A Segregating Inversion Generates Fitness Variation in Yellow Monkeyflower (*Mimulus guttatus*)

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ABSTRACT Polymorphic chromosomal rearrangements can bind hundreds of genes into single genetic loci with diverse effects. Rearrangements are often associated with local adaptation and speciation and may also be an important component of genetic variation within populations. We genetically and phenotypically characterize a segregating inversion (*inv6*) in the Iron Mountain (IM) population of *Mimulus guttatus* (yellow monkeyflower). We initially mapped *inv6* as a region of recombination suppression in three F_2 populations resulting from crosses among IM plants. In each case, the F_1 parent was heterozygous for a derived haplotype, homogenous across markers spanning over 5 Mb of chromsome 6. In the three F_2 populations, *inv6* reduced male and female fitness components. In addition, *inv6* carriers suffered an ~30% loss of pollen viability in the field. Despite these costs, *inv6* exists at moderate frequency (~8%) in the natural population, suggesting counterbalancing fitness benefits that maintain the polymorphism. Across 4 years of monitoring in the field, *inv6* had an overall significant positive effect on seed production (lifetime female fitness) of carriers. This benefit was particularly strong in harsh years and may be mediated (in part) by strong positive effects on flower production. These data suggest that opposing fitness effects maintain an intermediate frequency, and as a consequence, *inv6* generates inbreeding depression and high genetic variance. We discuss these findings in relation to the theory of inbreeding depression and the maintenance of fitness variation.

KEYWORDS inbreeding depression; polymorphism; variation; chromosomal rearrangement; *Mimulus guttatus*

POLYMORPHIC chromosomal inversions are an important component of genetic variability (Sturtevant and Mather 1938; Hoffmann and Rieseberg 2008). They are associated with species differentiation in both plants (Rieseberg *et al.* 1999; Fishman *et al.* 2013; Hermann *et al.* 2013) and animals (Noor *et al.* 2001). Within species, inversions often exhibit clines suggestive of adaptation to latitudinal environmental variables (Balanyà *et al.* 2003; Hoffmann and Rieseberg 2008; Cheng *et al.* 2012; Fang *et al.* 2012). Similarly, putatively adaptive trait differences cosegregate with inversions within many species, including *Drosophila* (Krimbas and Powell 1992), *Anopheles* (Coluzzi *et al.* 2002), *Rhagoletis* (Feder *et al.* 2003), seaweed flies (Gilburn and Day 1999), monkeyflowers (Lowry and Willis 2010), and sticklebacks (Jones *et al.* 2012). These patterns support the idea that inversions contribute to local adaptation and speciation because they suppress recombination among multiple genetic variants with context-dependent effects on fitness (Kirkpatrick and Barton 2006).

The same evolutionary processes that generate differences among geographical populations can also maintain chromosomal polymorphisms within populations. Environmental fluctuations or frequency dependence could allow the persistence of alternative arrangements containing sets of coadapted alleles, each set being optimal under different conditions (Dobzhansky 1970). This hypothesis may account for segregating "supergenes" such as those determining mating types in heterostylous plants or wing mimicry polymorphisms in *Heliconius* butterflies [reviewed in Schwander *et al.* (2014) and Thompson and Jiggins (2014)]. An alternative scenario for polymorphism is that a newly arising chromosomal rearrangement may happen to contain one or more intrinsically detrimental alleles along with an advantageous allele (Kirkpatrick and Barton 2006). Recessive or partially

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recessive deleterious alleles should be individually rare, but they are common as a class of variant (Muller 1918; Kondrashov 1988) and likely the major cause of inbreeding depression (Charlesworth and Willis 2009). A stable polymorphism may result if the beneficial variants caught by a new inversion increase fitness as heterozygotes (*i.e.*, are at least partially dominant), whereas the detrimental alleles are at least partially recessive. The inversion will increase in frequency initially, as its deleterious recessive alleles will be rare and thus hidden in heterokaryotypes. However, as the inversion frequency rises, recessive costs are expressed more often. Eventually, the benefits and costs balance, resulting in an equilibrium frequency. Thus, rather than promoting adaptation, the inversion brakes the spread of a beneficial allele and leads to elevated frequencies of deleterious alleles.

Here, we describe a polymorphic inversion (hereafter *inv6*) with strong but conflicting effects on different fitness components. We initially mapped this feature in 2003. The results of that study, presented for the first time in this article, were paradoxical. *inv6* segregated in each of three independent QTL mapping crosses, suggesting an intermediate population frequency (it was present in at least three of six plants sampled). However, the fitness effects of *inv6* were estimated to be entirely negative in the greenhouse. The Iron Mountain (IM) population, in which *inv6* segregates, is a large, stable population and it is extremely unlikely that a genetic variant with uniformly detrimental fitness effects would rise to high frequency. To address this mystery, we conducted five subsequent experiments using a combination of genetic, genomic, and ecological methods.

The original experiment mapped major QTL for multiple fitness components to *inv6* in each of three F_2 populations, demonstrating substantial negative effects on both male and female fertility under greenhouse conditions. We then generated crosses within *inv6* karyotypes to demonstrate that the recombination-suppressed region exhibits normal levels of recombination in crosses with collinear genomes. We then genotyped a random sample of plants from the natural population at markers diagnostic of *inv6*, which provided an initial estimate of frequency within IM. This experiment also revealed a distinctive haplotype structure for *inv6*, suggesting that it is recently derived. Next, we conducted a genomic study of eight IM lines with the standard karyotype in combination with two that are homozygous for *inv6*. These data confirm the recent origin of *inv6* and identify several genic regions of elevated sequence divergence. Finally, we performed two different field studies, a one-generation study of male fitness (conducted in 2007) and a four-generation study of female fitness variation (2010–2013). The first demonstrated that the negative *inv6* effect on pollen viability, first observed in the greenhouse, is reiterated in the field. Surprisingly, however, the second field study revealed a net positive effect of *inv6* on lifetime female fitness, mediated in part by effects on flower production. These estimates are the first compelling evidence of a fitness advantage associated with *inv6* and a possible resolution to its unexpected abundance in nature.

When an allele (or karyotype) has both positive and negative effects, gene action (dominance or recessivity) is a critical evolutionary factor. A limitation of our field studies is that they are based on observed genotype frequencies in nature, where *inv6* occurs almost entirely as a heterozygote. However, key corroborative evidence comes from two other studies (Scoville *et al.* 2009; Bodbyl Roels and Kelly 2011) showing that *inv6* is strongly deleterious in homozygotes because it is rapidly "purged" when a population is experimentally inbred. *inv6* increased in frequency within a third study (Kelly 2008; Kelly *et al.* 2013), but evolving populations of this experiment were prevented from inbreeding, and homozygous fitness costs were directly ameliorated. Given the aggregate of results from these many experiments, we argue that the positive heterozygous effects on female fecundity have allowed *inv6* to increase from a rarity to a substantial population frequency within IM (≈8%). However, its strongly negative homozygous effects likely prevent this variant from fixing in the population. We discuss the possibility that inversions under balancing selection may be common and potentially important contributors to both genetic variation and inbreeding depression in natural populations.

Materials and Methods

Study system

Mimulus guttatus ($2n = 28$, Phrymaceae) is a self-compatible wildflower that grows throughout western North America. It is the most common member of an eponymous species complex composed of highly polytypic, partially interfertile subspecies (Vickery 1978; Wu *et al.* 2007). We focus on a population located on Iron Mountain in the Western Cascades of Oregon. The site is an alpine meadow composed of a steep north-facing slope at an elevation of 1470 m over an area of ~600 m^2. The population usually comprises hundreds of thousands of flowering individuals each year, is bee-pollinated, and has a mixed mating system with an estimated selfing rate of 0–25% (Willis 1993; Sweigart *et al.* 1999). The population shows no evidence of spatial genetic structure (Sweigart *et al.* 1999) or biparental inbreeding (Kelly and Willis 2002).

Mapping populations

As part of our study to investigate the genetic basis of complex trait variation and inbreeding depression within the IM population, we crossed six plants to produce three mapping populations (hereafter, the replicated F_2 experiment). The grandparents of the replicated F_2 were sampled from a selection experiment on flower size (Kelly 2008; Kelly *et al.* 2013). Selection was sustained for six generations (bidirectional on corolla width) within populations that were maintained at large size. After a generation of random mating without selection within each population, three Low parents (sampled from the low-selected population) were selected from the Low population, and each was randomly paired to a distinct High parent. We crossed the plants within each High–Low pair and

randomly selected a single F_1 offspring. Three mapping populations, each consisting of 378–384 F_2 individuals, were derived from selfing the three F_1 progenitors. These are called the c2, c3, and c4 mapping populations, and each F_2 was scored as High–High, High–Low, or Low–Low at each locus according to the grandparental origin of a marker allele.

Following discovery of the inversion on chromosome 6 (see below), we generated an additional cross between two inbred lines from Iron Mountain (IM179 and IM767) with the same orientation of markers on chromosome 6. We selfed a single F_1 from this cross to produce 86 F_2 plants that we grew and then genotyped at 18 marker loci.

Genotypes and phenotypes in the replicated F_2 experiment

As part of our original QTL mapping design, we measured days to flower, pollen viability, pollen number, and supplemented seed set on each F_2 individual in the University of Kansas greenhouses in the Spring of 2003. On each plant, the first and second flower were sampled for pollen while the third and fourth were hand-pollinated. We later harvested and counted seed to estimate supplemented seedset. We used a common pollen donor (IM62, a standard inbred line derived from Iron Mountain) for all flowers. Pollen number and viability were measured using a Coulter Counter following the protocol of Kelly *et al.* (2002). We collected bud meristem tissue from each of 378, 384, and 384 individuals of mapping population c2, c3, and c4, respectively, and extracted DNA using a CTAB procedure (Kelly and Willis 1998).

Nearly all of the markers used for this study (prefixed with MgSTS for *M. g*uttatus *s*equence *t*agged *s*ite or simply "e" for *e*xpressed sequence tag) are exon-primed markers spanning introns (MgSTS markers are available from http://www.mimulusevolution.org). To identify informative markers in each cross, the High and Low outbred parents and progenitor individuals were screened at 748 MgSTS markers that had been successfully amplified in IM62. The forward primer was tagged in the 5′ end with fluorescent dye, and the resulting labeled PCR products were run on ABI 3730 or 3700 Genetic Analyzers (Applied Biosystems, Foster City, CA). PCR amplification followed a touchdown protocol (see Fishman *et al.* 2001) with multiplexing and pooling based on expected allele sizes. We scored genotypes using Genemarker software (Softgenetics, State College, PA) based on the segregation of length-variable alleles. These markers are usually codominant and single copy, allowing genetic maps from different experiments to be tied together through markers in orthologous genes. We also genotyped several microsatellite markers [prefixed by "aat" (Fishman *et al.* 2001)] and custom-designed markers (prefixed by "yw") in each mapping population to fill large gaps in the maps (>30 cM).

Linkage map construction

We built the linkage maps following three iterative rounds of data quality control. We used JOINMAP 4.0 (Stam 1993) to construct preliminary maps by regression mapping and examined each marker for amount of missing data and for incidence of double crossovers. Markers with significant missing data (20% or more) were re-amplified depending on whether the marker was critical to fill a gap; otherwise, it was discarded. We discarded questionable markers. Special effort was made to identify and place markers that filled in large gaps and markers that were shared between crosses. The final missing data proportions were 4.7, 2.8, and 3.3% for c2, c3, and c4, respectively.

We finalized linkage maps for each mapping population with the Kosambi mapping function using the maximum-likelihood algorithm in JOINMAP 4.0 run with default settings. We made two versions of linkage group 6 for each map as this chromosome exhibited extensive recombination suppression involving 20–30 markers in each map. One version was made using the maximum-likelihood algorithm as above, with all but one representative marker in the suppressed recombination region deleted. Deleting excess markers in the region of suppressed recombination was necessary for permutation tests of significance. The second version of chromosome 6 was made to illustrate recombination suppression. We included all genotyped markers on chromosome 6 and performed regression mapping. Taking the ends of linkage groups into account, total map length was calculated by adding 2*s* to the estimated length of each linkage group, where "s" equals the average intermarker distance for that linkage group. Assuming random distribution of markers, estimated genome coverage was calculated as $c = 1 - (e - 2dn/L)$, the proportion of the genome within distance "d" of a marker, where "n" is the number of markers and "L" is total map length.

QTL mapping and inv6 phenotypic effects

For genome-wide mapping of fertility traits in the three F_2 populations, we used composite interval mapping in Windows QTL Cartographer 2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm) to set priors for Bayesian mapping as implemented in Rqtlbim (Yandell *et al.* 2007; Yi *et al.* 2007). Details of the QTL mapping methods are given in File S1. To estimate the effects of *inv6* on fertility traits in the mapping populations, we used ANOVA with the *inv6* genotype inferred from diagnostic markers with ambiguous genotypes treated as missing data.

Identification of the inversion haplotype

We grew and extracted DNA from a sample of 96 outbred individuals from the Zia-1 base population, the source population for the artificial selection experiment (Kelly 2008). We genotyped each sample for 18 markers spanning chromosome 6, in both recombination-suppressed and freely recombining regions.

Sequencing of IM664 and analysis of IM genomic data

Flagel *et al.* (2014) fully genome-sequenced nine inbred lines from a large collection of homozygous lines formed from randomly sampled IM plants (Willis 1999b). Genotyping of

the lines at diagnostic markers by Scoville *et al.* (2009) indicates that eight of the nine lines sequenced by Flagel *et al.* (2014) have the standard karyotype for chromosome 6 (lines IM109, IM1145, IM320, IM479, IM62, IM624, IM693, IM767), while one is *inv6* (IM835). To examine patterns of sequence variation within *inv6*, we resequenced an additional *inv6* line (IM664). We extracted genomic DNA from a single plant of IM664 using a CTAB extraction protocol (Kelly and Willis 1998) followed by column purification (MoBio PowerClean DNA kit, MoBio Laboratories, Carlsbad CA). We prepared a genomic DNA library with the Illumina Nextera kit (Illumina Inc., San Diego), following standard protocols. The IM664 library was sequenced (along with 19 other libraries unrelated to this project) in one 150-bp PE run of Illumina NextSeq.

Sequence data from IM664 was combined with the data from the nine previously sequenced IM lines (reads downloaded from the JGI Short Read Archive). We processed reads from each line with Scythe (https://github.com/vsbuffalo/scythe/) to remove adaptor contamination and then with Sickle (https://github.com/najoshi/sickle/) to trim low-quality sequence. Using BWA (http://bio-bwa.sourceforge.net/) with default parameters, we mapped read pairs to the v2.0 draft of the *M. guttatus* genome (http://www.phytozome.net/) after masking repetitive sequence. We used Picard tools (http://broadinstitute.github.io/picard/) to add ReadGroups to the resulting bam files and then indexed these files using SAMtools (http://samtools.sourceforge.net/). Finally, we called SNPs across the 10 lines simultaneously using the UnifiedGenotyper of GATK v2.5 (https://www.broadinstitute.org/gatk/). We analyzed the resulting VCF file using custom python scripts (Supplemental material *Materials*). We filtered SNPs to include only sites within coding regions of the 14 main chromosomes (32,579,125 bp of the genome) with a minimum mapping quality score of ≥30. We suppressed all sites that exhibited heterozygosity in any line and required at least five reads at a site to call a plant as homozygous for either the reference or alternative base. With these filters, we found a total of 791,416 SNPs. We calculated pairwise differences (π) among all lines across the genome within 50-kb windows.

Field measures of male and female fitness components

In 2007, we collected the pollen from all four anthers of newly opened flowers (one per plant) at IM and then harvested plants for later DNA extraction (Fishman and Saunders 2008). Pollen was stained with aniline blue, and a subset of fertile and sterile grains was counted using a hemocytometer. Individuals (n = 187) were genotyped at e423 and e723, markers diagnostic for *inv6*. Genotypic effects on male fertility traits were analyzed with *t*-tests in JMP. In 2010–2014, we collected entire senescing *M. guttatus* plants from the Iron Mountain population and then recovered and counted their fruits, seeds, and (in 2012 and 2013) flowers (Fishman and Kelly 2015). We then extracted genomic DNA from the remaining tissue, and genotyped individuals at the *inv6* diagnostic marker e423 (n = 1248 over the 4 years). Because we collected only plants that survived to mature at least one fruit, our measures of female fitness do not include survival-to-reproduction.

There were too few *inv6* homozygotes per year to include all three genotypes in the analyses. Thus, we coded individuals as *inv6* carriers if they were either heterozygotes or *inv6* homozygotes. This allows us to evaluate dominant and/or additive effects of the inversion, but not recessive ones. We examined female reproductive trait variation (flowers, fruits, seeds/fruit, and total seeds) using generalized linear models in JMP 11 (SAS Institute, Cary NC) with year and *inv6* as main effects. For the first three traits, we used an overdispersed Poisson distribution (log-link function), whereas we analyzed log-transformed seed number (+1) using a normal distribution and identity link (as in Fishman and Kelly 2015).

The plants genotyped for *inv6* only partially overlapped those genotyped at the *D* female meiotic drive locus, which affected several female fitness traits over the same seed collections (Fishman and Kelly 2015). To maximize sample size per year, we did not include *D* genotype in these analyses, but did verify that there was no statistical association between the two polymorphisms (Pearson χ^2, $P = 0.61$). There were never significant year × genotype interactions [lowest $P = 0.09$ for log(total seeds)], so we present analyses without interaction effects. However, our study period spanned two fairly standard growth years (2010 and 2012: one to two fruits, 40–60 seeds per plant) and two relatively benign years (2011 and 2013). Using mean seedset as a proxy for year quality, we then asked whether the inversion had different effects in good (mean seedset 100–250) and poor (mean seedset 40–55) years in an analysis with quality, year (quality), *inv6*, and *inv6* × quality interaction.

Data availability

The genetic, phenotypic, and fitness data specific to inv6 are contained in File S2. The SNP data (within genes) for the 10 resequenced IM lines is reported as Table S3. The raw sequence data is available from the Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra).

Results

Discovery of inv6 region by recombination suppression

All three of the replicate High × Low F_2 genetic maps (Supplemental Material, Figure S1) revealed a large cluster of markers on chromosome 6 that appeared to be completely linked. This nonrecombining region, spanning marker e25 to e804 on the consensus map (Figure 1), corresponds to ~4.2 Mb of the v2.0 *M. guttatus* physical map (http://phytozome.jgi.doe.gov/). In contrast, the same set of markers corresponds to 40 cM in the IM79 × IM767 cross (Figure 1), as well as in other *M. guttatus* linkage maps (Lowry and Willis 2010; Fishman *et al.* 2014; Holeski *et al.* 2014). Each High × Low F_2 segregated for a particular shared haplotype (*i.e.*, a

specific set of alleles) at contiguous MgSTS loci across the recombination-suppressed region. *inv6* was contributed by the Low grandparent (small flower size) in c2, but from the High grandparents in c3 and c4.

QTL for fitness traits map to Inv6

inv6 affected trait means differently in each of the three mapping populations, but all significant effects were negative (Figure 2). In c2, the inversion reduced total pollen ($F_{2,369} = 11.26$, $P < 0.001$), pollen viability (proportion of grains viable; $F_{2,369} = 40.32$, $P < 0.001$), and supplemented seedset (female reproductive capacity; $F_{2,364} = 14.87$, $P < 0.001$) per flower. For all three measurements, gene action was recessive with the negative effect limited to *inv6* homozygotes (Figure 2). In c3, there were no effects on female fertility, but a significant negative effect on pollen viability ($F_{2,379} = 9.43$, $P < 0.001$). Gene action is more nearly additive in c3 (Figure 2). In c4, Inv6 had strongly deleterious effects on female fertility ($F_{2,343} = 10.40$, $P < 0.001$), total pollen ($F_{2,345} = 10.04$, $P < 0.001$), and pollen viability ($F_{2,345} = 92.67$, $P < 0.001$). Heterozygotes were intermediate for all three measurements in c4; *inv6* was partially recessive its effects on female fertility and total pollen but slightly dominant for pollen viability.

The heterogeneity of *inv6* effects among crosses could be due to variation in the non-*inv6* alleles and/or differences in the remainder of the genetic backgrounds. Trait means differed substantially among the three mapping populations (Table S1). Consistent with a highly polygenic basis for fertility variation, we mapped 30 QTL for pollen number or pollen viability in other parts of the genome, as well as two QTL for supplemented seed set (Table S2). The chromosome 11 meiotic driver *D* (Fishman and Saunders 2008) also segregated in each cross and, as expected, the drive allele reduced male fitness. Most fertility QTL exhibit intermediate dominance, but we did map one under-dominant QTL (for viable pollen number) and three overdominant QTL (two for viable pollen number and one for supplemented seedset). However, none of these were present in more than one mapping population. Excluding the two major QTL (*inv6* and D) as well as the over/underdominant QTL, low alleles were moderate in effect (average $s = 0.31$) and partially recessive (average $h = 0.18$). These estimates, combined with the fact that these QTL were always segregating in only one of the three crosses, suggests that they may be deleterious alleles segregating at low frequencies in the natural population.

Frequency of inv6 in nature

In the Zia-1 base population, which was derived via two generations of hand-outcrossing from a wild IM sample of >1000 plants, the *inv6* haplotype had an overall frequency of 15% (Figure 3). In the flowering plants sampled from the field, the estimated frequency of *inv6* was slightly lower (7–8%; see *Genome sequence variation within and between inv6 carriers*). Allelic variation in this population suggests that *inv6* is a derived haplotype, as it shares alleles with the alternative arrangement at many loci. However, in this sample, *inv6* has private alleles at a few apparently diagnostic loci (*e.g.*, e723 and e423). This pattern is confirmed with enormously increased replication of polymorphism in the genomic survey.

Genome sequence variation within and between inv6 carriers

The two *inv6* lines (IM664 and IM835) are nearly identical over 5.4 Mb of chromosome 6 (Figure 4). They differ at only six nucleotide positions within the interval from location 1,336,275 to 6,751,183. This is not generally a region of low variation. Average divergence among standard lines (gray points in Figure 4) is greater than the genome-wide average $\pi = 0.0063$ for all lines and over a thousand times the divergence between IM664 and IM835 across this region ($\pi = 0.0000051$). Importantly, these two lines are not especially similar outside of the *inv6* region. The divergence between IM664 and IM835 across the other 13 chromosomes is typical of the distance between any two random lines from IM ($\pi = 0.0058$ for IM664 *vs.* IM835, the overall nonchromosome 6 average $\pi = 0.0061$ between lines). At each of the six sites differing between IM664 and IM835 within *inv6*, one of these two lines harbors a "singleton." In other words, all lines have the reference base except IM664 (which has the alternative base at two SNPs) or IM835 (which has the alternative at the other four SNPs).

At SNPs within the inverted region, the base exhibited by *inv6* lines is usually, but not always, segregating within the other eight lines (reiterating pattern of Figure 3). About 15% of the SNPs in the inverted region represent "fixed differences" (the two *inv6* lines exhibit one base and all other lines are homozygous for the alternative), and these SNPs exhibit some clustering (note peaks of orange trajectories in Figure 4). This divergence (15%) is elevated relative to the genome-wide average; outside of chromosome 6, IM664 and/or IM835 differ from all other genotyped lines at 4.3% of SNPs. However, given that the sample includes only eight standard lines, a fixed difference in the sample does not imply that the *inv6* base is absent from the larger population of standard karyotypes. The full set of genotype calls (10 lines scored at 791,416 SNPs) is reported in Table S3.

Inv6 fitness effects in the field

Given strong negative effects on fertility traits in the greenhouse, the nontrivial frequency of *inv6* in the IM population is surprising. To evaluate both the frequency and the fitness effects of *inv6* more directly, we capitalized on two existing samples of wild IM plants. First, we examined pollen viability and pollen number in field samples collected in 2007. As in the greenhouse experiments (particularly c4, where it was additively deleterious), carriers of *inv6* had 30% lower pollen viability than noncarriers ($P < 0.0001$, $n = 187$; Figure 5). Homozygotes are too rare in the field to estimate dominance effects. These deleterious effects in heterozygotes make the

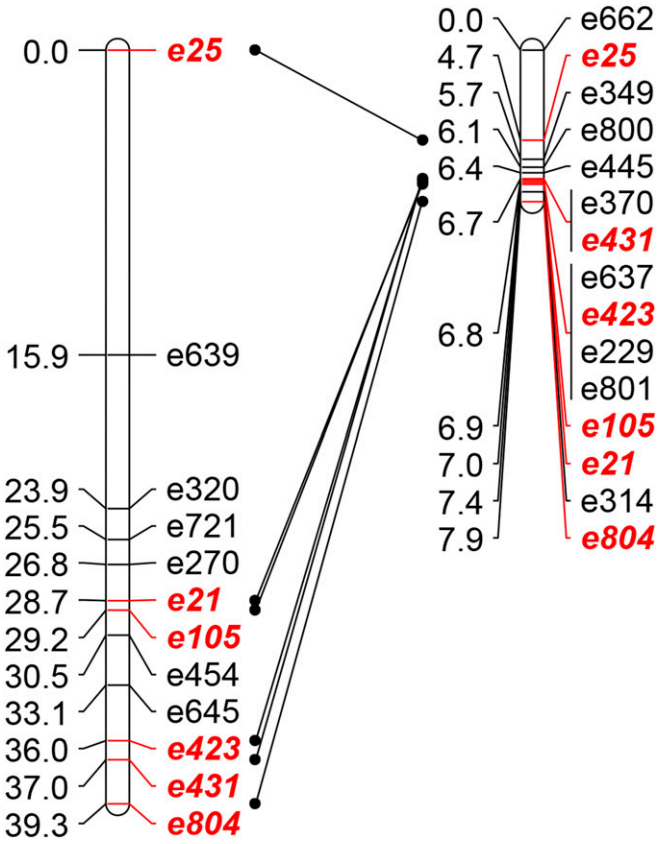

Figure 1 Comparative linkage mapping of the upper end of chromosome 6 in Iron Mountain *M. guttatus* hybrids. In a freely recombining cross (left: IM179 × IM767 F_2; n = 86), this region spans ~40 cM, whereas recombination is highly suppressed in all three F_2 mapping populations segregating for the *inv6* haplotype (right; c3 map shown). Marker names are to the right, and centimorgans are to the left of bar. Shared markers are highlighted in italic red type.

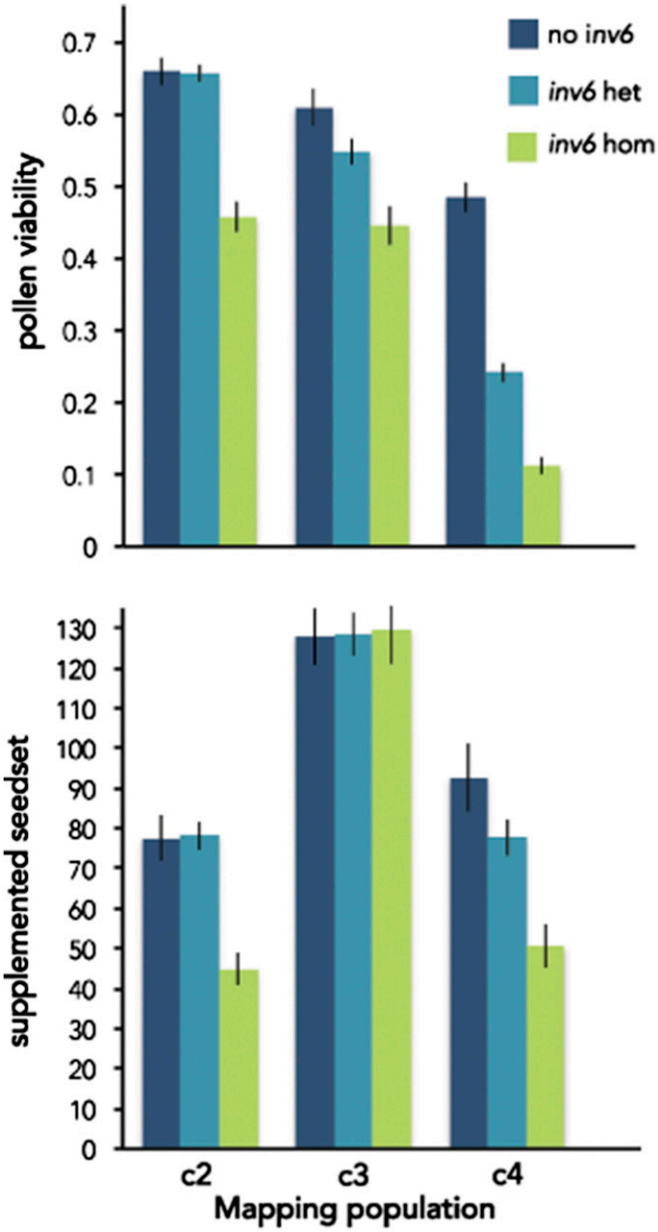

Figure 2 Effect of *inv6* genotype on pollen viability (mean ± 1 SE) and supplemented seedset within each of the F_2 mapping populations.

observed frequency of *inv6* (8% in the 2007 sample of wild plants) extremely unlikely without counterbalancing benefits. Second, we examined effects of the *inv6* genotype on female fertility traits (flower number, fruit number, seeds/fruit, and total seed number) from 2010 through 2013. There was strong year-to-year variation (all $P < 0.0001$) in all these traits, indicating that this sample captures the breadth of natural environmental variation in female fertility. Similar to 2007, the frequency of *inv6* was ~7% (~14% heterozygous plants) and was fairly constant across years in the 2010–2013 samples.

In contrast to *inv6*'s negative effects on seedset per flower in the greenhouse and on male fertility in both environments, we detected only positive effects of *inv6* on female reproductive traits (flower, fruit, and total seed number) in the field (Figure 6). Most strikingly, for the 2 years in which we had flower counts (2012 and 2013), *inv6* carriers produced significantly more flowers than alternative genotypes (GLM, LR $\chi^2 = 7.63$; $P = 0.006$, $n = 889$). In those 2 years, increased flower production translated into increased fruit set ($P = 0.04$); however, the effect on fruit number was marginally nonsignificant across all 4 years ($P = 0.12$, $n = 1242$). In contrast to the greenhouse experiments, we saw no *inv6* effect on seeds per fruit ($P = 0.78$). However, across all 4 years, there was a significant positive effect of *inv6* on log(total seeds) ($P = 0.047$). Although the year × *inv6* interaction was not significant for seed number ($P = 0.13$), there did seem to be a pattern. In 2010 and 2012, which had relatively low fecundity (population mean = 43 and 53 seeds, respectively), *inv6* appeared beneficial, whereas it had slightly negative effects in 2011 and 2013 (population mean = 110 and 227, respectively) (Figure 6). Grouping years by quality (2011 and 2013 good, 2010 and 2012 bad), there was a significant quality × *inv6* interaction ($P = 0.02$), as well as a significant effect of *inv6* ($P = 0.03$), in the full model.

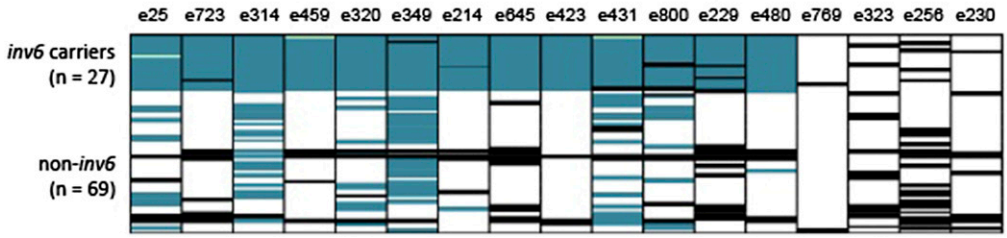

Figure 3 Delineation of *inv6* haplotype block in *M. guttatus* individuals derived from wild IM plants by one generation of outbreeding (n = 96). Each cell represents the genotype of an individual genotyped at 17 markers across chromosome 6. Genotypes carrying at least one *inv6*-associated allele are shown in blue (for the 13 *inv6*-spanning markers) and non-*inv6* genotypes are shown in white. Black indicates missing data, and three non-*inv6* genotypes (likely genotyping errors or double crossovers) within the *inv6* block are shown in green.

Discussion

Chromosomal rearrangements such as inversions are increasingly recognized as contributors to local adaptation and speciation. Inversion polymorphisms may also be an important component of complex trait variation within populations, but have been little explored beyond a few supergenes and selfish elements (Ford 1971; Thomas *et al.* 2008; Thompson and Jiggins 2014). Here, we genetically and phenotypically characterize an intermediate-frequency inversion polymorphism in the Iron Mountain population of *M. guttatus* (yellow monkeyflower). We identified *inv6* on the basis of no recombination among markers on chromosome 6 spanning over 40 cM in a freely recombining cross (Figure 1). Genomic data confirms it to be a distinct and apparently recently derived haplotype. We demonstrate that *inv6* has strong and partially recessive deleterious effects on male and female fertility traits in the greenhouse and negative effects on pollen fertility in wild plants, but positive effects on lifetime female fitness likely mediated through increases in flower production. These results contribute to the evidence that inversion polymorphisms may commonly segregate within populations because they bind deleterious and beneficial variants into genetic loci with balanced fitness effects.

Why is inv6 polymorphic?

The mapping experiment that first identified *inv6* revealed only strongly negative fitness effects (Figure 2). Despite this, extensive field sampling indicates that *inv6* is surprisingly common in the largely outbred IM population. The estimated frequency of 7–10% translates to ~50,000 copies of *inv6* among the ≈300,000 flowering adults in a typical year at IM. IM exhibits very high levels of nucleotide variation (~2% genome wide), and there is no evidence of a population bottleneck that could have recently inflated the frequency of an unconditionally deleterious mutation (J. Puzey, J. Willis, J. Kelly, unpublished results). Although we do not yet know the full phenotypic effects of genes within *inv6*, the synthesis of data from this and several other experiments provides a partial explanation for this paradoxical abundance. Hardy–Weinberg proportions are immediately relevant to this explanation. In the field, there are 10–20× as many *inv6* heterozygotes as homozygotes. As a consequence, a slight advantage of *inv6* in heterozygotes may allow it to increase when rare even if it is strongly deleterious when homozygous.

The balance of evidence suggests variable, but on average, partially recessive deleterious effects for *inv6*. We see no evidence for the underdominance expected when sterility is caused by chromosomal differences *per se*, *i.e.*, gametes with duplications/deletions resulting from crossovers in inversion loops (White 1969). In the replicated F_2, one of three crosses (c2) revealed recessive gene action, while the other two were more nearly additive (Figure 2). This variability is not surprising, given that the alternative to *inv6* is not a single allele but many distinct haplotypes (Figure 3 and Figure 4). Previous experiments conducted on the IM population corroborate average recessive deleterious effects for *inv6*. Willis (1999b) initiated >1000 independent inbred lines, starting each from an outbred IM plant. Scoville *et al.* (2009) genotyped 138 of these lines after six or more generations of self-fertilization (predicted inbreeding coefficient >0.98). Only 4 of 138 (≈3%) carried *inv6*, which is much reduced from the frequency in the initial sample. In a distinct experiment, Bodbyl Roels and Kelly (2011) synthesized large synthetic populations by intercrossing the F_2 populations described here for subsequent experimental evolution. Two replicate populations were maintained at large size but compelled to self-fertilize. The initial frequency of *inv6* was 37% in each replicate, but declined to 1 and 16%, respectively, after five generations. In two other populations that were supplied with bumblebee pollinators, *inv6* declined to a lesser extent (14 and 21%, respectively).

The field data for *inv6* (Figure 5 and Figure 6) paint a different picture, but these estimates are based almost entirely on the heterozygous effects of the inversion (very few *inv6* homozygotes were sampled). The single year that male fitness was estimated (2007) suggests a 30% pollen viability cost in wild heterozygotes (Figure 5). This estimate is intermediate to that obtained from c3 and c4 of the replicated F_2 experiment (Figure 2). In contrast, field data on female fitness show that, particularly in poor years, *inv6* carriers set significantly more seeds than noncarriers. This appears to be primarily mediated through increases in flower and fruit number.

inv6 would not occur primarily in heterozygotes if its natural population frequency was near 50% as initially suspected. However, the estimated 7–10% frequency suggests it unlikely that *inv6* would have segregated in each of the three F_2 mapping populations if the parents were directly

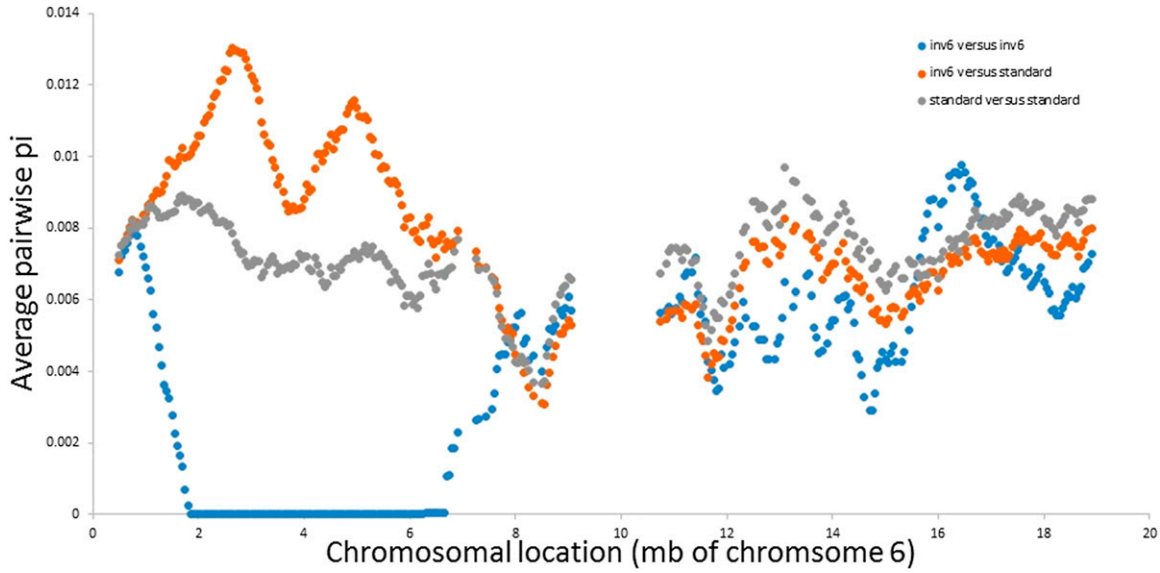

Figure 4 The average pairwise sequence divergence (π / bp) is reported across chromosome 6 for three distinct contrasts: blue indicates *inv6*/*inv6*, orange indicates *inv6*/standard, and gray indicates standard/standard. Points are based on a 1-Mb moving average (each is calculated by averaging π/bp estimates from contiguous 50-kb windows). The absence of data from 9 to 11 Mb corresponds to the putative centromere.

sampled from IM. Instead, the parents of the QTL study were sampled from the diverging populations of an artificial selection experiment (Kelly 2008). Large experimental populations were founded from IM and maintained with enforced outcrossing for 10 generations (the parents were sampled from generation 6). Over 10 generations, *inv6* rose from an initial frequency of 15 to ~65% in *each* of three independent populations, one that experienced selection for larger flowers, one for smaller flowers, as well as the unselected control (Kelly *et al.* 2013). Even though *inv6* would routinely occur in homozygotes once at high frequency, this would not translate into a fitness disadvantage given the methods of propagation within that experiment. All adult plants were randomly paired to another survivor from the same population, one assigned as sire and the other as dam. We repeatedly hand-pollinated dam from sire to ensure sufficient seedset. Even plants with 30–40% reduced pollen viability would still sire far more seed than required to found a family of the next generation. Because we equalized the contribution of each parent pair to the next generation (each contributed ~10 progeny), any intrinsic difference in female fecundity would also be inconsequential. The parallel increase of *inv6* within up, down, and unselected populations suggests that *inv6* conferred some unmeasured fitness benefits common to all populations in that experiment. One possibility is germination requirements/timing, which could both respond to inadvertent greenhouse selection and underlie the genotypic differences in flower number and seedset seen in the wild. Seed germination was one of the few life stages in the selection experiment with opportunity for uncontrolled selection.

The internal homogeneity of *inv6* is relevant to its origin and frequency. Figure 3 indicates genotype matching of 27 *inv6* carriers at 13 markers. Figure 4 shows near identity of two *inv6* homozygotes across millions of bases. Thus, *inv6* appears recently derived and exhibits the molecular pattern of a partial sweep, similar to the pattern evident at the *D* locus on chromosome 11, which is strongly indicated as a balanced polymorphism (Fishman and Saunders 2008; Fishman and Kelly 2015). Importantly, *inv6* alleles/bases are usually, but not always, present in non-*inv6* haplotypes. The latter exhibit many different combinations of alleles, indicative of free recombination in the majority of the population. Together, these observations indicate that *inv6* is the derived arrangement, but its absolute age is difficult to infer. Under the neutral infinite sites model, the expected π between two lines is $2\mu T_{\mathrm{mrca}}$ (Tajima and Nei 1983), where μ is the mutation rate per generation and T_{mrca} is the number of generations since the common ancestor of the two lines. The strict validity of this model for the present data is certainly questionable, but it provides a useful guide regarding the relative age of the feature. The estimated average T_{mrca} for standard karyotypes in the region is ~1500 times the T_{mrca} of the two *inv6* sequences. This may be due to the fact that the inversion occurred relatively recently within IM, allowing minimal time to accumulate new "internal" variation via mutation. Alternatively, a selective sweep may have occurred within *inv6* (but not the larger population), eliminating any internal variation previously accumulated.

The observation that *inv6* increased from rarity to a population frequency of nearly 10% does not imply that it is a balanced polymorphism. The evidence for balance is the low fitness estimates for *inv6* homozygotes in Figure 2, as well as the negative effect of inbreeding evident in previous experiments (Scoville *et al.* 2009; Bodbyl Roels and Kelly 2011). The evidence is not sufficient to determine if the IM population is currently at, or close to, an equilibrium frequency for

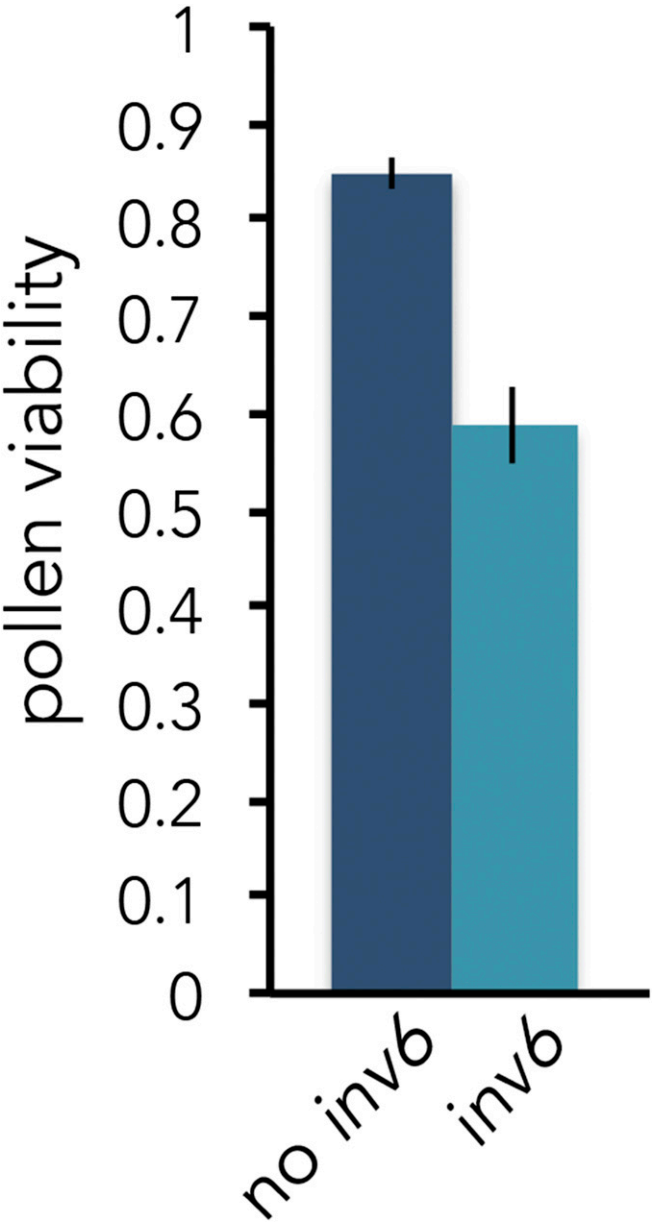

Figure 5 Effect of *inv6* genotype on pollen viability (mean ± 1 SE) of wild Iron Mountain *M. guttatus* plants (2007; n = 177). Individuals were assigned to genotypic categories (*inv6*, no *inv6*) based on their alleles at the diagnostic markers e423 and e723. An *inv6* assignment indicates a heterozygous individual, as the two *inv6* homozygotes in the dataset were excluded.

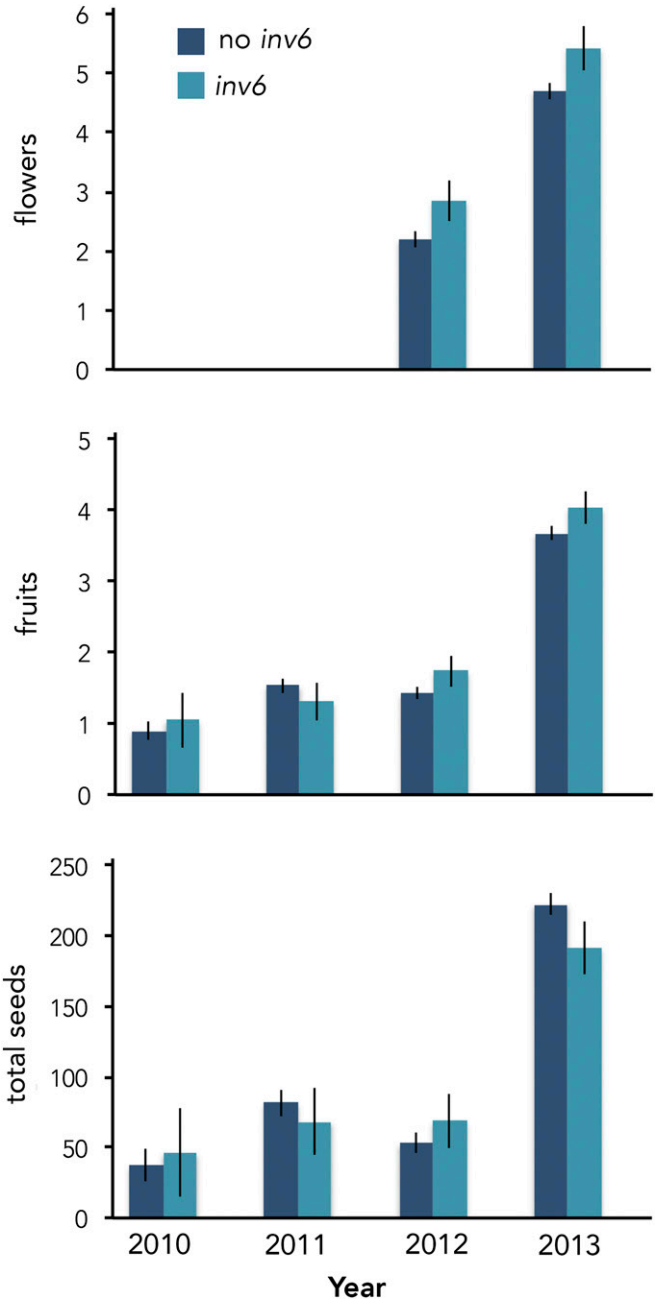

Figure 6 Effects of *inv6* genotype on female fitness components (mean ± 1 SE) of wild Iron Mountain *M. guttatus* plants (n = 1248). Individuals were assigned to genotypic categories (*inv6*, no *inv6*) based on their alleles at the diagnostic marker e423. There were only a few *inv6* homozygotes in the entire 4-year dataset (not enough to include in the statistical analyses), so an *inv6* assignment indicates a heterozygous individual. We show raw means and standard errors here, but the statistical tests in the text were done in a GLM framework (Poisson, log link for fruits and flowers) or with log-transformed values (normal, identity link for seeds).

inv6, given conflicting positive and negative effects. Moreover, a balanced polymorphism does not even necessarily predict a fixed allele frequency. The fluctuating selection suggested by Figure 6 does not predict a fixed equilibrium.

Genetic basis of inbreeding depression

Inbreeding depression is the decrease in fitness that occurs with increased homozygosity caused by mating between relatives or self-fertilization. It has been a focus of research in genetics for >150 years (Darwin 1876; East 1908; Crow 1993). Two models have dominated thinking about inbreeding depression: dominance and overdominance. The dominance model posits that inbreeding depression is caused by recessive or partially recessive alleles with deleterious effects on fitness. Recessivity of segregating deleterious mutations is

predicted because selection more rapidly eliminates additive and dominant mutations from a population. In contrast, the overdominance model states that heterozygotes have superior fitness compared to either alternative homozygote, such that fitness declines as homozygosity increases with inbreeding. At present, a preponderance of data favors deleterious, partially recessive mutations rather than overdominance to explain the bulk of inbreeding depression (Charlesworth and Willis 2009).

inv6 is the second major chromosomal polymorphism to have been mapped within IM, following the drive locus on chromosome 11 (*D*). The latter is a structural variant of the centromeric region of chromosome 11 that exhibits centromere-associated drive over the alternative chromosomal type. *D* gains an ~60:40 transmission advantage in heterozygote individuals by driving through female meiosis (Fishman and Saunders 2008). Consistent with this selective advantage, patterns of nucleotide diversity suggest a recent and rapid spread of the *D* variant at Iron Mountain. However, *D* is prevented from reaching fixation because it exhibits recessive negative effects on both male (pollen viability) and female (seed set) fitness components in nature. Together, these linked recessive costs maintain the *D* chromosomal variant at intermediate frequency (30–40%) near the predicted equilibrium (Fishman and Kelly 2015). Although we do not know as much about *inv6* yet, its shared features with *D* suggest a general alternative model for inbreeding depression.

inv6 and *D* each generate substantial inbreeding depression, but neither polymorphism conforms to either the dominance or overdominance model. These loci exhibit partially recessive deleterious effects (like the dominance model) but intermediate allele frequencies (like the overdominance model). Because of the latter feature, these polymorphisms generate considerable inbreeding depression and also genetic variance for fitness. For both *D* and *inv6*, the existence of such intermediate frequency deleterious variation depends on structural variants that prevent (at least in the short-term) recombination from breaking up the association between alleles with positive and negative effects. Otherwise, the alleles with deleterious effects (particularly if not entirely recessive) should be driven to low frequency by selection. Both deleterious recessive mutations and structural mutations are common; in combination with rare beneficial variants or driving selfish elements, they may often contribute to balanced polymorphism.

Apart from *D* and *inv6*, the data from the replicated F_2 experiment are consistent mainly with the dominance model; inbreeding depression maintained by mutation-selection balance. We mapped >20 additional QTL for male fertility (Table S2). Nearly all were mapped in only one of the three crosses. This is consistent with the prediction that deleterious alleles should be rare in the population, and as a consequence, each such allele was sampled into only one founding parent of the six used to generate the mapping populations. A few QTL did exhibit apparent overdominance. However, these QTL were also unique to a mapping population, contrary to expectations for alleles maintained at intermediate population frequencies by balancing selection. The level of mapping resolution in the replicated F_2 experiment cannot distinguish true overdominance from associative overdominance (deleterious alleles linked in repulsion phase, also known as "pseudo-overdominance"). The remaining QTL exhibit average partially recessive gene action of the low allele. The average dominance coefficient (excluding the chromosomal rearrangements) of $h = 0.18$ is very close to the previous estimate of $h \sim 0.15$ (Willis 1999a). It is likely that many additional deleterious mutations are segregating in IM, but are yet unmapped because the individual effects are below our detection limit.

Conclusion

Modern tools of genetic mapping, combined with population sampling, provide a direct means to investigate the maintenance of genetic variation in fitness. This work contributes to a larger effort to identify genetic components of fitness variation within the Iron Mountain population of *M. guttatus* and reveals their individual (and apparently complex) histories. Balancing selection facilitated by recombination suppression in inversions may be an unexpectedly significant and general factor in the maintenance of fitness variation within populations, in keeping with the prominent role of inversions in speciation and divergence.

A great deal remains unknown about the fitness costs and benefits of *inv6*. It is reasonable to hypothesize that the negative effects of *inv6* are due to partially recessive deleterious mutations captured within a novel inversion. This inversion has reached an unexpectedly high frequency owing to (perfectly) linked alleles with beneficial heterozygous effects. This interpretation is generally congruent with theory (Sturtevant and Mather 1938; Kirkpatrick and Barton 2006). An inversion polymorphism that has captured both advantageous and deleterious alleles is maintained if the advantageous effect of the inversion is (i) greater than its disadvantageous effects in the heterozygote and (ii) smaller than the disadvantageous effects in the homozygote. Of course, these are conditions for polymorphism under almost any diploid model, inversion or not. However, inversions might greatly increase the likelihood that the conditions are met; advantageous and deleterious effects are bundled together by recombination suppression in heterokaryotypes.

The genetic basis and the selective factors underlying the positive heterozygous effect of *inv6* are presently unknown. The inverted genomic region contains >1200 annotated genes. The advantageous effect could owe to one mutation (producing associative overdominance in combination with linked deleterious alleles) or to multiple genetic changes. In the latter case, it is plausible that *inv6* has captured a constellation of alleles that are locally advantageous within IM, as postulated by Kirkpatrick and Barton (2006). However, it is also possible that co-adaptation among alleles within *inv6* underlie its beneficial effects. Linked with deleterious recessive alleles, *inv6* could represent a "supergene with baggage." Epistasis for fitness-related traits is common in *M. guttatus*

(Kelly 2005; Monnahan and Kelly 2015); if positively interacting alleles are located on the same chromosome, inversions may often be favored because they suppress recombination among them. The environmental dependence of *inv6* fitness effects, evident from differences between greenhouse and field and between years at the field site, is also likely important. Finally, the inversion itself (*e.g.*, mutations caused by the breakpoints rather than genic variants within the rearranged region) may cause either positive or negative fitness effects (*e.g.*, Küpper *et al.* 2016). Much remains to be learned.

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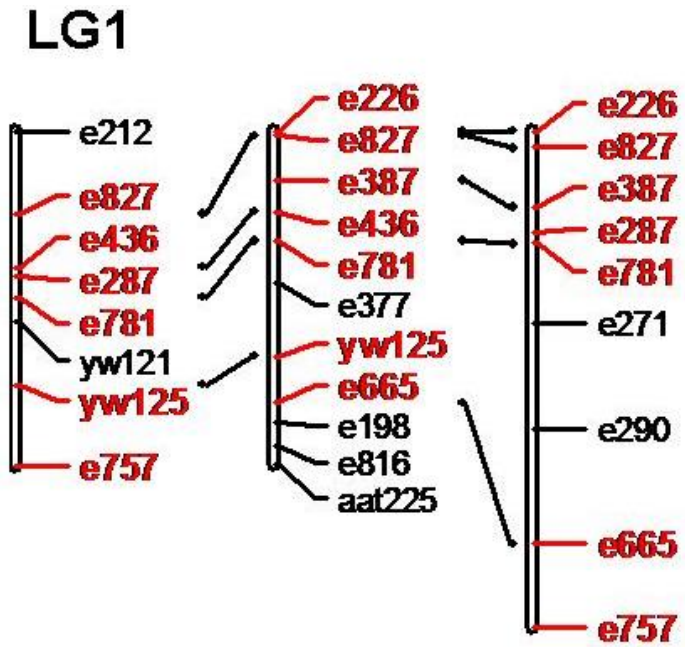
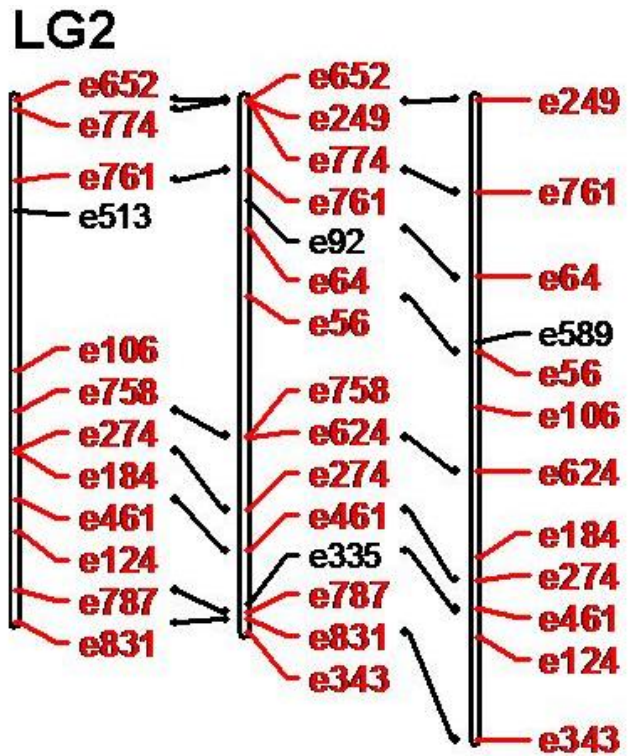
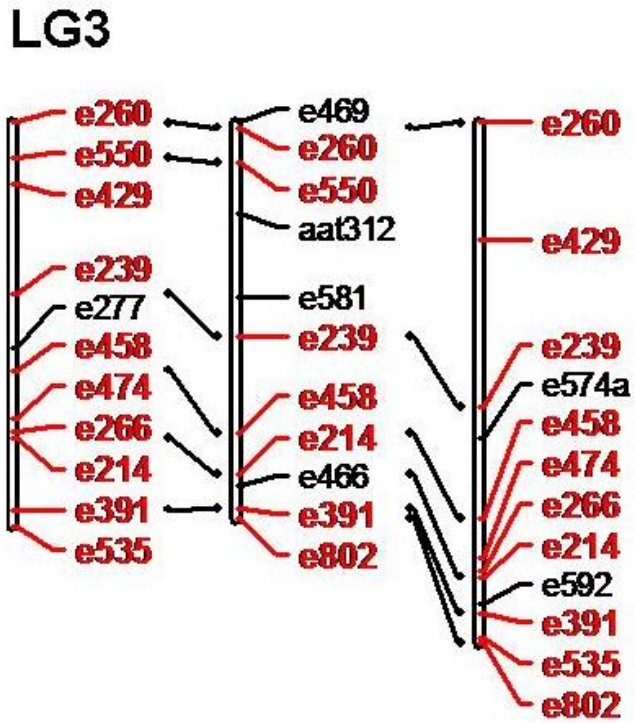
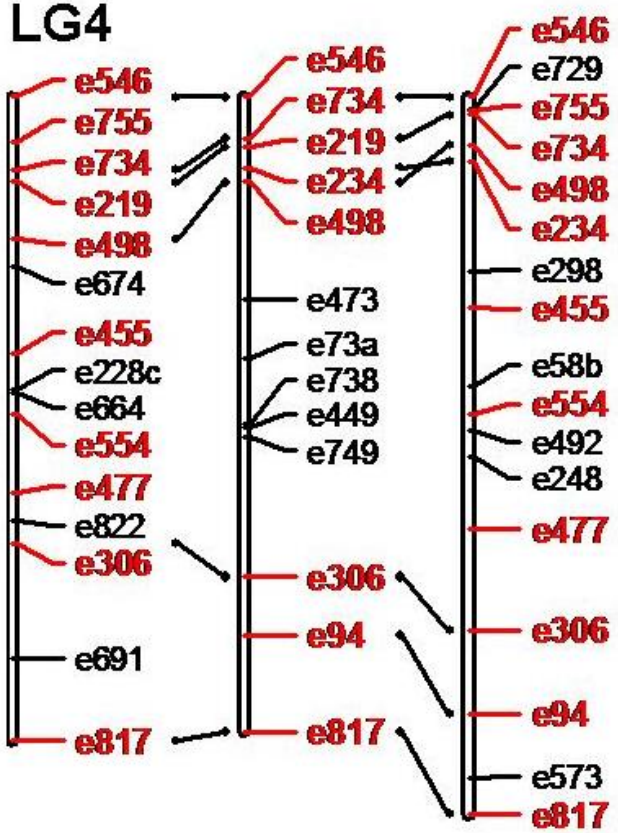
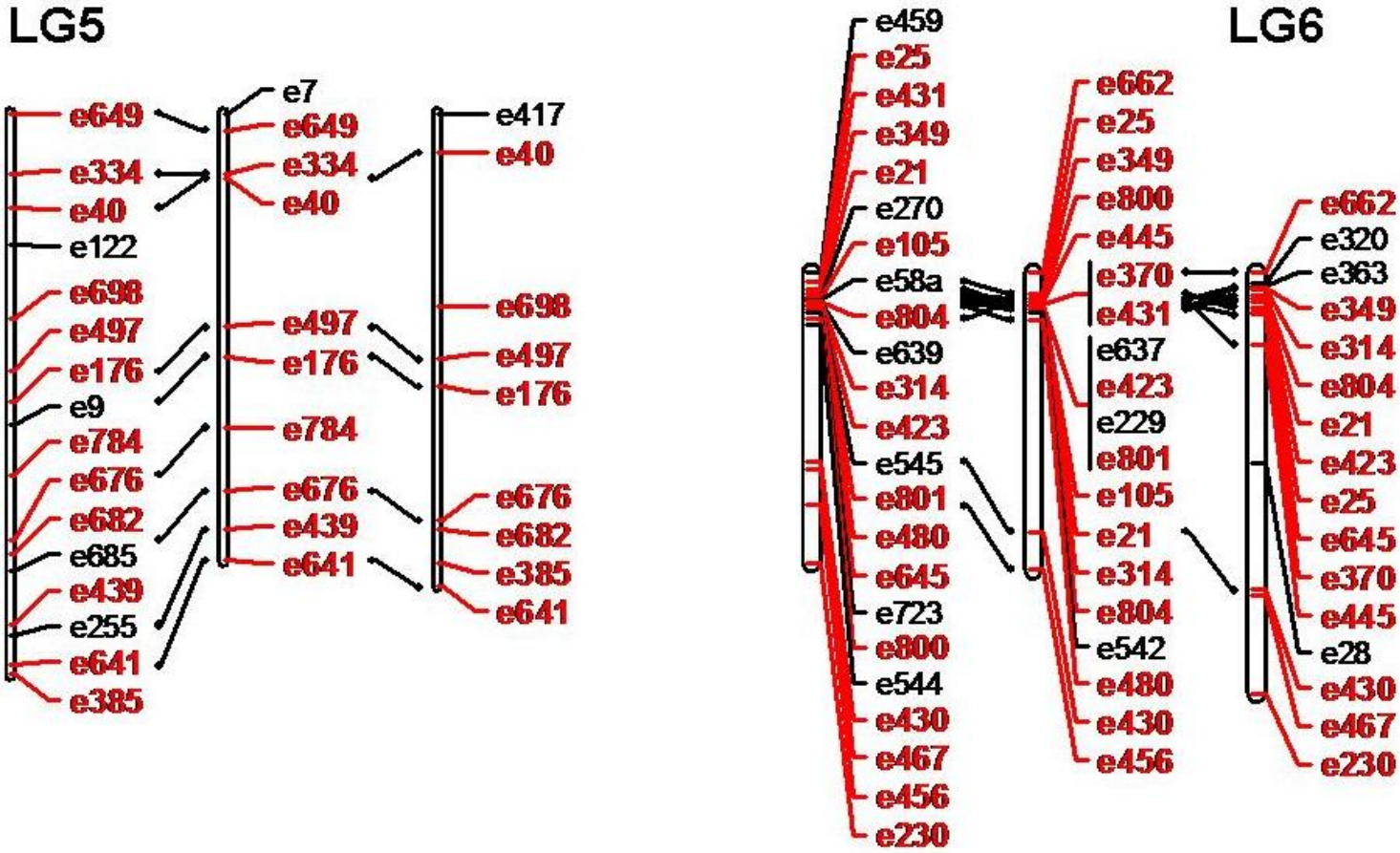
Figure S1. Three F2 linkage maps of the Iron Mountain *M. guttatus* population are aligned together by linkage group. From left to right: mapping population c2, c3, and c4. Marker names in black are unique to a single cross. Marker names in red are shared across at least two of the three mapping populations. Black lines connect shared markers in adjacent linkage groups (i.e. shared markers between c2 and c3, and shared markers between c3 and c4).

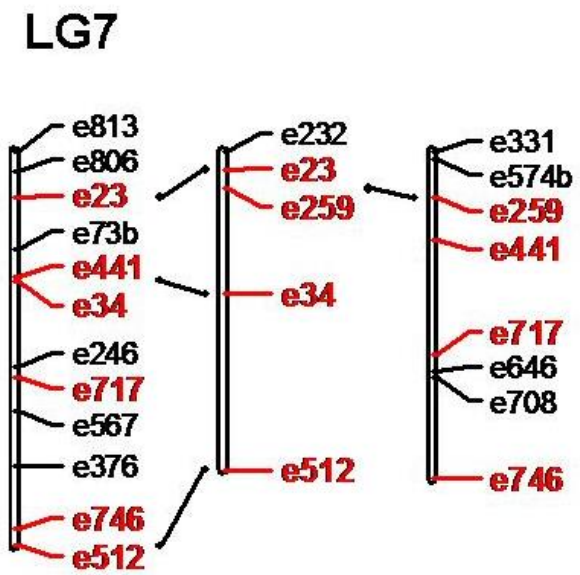


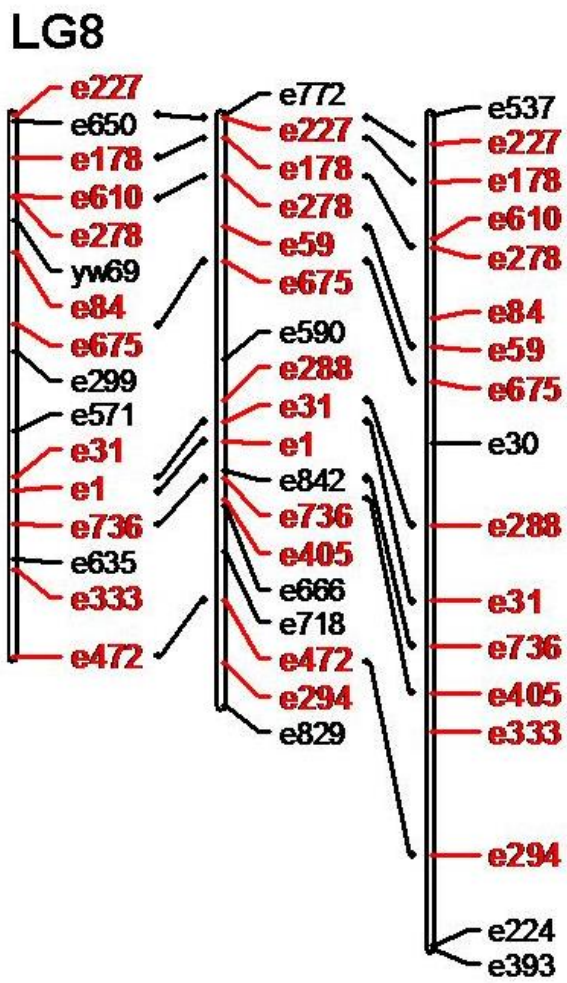


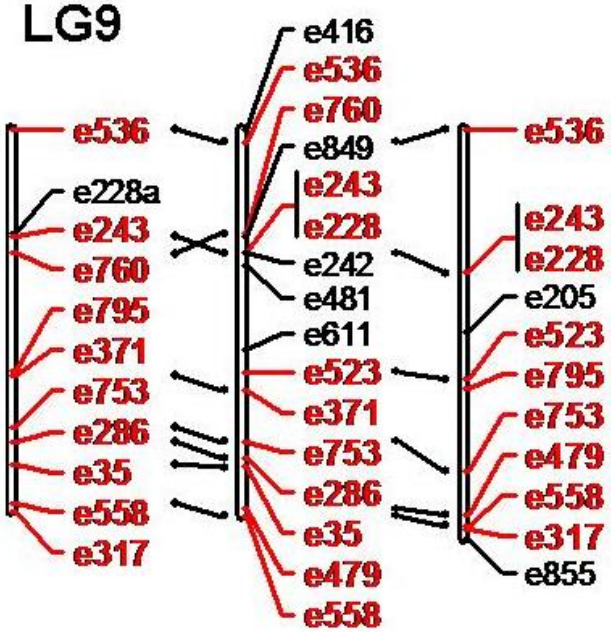


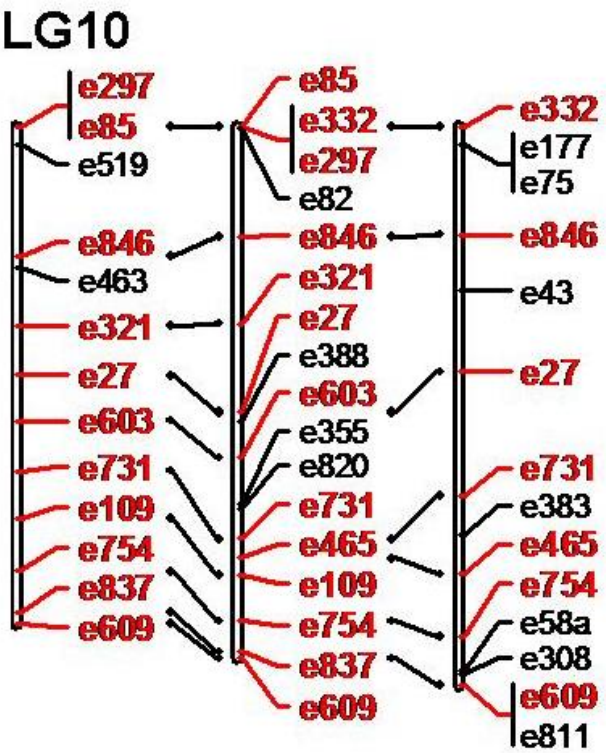


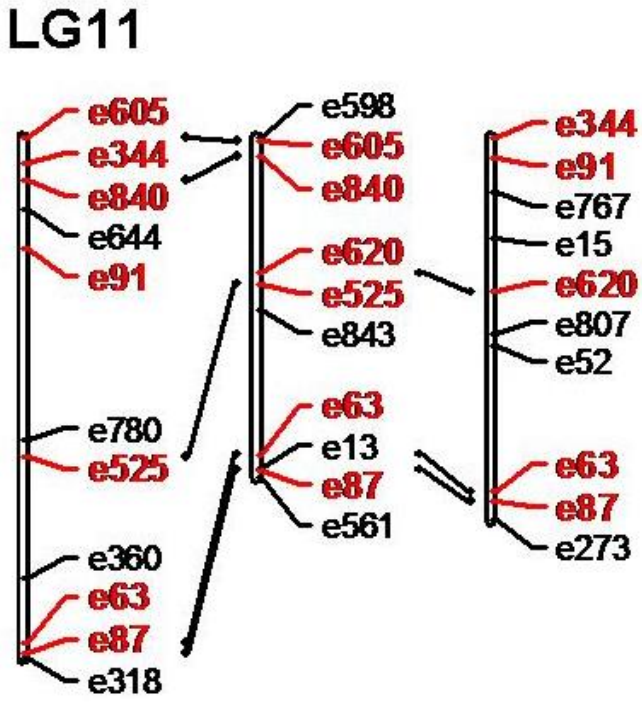


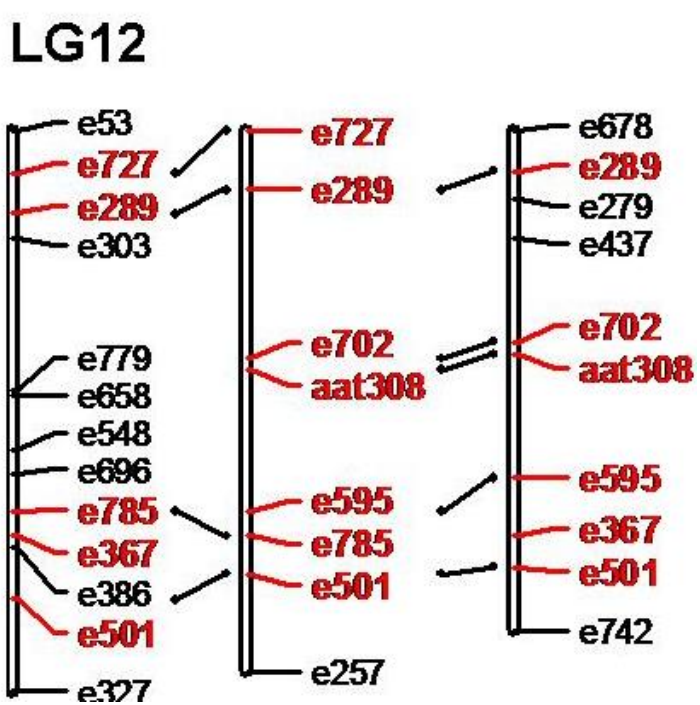












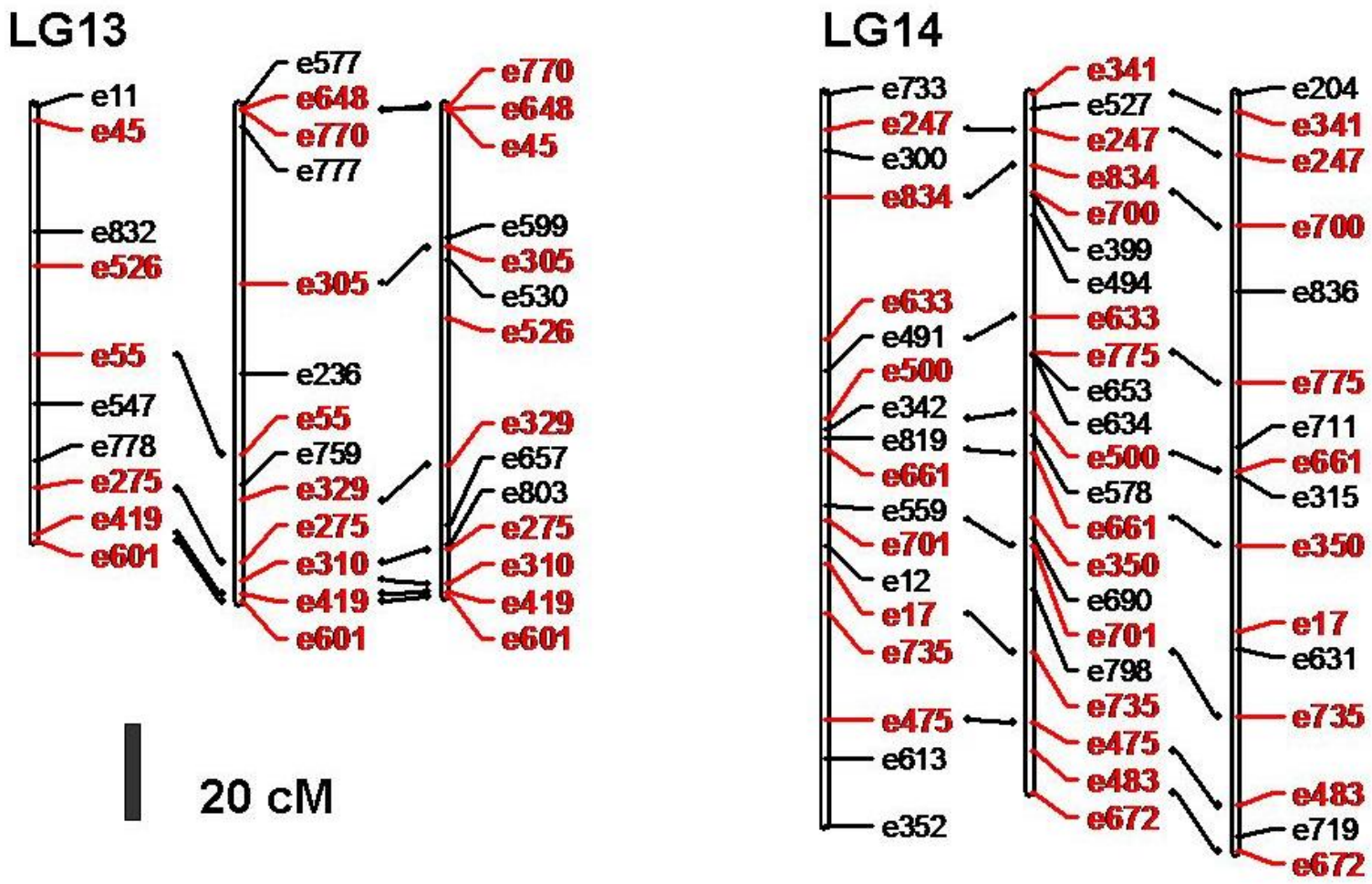


Table S1: Summary of male and female fertility trait means and distributions. Pollen viability is proportion viable pollen, total pollen and supplemented seedset are counts per flower. Trait means are presented with phenotypic standard deviations.

cross	pollen viability	total pollen	supplemented seed set
c2	0.55(.17)	8598(3192)	69.2(49.8)
c3	0.48(.24)	8553(3850)	128.3(75.7)
c4	0.22(.19)	7907(3805)	72.8(64.2)

Table S2: The cross and location is reported for each QTL for Supplemented Seed Set (s), Pollen Viability (p), and Pollen Number (n). Origin of alleles are reported (H = high parent, L = low parent) with the raw mean values for each genotype among F2s. Excepting QTLs with over/under-dominance, we standardized phenotype as 1, 1-hs, and 1-s (raw means divided by higher homozygote mean).

cross	lg	position	marker	trait	HH	HL	LL	Parental origin of deleterious allele	s	h
c2	4	102.6	e691	p	0.4997	0.5663	0.5739	H	0.13	0.1
c2	6	0	inversion	n	5552.3	5274.6	3115.9	L	0.44	0.11
c2	6	0	inversion	p	0.5914	0.5934	0.4111	L	0.3	-0.01
c2	6	0	inversion	s	79.522	78.579	42.684	L	0.46	0.03
c2	7	41.4	e246	p	0.5071	0.5629	0.5789	H	0.12	0.22
c2	8	9.28	e178	n	4024.4	4985.7	5562.2	H	0.28	0.37
c2	11	87.4	driver	p	0.5059	0.5539	0.6124	H	0.17	0.55
c2	13	96.14	e601	n	5390.5	5023	4220.6	L	0.22	0.31
c2	14	61.48	e491	n	4202.7	5054.6	5150.1	H	0.18	0.1
c2	14	61.48	e491	p	0.4993	0.5739	0.5659	H	0.12	-0.12
c3	2	25.2	e64	n	4226.6	5156.9	4015.7	NA	OVERDOM	
c3	2	78.1-80.7	e274	n	1244.8	4616.4	4944.6	H	0.75	0.09
c3	2	78.1-80.7	e274	p	0.2359	0.4825	0.5329	H	0.56	0.17
c3	2	78.1-80.7	e274	s	33.5	139.19	118.74	H	0.72	-0.24
c3	3	52.4	e239	n	5007.8	4752.4	3910.6	L	0.22	0.23
c3	6	0	inversion	p	0.3997	0.492	0.5472	H	0.27	0.37
c3	8	9.9-18.6	e178	n	4218.6	4326.2	5316	H	0.21	0.9
c3	8	110.6	e472	n	5864.6	4976.4	2739.3	L	0.53	0.28

c3	8	110.6	e472	p	0.5586	0.5322	0.3387	L	0.39	0.12
c3	9	50.2	e523	n	5576	4685.7	3695.1	L	0.34	0.47
c3	9	69.6	e35	p	0.5442	0.4849	0.4486	L	0.18	0.62
c3	10	17.2	e846	n	4632.6	5325.4	3147.7	L	0.32	-0.47
c3	10	17.2	e846	p	0.4825	0.5424	0.3914	L	0.19	-0.66
c3	11	66.2-67.4	driver	n	2155.6	5156.1	6517.2	H	0.67	0.31
c3	11	66.2-67.4	driver	p	0.298	0.5398	0.6237	H	0.52	0.26
c3	12	57.2	aat308	n	4151.6	4973.2	4160.4	NA	OVERDOM	
c3	13	0	e648	n	4475.7	5147.9	3618.3	L	0.19	-0.78
c4	4	0-9.55	e546	n	2206.5	2001.6	1235.5	L	0.44	0.21
c4	4	0-9.55	e546	p	0.2546	0.2361	0.1744	L	0.31	0.23
c4	5	0	e417	n	2278	1769	1889	L	0.17	1.31
c4	5	102.18	e641	n	1889.7	1578.8	2018.5	NA	UNDERDOM	
c4	6	4.5-14.8	inversion	n	665.35	1950.3	3738.5	H	0.82	0.58
c4	6	4.5-14.8	inversion	p	0.1016	0.2288	0.4242	H	0.76	0.61
c4	6	4.5-14.8	inversion	s	50.847	77.055	91.067	H	0.44	0.35
c4	7	71	e746	p	0.2515	0.2241	0.1964	L	0.22	0.5
c4	10	22-26	e846	p	0.1632	0.2215	0.2773	H	0.41	0.49
c4	10	22-26	e846	s	64.485	79.443	71.889	NA	OVERDOM	
c4	10	50.15	e27	n	1082.7	1948.5	2293.9	H	0.53	0.29
c4	10	97.6	e465	p	0.1787	0.2327	0.2603	H	0.31	0.34
c4	11	63-65	driver	n	1316	1822	3091.1	H	0.57	0.71
c4	11	63-65	driver	p	0.1773	0.2226	0.332	H	0.47	0.71

Table S3: The base calls at 10 IM lines are reported for each location (reference base=0, alternative base = 1, no call = .). (.zip, 4 MB)

Available for download as a .zip file at :

http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.183566/-/DC1/TableS3.zip

File S1: Supplemental Methods

We used QTL Cartographer 2.5 (Wang et al 2007) for the initial mapping of QTLs by composite interval mapping (Zeng 1994). Model selection for background markers in QTL cartographer proceeded by forward backward selection with window sizes of 5 cM, 10 cM , and 15 cM and background marker numbers 15, 5, 10 for c3, c2, c4 respectively. We ran 1000 permutations per trait to get a genome wide 5% threshold (generally LR=14-17) and identified significant QTLs for each trait in each cross (results not presented). The QTL cartographer CIM results were used to set the prior in Bayesian mapping as implemented in Rqtlbim (Yandell et al 2007, Yi et al 2007), and each trait was analyzed singly. In the Bayesian mapping framework, Markov chain Monte Carlo (MCMC) samples are drawn to estimate the posterior distribution of the genetic architecture of the trait (i.e. the number of loci, their locations, effects at each locus, epistatic interactions). The "best" model is estimated from the entire posterior sample by weighting each model with its posterior probability. The strength of evidence of inclusion of a particular QTL in the model can be seen by the Bayes factor for a locus, or the ratio of posterior to prior odds for the model with and without that QTL (by convention, 2logBF = 2.1 is "high"). The estimated model is further refined in an ANOVA framework by stepwise backward elimination of QTLs, and only significant QTLs ($p<0.05$) were retained. Using model selection tools in Rqtlbim, we then did explicit comparisons of the resulting model with reduced models where the QTL with the lowest p-value and/or any epistatic interactions were dropped. MCMC runs were assessed for convergence and standard defaults sufficed. While this is simply observation and not a formal test for pleiotropy, QTLs were called as pleiotropic if the 50% highest posterior probability regions overlapped by containing one shared marker.

File S2: Analysis of the resulting VCF file using 13 custom python scripts. (.tar, 110 KB)

Available for download as a .tar file at:

http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.183566/-/DC1/FileS2.tar