dates reflected earlier snow melt at the native site. Two weeks following germination, seedlings were transplanted into Hummert Jiffy Strips arranged into 98 well flats and filled with a mixture of soil from the transplant site (44.373238 N, 122.130675 W) and potting soil. The transplant site was the Browder Ridge (BR) trailhead near IM. Planting into the original IM site has been avoided due to concerns of contaminating the focal IM population. Previous studies have found nearly identical conditions at IM and BR (*20*). Flats were placed flush with the surrounding soil in four transects of roughly equal length following transplant protocols used previously (*18, 19*). Seedlings that died or were flushed from their cells within the first seven days were replaced. Subsequently lost seedlings were removed from further analyses. 2014 was a smaller trial year with fewer F1 lines (roughly six individuals per line and 79 lines). Roughly 40 individuals per F1 line across 63 and 83 lines were grown in 2015 and 2016, respectively. Plants were watered for part of the pre-flowering period in 2015 to prevent death due to desiccation.

On the day of first flower for each plant, day of flower, corolla width, corolla length, floral tube length, floral throat width, widest leaf, height at flowering, and flowering node were recorded. Following senescence, all plants were collected. For 2014 and 2015, total number of flowers and number of seeds from the first flower were recorded. For 2016, total flower count and seed count of each flower were recorded as proxies for fitness. To establish whether the mean fitness effect of minor alleles (Fig. 3) or the association between estimated effect on flower size and on fitness (Fig. 4) are significantly non-zero, we permuted whole genome genotypes against fitness data within each year. For each randomized dataset (1000 replicates), we calculated the mean difference (first test) and slope (second test) within each year and also the difference among years. The distributions for the within year tests provide a null distribution (and thus p-values) for selection; the distributions for differences among years for heterogeneity in selection.

Table Legends

Table S1. The 45 polymorphisms (SNPs and small indels) that were genome-wide significant from permutation are reported. These are distilled into 27 distinct loci (Locus ID column) by combining closely linked and strongly correlated sites. Four of the 27 loci exhibit strong correlation despite being unlinked (see Supplemental Table 2) and so only one was used in Figure 1 of main paper. (n=24 there).

Table S2. Associations are reported among the 45 polymorphisms (SNPs and small indels) that were genome-wide significant from permutation. Calculations of the LD (as both D and r2) were based only on lines where both loci are scored. The count of these informative lines (No.

haplotypes) was required to be >=100. p1 and p2 refer to frequency of the reference base at each locus among scored haplotypes.

Table S3. The description of structural polymorphisms (from Lumpy) that were significant ($p < 10^{-5}$) for effects on greenhouse phenotypes. If more than one trait is effected by a single locus, the results for each trait are reported on a different line.

Table S4. Estimates of phenotypic effect in the greenhouse are reported for the meiotic drive locus on chromosome 11. Across lines, genotypes counts are 111 dd, 6 Dd, and 48 DD, where D refers to the driving allele.

Table S5. Estimates of phenotypic and fitness effect in the field experiment (2014-2016) are reported for the meiotic drive locus on chromosome 11. The same conventions are used as in Table S4.

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