Fitness variation is generated by a segregating inversion 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. They 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 F2 populations resulting from crosses among IM plants. In each case, the F1 parent was heterozygous for a derived haplotype, homogenous across markers spanning over 5 Mb of chromosome 6. In the three F2 populations, inv6 reduced male and female fitness components. In addition, inv6 carriers suffered a ~30% loss of pollen viability in the field. Despite these costs, inv6 exists at moderate frequency (~8%) in the natural population, suggesting counter-balancing fitness benefits that maintain the polymorphism. Across four 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 (5)
inbreeding depression, polymorphism, variation, chromosomal rearrangement, *Mimulus guttatus*
Polymorphic chromosomal rearrangements may contribute importantly to genetic variation within populations. Here, we characterize a segregating inversion (inv6) that generates fitness variation in a natural population of *Mimulus guttatus* (yellow monkeyflower). Greenhouse studies demonstrate negative inv6 effects on male and female fitness components. Despite these costs, inv6 exists at moderate and apparently stable frequency (~8%) in nature. Four years of field data indicate significant positive effects on female fecundity. Opposing fitness effects apparently maintain an intermediate frequency, and as a consequence, inv6 generates substantial genetic variance in fitness.


Introduction

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; Fang et al. 2012; Cheng et al. 2012). Similarly, putatively adaptive trait differences co-segregate 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 co-adapted alleles, each set being optimal under different conditions (Dobzhansky 1970). This hypothesis may account for segregating “supergenes” such as those determining mating types in heterostyloous 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 & Barton, 2006). Recessive or partially 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, which spans over five megabases on the upper end of chromosome 6, in 2003. The results of that study, presented for the first time in this paper, 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 QTLs for multiple fitness components to *inv6* in each of three F₂ 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. Third, we 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. Fourth, 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 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 strong positive 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 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 comprised of highly polytypic, partially inter-fertile subspecies (Vickery 1978; Wu et al. 2007). We focus on a population located on Iron Mountain (IM) in the Western Cascades of Oregon. The site is an alpine xeric meadow composed of a steep north facing slope at an elevation of 1470 m, over an area of ~600m². 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 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 (bi-directional 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 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 HH, HL, or LL 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 with later harvest and counting of seed to estimate supplemented seed set. We used a common pollen donor (IM62, a standard inbred line derived from Iron Mountain) for all flowers. Pollen number and viability was 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 $c_2$, $c_3$, and $c_4$, respectively, and extracted DNA using a CTAB procedure (Kelly and Willis 1998).

Nearly all of the markers used for this study (prefixed with MgSTS – *M. guttatus* sequence tagged site – or simply “e” – for expressed sequence tag) are exon-primed markers spanning introns (MgSTS markers are available from [www.mimulusevolution.org](http://www.mimulusevolution.org)). To identify informative markers in each cross, the High and Low outbred parents and progenitor individuals were screened for 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 and Willis 2005) 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 co-dominant and single copy allowing genetics 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 2s to the estimated length of each
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.

**Quantitative Trait Locus (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 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, both in 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) indicate 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
re-sequenced an additional inv6 line (IM664). We extracted genomic DNA from a single plant
of IM664 using a CTAB extraction protocol (Kelly & Willis 1999) 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, CA), following
standard protocols. The IM664 library was sequenced (along with 19 other libraries unrelated to
this project) in one 150bp PE run of Illumina NextSeq.

Sequence data from IM664 was combined with the data from the nine previously sequenced IM
takes (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 ten lines simultaneously using the UnifiedGenotyper of GATK v2.5
(https://www.broadinstitute.org/gatk/). We analyzed the resulting VCF file using custom python
scripts (Supplementary Materials). We filtered SNPs to include only sites within coding regions
of the 14 main chromosomes (3,257,125bp of the genome) with a minimum mapping quality
score of 30 or greater. 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 50kb windows.

Field measures of male and female fitness components – In 2007, we collected the pollen from all four anthers of newly opened flowers (1 per plant) at IM, and then harvested plants for later DNA extraction (Fishman & Saunders 2008). Pollen was stained with aniline blue, and a subset of fertile and sterile grains counted using a haemocytometer. 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 at the Iron Mountain population, 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 only collected 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 (one *inv6*-diagnostic 236bp allele at e423) 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 a main effects. For the first
three traits, we used an over-dispersed 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 $\chi^2$, $P = 0.61$). There were never significant year x 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: 1-2 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 ask 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 x
quality interaction.

RESULTS

Discovery of inv6 region by recombination suppression—All three of the replicate High x Low
F2 genetic maps (Figure S1) revealed a large cluster of markers on chromosome 6
that appeared to be completely linked. This non-recombining 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 IM79xIM767 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 x Low F\textsuperscript{2} segregated for a particular shared haplotype (i.e. a specific set of alleles) at contiguous MgSTS loci across the recombination-suppressed region. \textit{inv6} was contributed by the Low grandparent (small flower size) in c2, but from the High grandparents in c3 and c4.

\textbf{QTLs for fitness traits map to Inv6—} \textit{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\textsubscript{2, 369} = 11.26, \(P < 0.001\)), pollen viability (proportion of grains viable; F\textsubscript{2,369} = 40.32, \(P < 0.001\)) and supplemented seedset (female reproductive capacity; F\textsubscript{2, 364} = 14.87, \(P < 0.001\)) per flower. For all three measurements, gene action was recessive, with the negative effect limited to \textit{inv6} homozygotes (Figure 2). In c3, there were no effects on female fertility, but a significant negative effect on pollen viability (F\textsubscript{2, 379} = 9.43, \(P < 0.001\)). Gene action is more nearly additive in c3 (Figure 2). In c4, \textit{Inv6} had strongly deleterious effects on female fertility (F\textsubscript{2, 343} = 10.40, \(P < 0.001\)), total pollen (F\textsubscript{2, 345} = 10.04, \(P < 0.001\)), and pollen viability (F\textsubscript{2, 345} = 92.67, \(P < 0.001\)). Heterozygotes were intermediate for all three measurements in c4; \textit{inv6} was partially recessive its effects on female fertility and total pollen but slightly dominant for pollen viability.

The heterogeneity of \textit{inv6} effects among crosses could be due to variation in the non-\textit{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 QTLs for pollen number or pollen viability in other parts of the genome, as well as two QTLs 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 QTLs exhibit intermediate dominance, but we did map one under-dominant QTL (for viable pollen number) and three over-dominant QTLs (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 QTLs ($\text{inv}6$ and $D$) as well as the over/under-dominant QTLs, 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 QTLs were always segregating in only one of the three crosses, suggests they may be deleterious alleles segregating at low frequencies in the natural population.

**Frequency of $\text{inv}6$ in nature**—In the Zia-1 base population, which was derived via two generations of hand-outcrossing from a wild IM sample of over 1000 plants, the $\text{inv}6$ haplotype had an overall frequency of 15\% (Figure 3). In the flowering plants sampled from the field, the estimated frequency of $\text{inv}6$ was slightly lower (7-8\%, see next section). Allelic variation in this population suggests that $\text{inv}6$ is a derived haplotype, as it shares alleles with the alternative arrangement at many loci. However, in this sample, $\text{inv}6$ 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 a region of low variation. Average divergence among standard lines (grey points in Figure 4) is greater than the genome wide average $\pi = 0.0063$ for all lines; 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 versus IM835, the overall non-chromosome 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 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 non-trivial 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 non-carriers (*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 observed frequency of *inv6* (8% in the 2007 sample of wild plants) extremely unlikely without counterbalancing benefits. Second, we examined effects of *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 two years in
which we had flower counts (2012 and 2013), *inv6* carriers produced significantly more flowers
than alternative genotypes (GLM, LR $X^2 = 7.63; P = 0.006, N = 889$). In those two years,
increased flower production translated into increased fruit set ($P = 0.04$); however, the effect on
fruit number was marginally non-significant across all four years ($P = 0.12, N = 1278$). In
contrast to the greenhouse experiments, we saw no *inv6* effect on seeds per fruit ($P = 0.78, N =
1278$). However, across all four years, there was a significant positive effect of *inv6* on log(total
seeds) ($P = 0.047$). Although the year x *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 x *inv6* interaction ($P = 0.02$), as well as a significant effect of *inv6* ($P = 0.03$), in the full
model.

**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 *Mimulus guttatus* (yellow monkeyflower). We
identified inv6 on the basis of no recombination among markers on chromosome 6 spanning over 40cM 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 about 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 (ca. 2% genome wide) and there is no evidence of a population bottleneck that could have recently inflated the frequency of an unconditionally deleterious mutation (Puzey, Willis, and Kelly, unpublished manuscript). Although we do not yet know the full phenotypic effects of genes within inv6, the synthesis of data from this and several other experiments provide a partial explanation for this paradoxical abundance. Hardy-Weinberg proportions are immediately relevant to this explanation. In the field, there are 10-20x 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 F2, 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 (Figures 3-4). Previous experiments conducted on the IM population corroborate average recessive deleterious effects for inv6. Willis (1999b) initiated over 1000 independent inbred lines starting each from an outbred IM plant. Scoville et al. (2009) genotyped 138 of these lines after 6 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 F2 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 5 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 (Figures 5-6) 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 non-carriers. 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 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 about 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 also 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 $2\mu T_{mrca}$ (Tajima and Nei 1983), where $\mu$ is the mutation rate per generation and $T_{mrca}$ is the number of generation 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 about 1500 times the $T_{mrca}$ of the two *inv6* sequences. This may 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 implied by 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 *inv6* given conflicting positive and negative effects. Moreover, a balanced polymorphism does even necessarily predict a fixed allele frequency equilibrium. The fluctuating selection suggested by Figure 6 does not predict a fixed equilibrium.

**The 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 over 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
mutation-selection balance of 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 a ~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 \( \text{inv6} \), the data from the replicated F\(_2\) experiment are mainly consistent with the dominance model; inbreeding depression maintained by mutation-selection balance. We mapped over 20 addition QTLs 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 QTLs did exhibit apparent overdominance. However, these QTLs 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 QTLs 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 \( h \approx 0.15 \) (Willis 1999a). It is likely that many additional deleterious mutations are segregating in IM, but are yet unmapped in 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 reveal 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 a 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 1) greater than its disadvantageous
effects in the heterozygote and 2) 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 \textit{inv6} are presently unknown. The inverted genomic region contains over 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 \textit{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 \textit{inv6} underlie its beneficial effects. Linked with deleterious recessive alleles, \textit{inv6} could represent a “supergene with baggage.” Epistasis for fitness-related traits is common in \textit{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 \textit{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. Kupper et al. (2015). Much remains to be learned.

Acknowledgements

We thank Arpiar Saunders, Tyler Huggins, Angela Stathos, Dan Crowser, Becky Fletcher, Katie Zarn, and Mariah McIntosh for assistance with field collections, plus counting of pollen and seeds and genotyping of markers. The research was supported by grant NIH GM073990 to JK and JW and by NSF DEB-0918902 to LF.
Cited:


Fishman, L., J. H. Willis, C. A. Wu, and Y. W. Lee. 2014. Comparative linkage maps suggest that fission, not polyploidy, underlies near-doubling of chromosome number within monkeyflowers (Mimulus; Phrymaceae). Heredity 112:562-568.


Willis, J. H. 1999b. The role of genes of large effect on inbreeding depression in Mimulus guttatus. Evolution 53:1678-1691.


Figure 1. Comparative linkage mapping of the upper end of chromosome 6 in Iron Mountain *Mimulus guttatus* hybrids. In a freely recombining cross (left: IM179xIM767 F$_2$; n = 86), this region spans ~40cM, whereas recombination is highly suppressed in all three F$_2$ mapping populations segregating for the inv6 haplotype (right; c3 map shown). Marker names to right, cM to left of bar. Shared markers are highlighted (red, italic).
Figure 2. Effect of inv6 genotype on pollen viability (mean +/- 1 SE) and supplemented seed set within each of the F2 mapping populations.
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 = missing data, and three non-inv6 genotypes (likely genotyping errors or double crossovers) within the inv6 block are shown in green.
Figure 4. The average pairwise sequence divergence ($\pi / \text{bp}$) is reported across chromosome 6 for three distinct contrasts: blue = inv6/inv6, orange = inv6/standard, and grey = standard/standard. Points are based on a 1 mb moving average (each is calculated by averaging $\pi / \text{bp}$ estimates from contiguous 50kb windows). The absence of data from 9-11mb corresponds to the putative centromere.
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 four-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).