On the nature of genetic variation in yellow monkeyflower (*Mimulus guttatus*): combining theoretical and empirical approaches

By
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On the nature of genetic variation in yellow monkeyflower (*Mimulus guttatus)*: combining theoretical and empirical approaches

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Abstract

Evolution via natural selection requires standing variation in a population while simultaneously diminishing it at loci under selection, a paradox that continues to vex evolutionary biologists. The work presented here contributes to our understanding of how variation can be maintained by selection, why decreasing genetic variation is detrimental on an individual level, and how genetic variation can have complicated effects on fitness-related phenotypes. Chapter one demonstrates that antagonistic pleiotropy caused by a fitness tradeoff between polygenic traits can maintain variation at a single locus by generating emergent overdominance. The results are surprisingly robust to perturbations in the underlying assumptions about random mating and environmental heterogeneity. Chapter two provides an explanation for how mating system can influence the maintenance of variation. Inbreeding depression in the Iron Mountain population of yellow monkeyflower (*Mimulus guttatus*) is potentially severe enough to maintain outcrossing and is also predicted by the load of rare alleles carried by a genotype. Chapter three illustrates the complex relationships between genotype and phenotype that are likely underappreciated in typical QTL mapping or GWAS approaches, due to tightly linked antagonistic effectors. It provides a mechanism for studying the influence of genetic variation on complex fitness-related phenotypes, like flower size.

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Introduction

One fundamental paradox in the theory of evolution is that natural selection requires standing variation in a population but diminishes that variation at loci under selection. New mutation and migration are not sufficient to guarantee enough standing variation for a population to either adapt to a changing environment or colonize a new one (Lewontin 1974).

Prior to the development of DNA sequencing, and its subsequent widespread use in diagnosing genetic variants in populations, three theories arose to predict the presence and extent of variation and allelic diversity we should expect. Those subscribing to the Classical school of population genetics expected genetic polymorphism to be a rare occurrence, and a transient property of a locus under selection (Muller 1950). In this case, selection would serve primarily to eliminate new deleterious mutations. Opposed to this view were the proponents of the Balance school, which predicted instead that heterozygosity within individuals and polymorphism within populations would be the norm (Dobzhansky 1955; Wallace 1958). Theodosius Dobzhansky, among others, suspected that selection should actively maintain allelic variation, based on the prediction that heterozygotes should be superior (a phenomenon called overdominance). Dobzhansky suggested that observing frequent signatures of balancing selection, measured by the preponderance of overdominance of mutant alleles, would support the Balance school of population genetics (Dobzhansky 1950). Certainly, there exists no shortage of examples of balancing selection of various types, including overdominance, in plants (Delph and Kelly 2014). This has many important consequences. In phylogenetics for example, the existence of balanced polymorphism in ancestral populations could also result in discordance between gene trees and species trees consistent with incomplete lineage sorting (ILS) (Hahn and Nakhleh 2016). As

such, the conditions appropriate for maintaining long term variation in natural populations are of great interest (Haldane and Jayakar 1963a).

Ambiguous experimental evidence beginning in the mid-1900's (Dobzhansky and Spassky 1953, 1954; Morton et al. 1956a; Greenberg and Crow 1960; Dobzhansky et al. 1963; Mukai et al. 1964, 1965), led to the rise in popularity of the Neutral Theory, which remains a favorite explanation for the in-between level of polymorphism typically observed. Under a model of neutral evolution, the majority of new mutations are neither substantially deleterious to be immediately removed by selection, nor adequately beneficial in heterozygous form to be maintained (Kimura 1983). The ultimate fate of most new mutations is random. While the neutral model suggests that most genetic variation present in natural populations is the result of random fluctuations in allele frequencies at neutrally evolving loci (called genetic drift), it does not prohibit either overdominance or purifying selection.

My dissertation contributes to our understanding of how selection maintains genetic and phenotypic variation in yellow monkeyflower (*Mimulus guttatus*). Chapter one is a theoretical demonstration that antagonistic pleiotropy can be a mechanism for balancing selection. Antagonistic pleiotropy is sufficient to maintain variation at a locus affecting the trade-off between flower size and fecundity without the unreasonably restrictive conditions, such as trait-specific dominance, that emerged from mathematical studies like Rose (1982). In the case of the Iron Mountain population of monkeyflower, overdominance emerges from the biology of the system, especially the pressure from the short growing season. The work in chapter two demonstrates that selection for outcrossing, which can generate new combinations of

polymorphisms, is facilitated by inbreeding depression. Further, it suggests that migration and local adaptation pair to maintain standing genetic variation, which could be an important source of variation, particularly in a changing climate. Chapter three provides evidence that a small part of the genome can affect traits like gene expression and life-history phenotypes in a strikingly complicated way. Importantly, this means that complex traits are governed by a much larger set of loci, which could all be targets for balancing selection, or for de novo mutations contributing variation that affects fitness.

Chapter 1: Antagonistic pleiotropy can maintain fitness variation in annual plants

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Abstract

Antagonistic pleiotropy (AP) is a genetic tradeoff between different fitness components. In annual plants, a tradeoff between days to flower (DTF) and reproductive capacity often determines how many individuals survive to flower in a short growing season, and also influences the seed set of survivors. We develop a model of viability and fecundity selection informed by many experiments on the yellow monkeyflower, *Mimulus guttatus*, but applicable to many annual species. A viability/fecundity tradeoff maintains stable polymorphism under surprisingly general conditions. We also introduce both spatial heterogeneity and temporal stochasticity in environmental parameters. Neither is necessary for polymorphism, but spatial heterogeneity allows polymorphism while also generating the often observed non-negative correlations in fitness components.

Introduction

Antagonistic pleiotropy (AP) occurs when a single gene affects multiple fitness components of an organism with conflicting effects on each (Caspari 1950; Williams 1957; Wallace 1958). For example, an allele that increases early life reproductive success might also reduce lifespan (Rose 1984; Sgrò and Partridge 1999; Leroi et al. 2005). Direct evidence for AP at the gene level is limited, although there are some compelling examples including disease alleles in humans (Carter and Nguyen 2011) and parasite defense in *Drosophila* (Hodges et al. 2013). AP has great potential as a mechanism for the maintenance of polymorphism because allocation tradeoffs naturally emerge through growth and development (Lande 1980; Stearns 1989).

Despite the intuitive appeal of AP, its general importance has been questioned on *two* major points, one theoretical and one empirical. Theoretical models predict that AP will lead to stable polymorphism only if it generates overdominance for *total fitness* (Rose 1982; Hedrick 1999). Heterozygotes that are intermediate in their effects on fitness components, say viability and fecundity, can still be superior for total fitness. However, this kind of emergent overdominance requires the apparently restrictive condition of trait-specific dominance (Rose 1982; Curtsinger et al. 1994; Hedrick 1999; Van Dooren 2006). For each fitness component affected by a locus, the heterozygotes must be closer to the more-fit homozygote. Why dominance should change from one affected fitness component to another is unclear, and thus so is the importance of AP as a mechanism for balancing selection (Hodges et al. 2013).

Empirically, correlations between measured fitness components are often non-negative, which seems inconsistent with AP as a cause of fitness variation. Many studies have found positive or

nonsignificant correlations between fitness components (Schemske 1984; Futuyma and Philippi 1987; Jaenike 1989; Ennos and Swales 1991; Carr and Fenster 1994; Fenster and Ritland 1994; Hall and Willis 2006). Locus-specific negative correlations generated by AP may be masked by other factors. Spatial heterogeneity in the environment or variation in general vigor can produce positive or nonsignificant correlations (Houle 1991; Fry 1993; Réale et al. 2003). Some individuals are just "lucky" in that a favorable environment affords them higher values for every measured fitness component (Rowe and Houle 1996; Ehrlén and Münzbergová 2009; Forrest 2014). Conditional fitness in natural populations can make identifying tradeoffs at the genetic level challenging.

In this paper, we develop a model that addresses both theoretical and empirical objections to AP. We consider flowering phenology in short-lived plants as a model. This trait naturally generates antagonistic pleiotropy owing to an inherent tradeoff between resource allocation to growth and reproductive effort. This can manifest as a survival/fecundity tradeoff in plants with a short growing season. Truncation selection on the rate of progression to flowering arises from a "hard" end to the growing season, where all individuals die owing to a severe stress such as drought or frost. Early flowering plants are then favored by viability selection. In contrast, reproductive capacity increases with size, which increases exponentially in time, conferring higher fecundity on later flowering plants. While gene action is specified in terms of effects on a quantitative trait (Days To Flower, DTF), the alternative alleles affect different components of fitness in opposing ways. Our flowering time model thus generates AP, at least as the term is typically used in evolutionary biology (e.g. aging (Williams 1957), sympatric speciation (Berlocher and Feder

2002), barriers to adaptation (Scarcelli et al. 2007), and human disease alleles (Carter and Nguyen 2011).

The specific features of our model are developed from genetic and field studies of the yellow monkeyflower, *Mimulus guttatus*, which provide multi-faceted support for a genetically determined tradeoff between timing of anthesis and fecundity (Kelly 2003a; Kelly and Arathi 2003; Kelly 2008; Mojica and Kelly 2010; Mojica et al. 2012a; Monnahan and Kelly 2015). In particular, mapping studies have identified individual loci that exhibit AP between DTF and fecundity (Scoville et al. 2011b; Mojica et al. 2012a). DTF is the primary determinant of the fitness component survival to flowering (survivorship). Growing season length is limited in alpine populations by summer drought and many plants fail to flower before dying. However, delayed flowering allows plants to produce larger flowers with greater fecundity, another component of fitness (Kelly 2008; Mojica and Kelly 2010). This system creates a tradeoff between two strategies: 1) a bet-hedging strategy involving flowering early, being more likely to survive to flower, and producing fewer seeds, and 2) a risk-taking strategy involving waiting later to flower, being less likely to survive to flower, and producing more seeds. The two components of fitness affected by DTF loci, survivorship and fecundity, represent a tradeoff on an individual plant level. Beyond Mimulus, correlations between fitness and phenology have been demonstrated in many other plants, including members of *Lactuca, Geranium, Rhododendron, Arabidopsis,* and *Boechera* (Marks and Prince 1981; Roach 1986; Kudo 1993; Mckay et al. 2003; Weinig et al. 2003; Juenger et al. 2005; Anderson et al. 2011; Anderson et al. 2013; Fournier-Level et al. 2013; Lovell et al. 2013; Anderson et al. 2014; Lee et al. 2014).

While our model is derived from observations on monkeyflower biology, its implications are broadly relevant to many species that exhibit fitness tradeoffs related to phenological traits.

The analysis of our model shows that protected polymorphism emerges naturally from the viability/fecundity tradeoff even with additive genetic inheritance of developmental timing. While polymorphism occurs only within a window of environmental parameter space, an evolving population will converge on a local fitness optimum, ensuring polymorphism at equilibrium. Finally, while the model imposes a negative genetic correlation between viability and fecundity, it is fully consistent with the non-negative correlations between (whole plant) fitness components that are often observed in field studies.

Theory

We consider a population with random mating and discrete generations. We initially assume that a single locus influences Days To Flower (DTF) and that DTF subsequently affects the probability that a plant survives to flower before the end of the finite growing season (x) and the fecundity of that plant if it flowers (y). Under this model, the fitness of an individual (F) is given by the product of the two fitness components:

$$F = x * y \tag{1}$$

For an individual plant, x is given by:

$$x = \begin{cases} 1; DDD - DTF \geq 0 \\ 0; DDD - DTF < 0 \end{cases} \tag{2}$$

where DDD is the Dry Down Date, the last day of the year on which a plant can flower and still reproduce prior to death. Fecundity is proportional to plant size. We assume exponential growth until flowering (see discussion and Supplemental Table 1.2 for variations on growth model). Thus, *y* is given by:

$$y = \beta m_0 e^{r*(DTF)} \quad (3)$$

Plant vegetative mass at time of flowering is proportional to seed set by a factor of β, m_0 is the initial vegetative mass, and *r* is the growth rate. We initially treat DDD, β, m_0, and r as constants and then relax this assumption to allow environmental effects on each. Equation 3 is the "big bang" model of plant reproductive allocation; all energy is allocated to growth until DTF, when it is all diverted to reproduction. This is the favored allocation model for short-lived plants (Cole 1954; Wenk and Falster 2015).

We now posit that DTF varies according to a normal distribution with a distinct mean for each genotype. Imagine a single diallelic locus with three genotypes: *AA* (early-flowering homozygote), *AB* (heterozygote), and *BB* (late-flowering homozygote). Letting μ_{ij} denote the mean DTF for diploid genotype *ij*, the average fitness of that genotype is:

$$W_{ij} = \int_0^{DDD} \left(\frac{1}{\sqrt{2\sigma^2\pi}} e^{\frac{-\left(t-\mu_{ij}\right)^2}{2\sigma^2}} \right) (\beta m_0 e^{rt}) dt \quad (4)$$

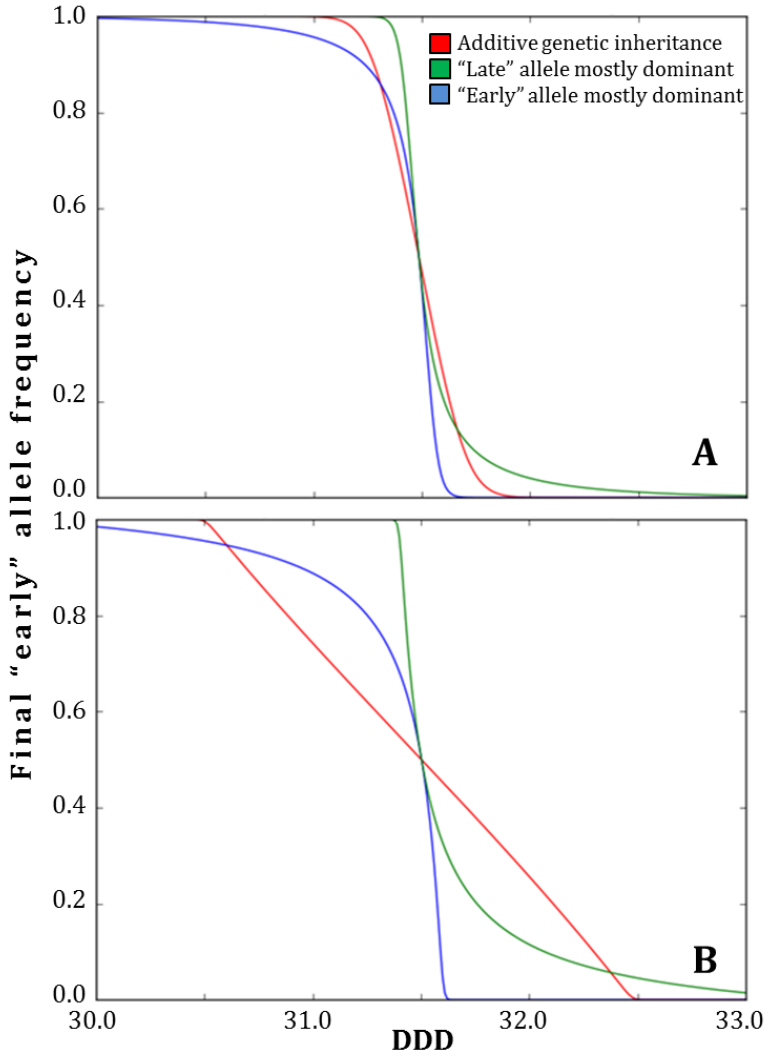
where σ^2 is the variance in DTF within each genotype. We integrate over time between the beginning of the growing season (t = 0, perhaps snow melt or first germination) and the end of

the growing season (t = DDD). This integral captures fecundity of the survivors; truncation selection occurs when drought concludes the growing season (Mojica and Kelly 2010). The fitness of a genotype is the sum of the fecundities of all individuals that survive to flower.

With additive inheritance and no environmental stochasticity, the "early" allele fixes when growing season is short, and the "late" allele fixes when growing season is long. In between, however, there is a span of values for DDD in which both alleles persist (red lines, Figure 1.1). In this region, polymorphism is protected; either allele increases when rare (Supplemental Figure 1.1). Protected polymorphism is due to emergent overdominance where the total heterozygote fitness exceeds that of either homozygote (Supplemental Figure 1.2).

As the difference between the homozygote genotype means (μ_{AA} and μ_{BB}) increases so does the span of growing season lengths that allows polymorphism (Figure 1.1, bottom panel). Partial dominance of either allele creates asymmetry. If the early allele is mostly dominant (i.e. the heterozygote mean, μ_{AB}, is closer to μ_{AA}), polymorphism can persist at shorter growing seasons than with additive genetic variance alone (Figure 1.1, blue lines). The reverse is true when the late allele is mostly dominant.

Figure 1.1 - Early allele frequency after 1000 generations under a range of growing season lengths. (A) An initial set of values for genotype mean DTF. Model parameters are as follows for additive inheritance (red line): AAm = 29.393712, ABm = 29.787871, BBm = 30.18203, σ = 3.85, β = 0.0723244012, m_0 = 2.0, r = 0.5. Green line ABm = 30.1426141. Blue line ABm = 29.4331279. (B) The result of increasing the difference in homozygote mean DTF five-fold. Red line: AAm = 27.817076, ABm = 29.787871, BBm = 31.758666. Green line ABm = 31.5615865. Blue line ABm = 28.0141555. Colored lines differ in whether the heterozygote mean is closer to the early homozygote (blue), late homozygote (green), or exactly intermediate (red).



The cause of emergent overdominance: Genotypic fitnesses are not frequency dependent in this model and polymorphism is stable if the average fitness of heterozygotes exceeds that of either homozygote (overdominance). Overdominance requires satisfying the following inequalities: $W_{AB} > W_{AA}$ and $W_{AB} > W_{BB}$

$$\int_0^{DDD}\left(e^{rt-\frac{(t-\mu_{AB})^2}{2\sigma^2}}\right)dt > \int_0^{DDD}\left(e^{rt-\frac{(t-\mu_{AA})^2}{2\sigma^2}}\right)dt \quad (5)$$

and

$$\int_0^{DDD}\left(e^{rt-\frac{(t-\mu_{AB})^2}{2\sigma^2}}\right)dt > \int_0^{DDD}\left(e^{rt-\frac{(t-\mu_{BB})^2}{2\sigma^2}}\right)dt \quad (6)$$

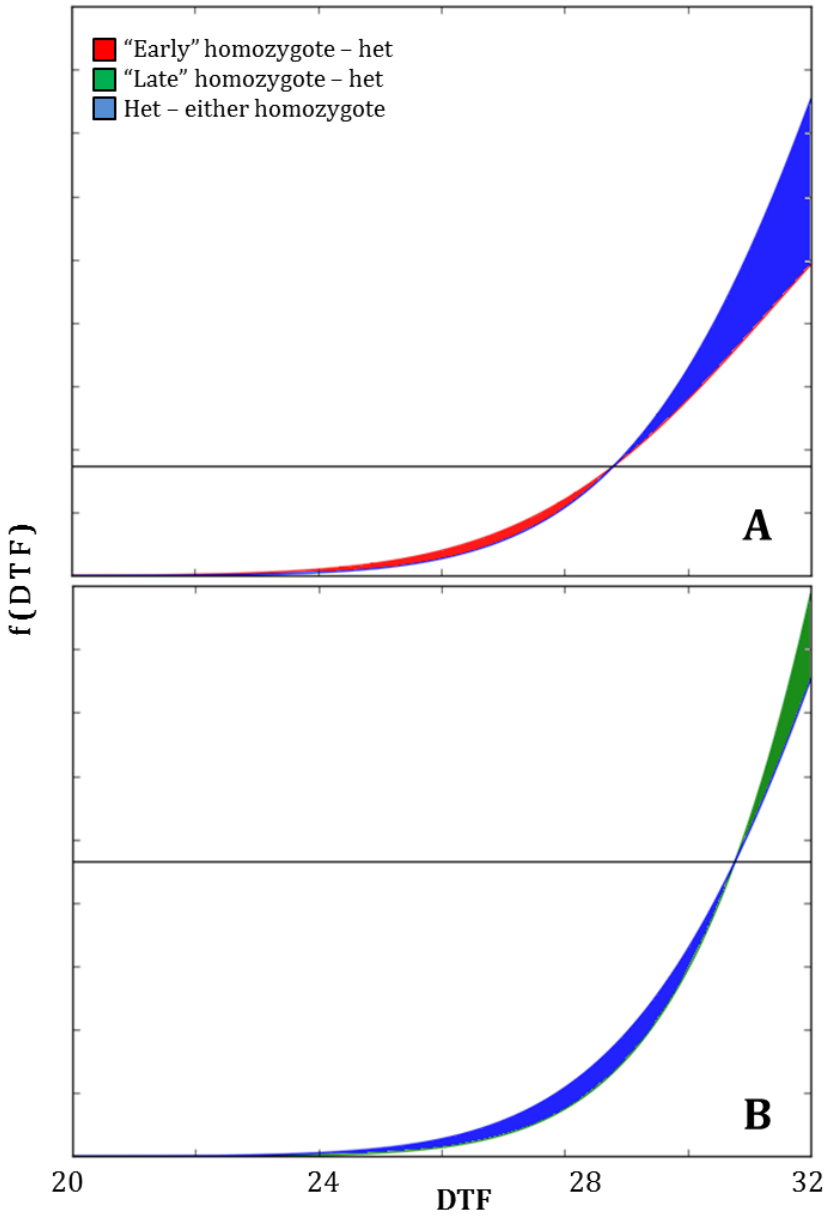
The inequalities above do not have simple, closed form solutions. To understand the conditions where equations 5 and 6 are true, it is useful to view the integrands graphically. Figure 1.2 depicts the functions defined by:

$$f(x) = e^{rt-\frac{(t-\mu_{ij})^2}{2\sigma^2}} \quad (7)$$

for a case with DDD=32, within the region of polymorphism (Figure 1.1b). The shaded areas represent the differences in fitness between two genotypes. The top panel compares the early homozygote to the heterozygote. As expected, the early homozygote enjoys a benefit because more plants reach flowering (red area). However, this is more than offset by the higher average

fecundity of heterozygotes that do flower (blue area exceeds red area). Roles are reversed in the lower panel where we compare the heterozygote to the late homozygote. Late homozygotes that flower do have higher fecundity, but this is not sufficient to overcome the much higher survivorship of heterozygotes (green area is less than blue area). For each fitness component, heterozygotes are intermediate, but with values slightly exceeding the midpoint (Supplemental Figure 1.5).

Figure 1.2- Function plots for equations 5 (A) and 6 (B) at DDD = 32. Shaded areas are the difference in area under the curve between the early homozygote and the het (red), the late homozygote and the het (green), and the het and either homozygote (blue). Parameter values are equivalent to Figure 1 panel B additive inheritance (red line). The black horizontal line indicates where f(DTF) of the either homozygote is equal to the heterozygote.



How likely is polymorphism? Heterozygotes will have the highest (total) fitness within a range of values for DDD (Figure 1.1, also see Supplemental Figure 1.2 for a calculation of this range). If DDD is below the lower bound of this range, the early allele fixes. If DDD is above the lower bound, the late allele fixes. While this range seems fairly narrow, a sequential analysis indicates that a population will "evolve towards" this region of polymorphism given recurrent mutation. If DDD is such that the mean DTF is below the optimum, new mutations that delay flowering will increase in frequency (Supplemental Figure 1.3). Depending on the effect of the new mutation, it may fix or remain stably polymorphic. In the former situation (fixation), the population mean DTF is brought closer to the optimum for that particular DDD and the population can be further invaded by new mutations. The same dynamic of convergence towards the region of polymorphism occurs in the other direction if an invariant population has a mean DTF that is substantially above the optimum (Supplemental Figure 1.3).

These results suggest that a population will evolve to become polymorphic as long as there is available mutation. DTF is a large mutational target and standing flowering time variation is usually polygenic, so variation is not likely to be limiting. To consider the consequences of recurrent mutation when the population is already polymorphic at a DTF locus, we developed a two locus model. The model structure is the same except with a second, unlinked locus affecting DTF (see Supplemental Appendix *S1* for details). The first locus is allowed to reach equilibrium before a new allele (either early or late) is introduced at locus 2. Although we find no circumstances under which both alleles at both loci are stably maintained, the second locus can remain polymorphic for extended periods. Additionally, the persistence time for locus 2 polymorphism is negatively correlated with the magnitude of effect on DTF (Supplemental

Figure 1.7). Fixation of the newly introduced allele at the second locus can disrupt the equilibrium of the first locus, which can stabilize to a new equilibrium (Supplemental Figure 1.7). Even without a complete treatment of the model, we conclude that additional mutations that affect DTF in other loci can exist as polymorphisms for many generations, indicating the possibility for transient heterozygosity in loci under this type of selection.

Allowing environmental effects on parameters: Micro-spatial environmental heterogeneity (within a population) can cause *r*, *b*, or m_0 to vary among plants within a genotype. Allowing *b* or m_0 to vary with environment does not alter the region of polymorphism because coefficients are factored out of the integral in equations 5 and 6. This is not true of *r*. We allowed growth rate to vary across a landscape by simulating 1000 individuals of each genotype and calculating their fecundity. DTF was drawn randomly from the normal distribution unique to each genotype, and the growth rate *r* was drawn randomly from a normal distribution common to all genotypes. When *r* is constant, the positive relationship between DTF and fecundity that the model imposes is unmistakable at the whole plant level (Fig. 3A). However, when *r* varies across a landscape, the relationship becomes nonsignificant (Fig. 3B). Importantly, in the presence of variation in growth rate where a tradeoff between flowering time and fecundity is not apparent, selection still maintains both alleles at intermediate frequency under a range of growing season lengths (panel B inset). Allowing the product $b*m_0$ to vary across a landscape does weaken the correlation between flowering time and fecundity, but not as strongly as a variable growth rate (Supplemental Figure 1.4).

Within a population, DDD necessarily has a single value for a specific year. However, temporal environmental fluctuations can cause DDD to vary among generations through time. We consider environmental fluctuations by choosing DDD randomly from a normal distribution each generation. Increasing the variance of this distribution shifts the region of polymorphism slightly toward longer growing seasons, but does not significantly increase the width of the region (Supplemental Table 1.1). We also simulated high autocorrelation (both positive and negative) in DDD from generation to generation, as might be expected for an environmental variable in time. It does not increase the width of the polymorphic region (Supplemental Table 1.1).

Allowing assortative mating: Our preceding analysis assumed random mating to recursively calculate allele frequency changes between generations. However, variation in flowering time can result in assortative mating (AM). Individuals can only transfer pollen to other plants with flowers open concurrently (Ennos and Dodson 1987; Hartl and Clark 1997; Lynch and Walsh 1998; Conner and Hartl 2004; Hedrick 2011). To consider AM, we revised the model allowing plants only to mate with other individuals that flower at exactly the same time (t). We calculate the production of seeds of each genotype given the proportions of plants flowering at that time and their relative fecundity. This is the most extreme form of AM. In most species, plants will have a window of time in which it can overlap with other plants. In *M. guttatus*, flowers stay open and receptive to pollen for a few days to a few weeks (Dudash and Ritland 1991; Arathi et al. 2002). The AM model (eqs 8-14 below) and the random mating model (eqs 4-6) essentially 'bracket' intermediate levels of assortative mating.

Assortative mating violates Hardy-Weinberg equilibrium and so we write recursions for genotype frequencies, z_{ij}. Each individual that flowers prior to DDD produces zygotes by mating with other individuals that flower at the same moment. The total number of individuals of genotype *ij* that flower at time t is proportional to:

$$F_{ij} = \frac{Z_{ij}}{\sqrt{2\sigma^2\pi}} e^{-\frac{(t-\mu_{ij})^2}{2\sigma^2}} \quad (8)$$

The frequency of the fast allele among flowering plants at time t:

$$P'_t = \frac{F_{AA}+\frac{1}{2}F_{AB}}{F_{AA}+F_{AB}+F_{BB}} \quad (9)$$

Because mating is random among plants that flower at time t, we can then calculate the number of zygotes of each genotype produced at time t using allele frequencies, and integrate over time for the total number of seeds of each genotype produced in a generation:

$$S_{AA} = \int_0^{DDD} n_t P'^2_t \, dt \quad (10)$$

$$S_{AB} = \int_0^{DDD} 2n_t P'_t (1 - P'_t) \, dt \quad (11)$$

$$S_{AA} = \int_0^{DDD} n_t (1 - P'_t)^2 \, dt \quad (12)$$

Where n_t is the time dependent fecundity across all plants flowering:

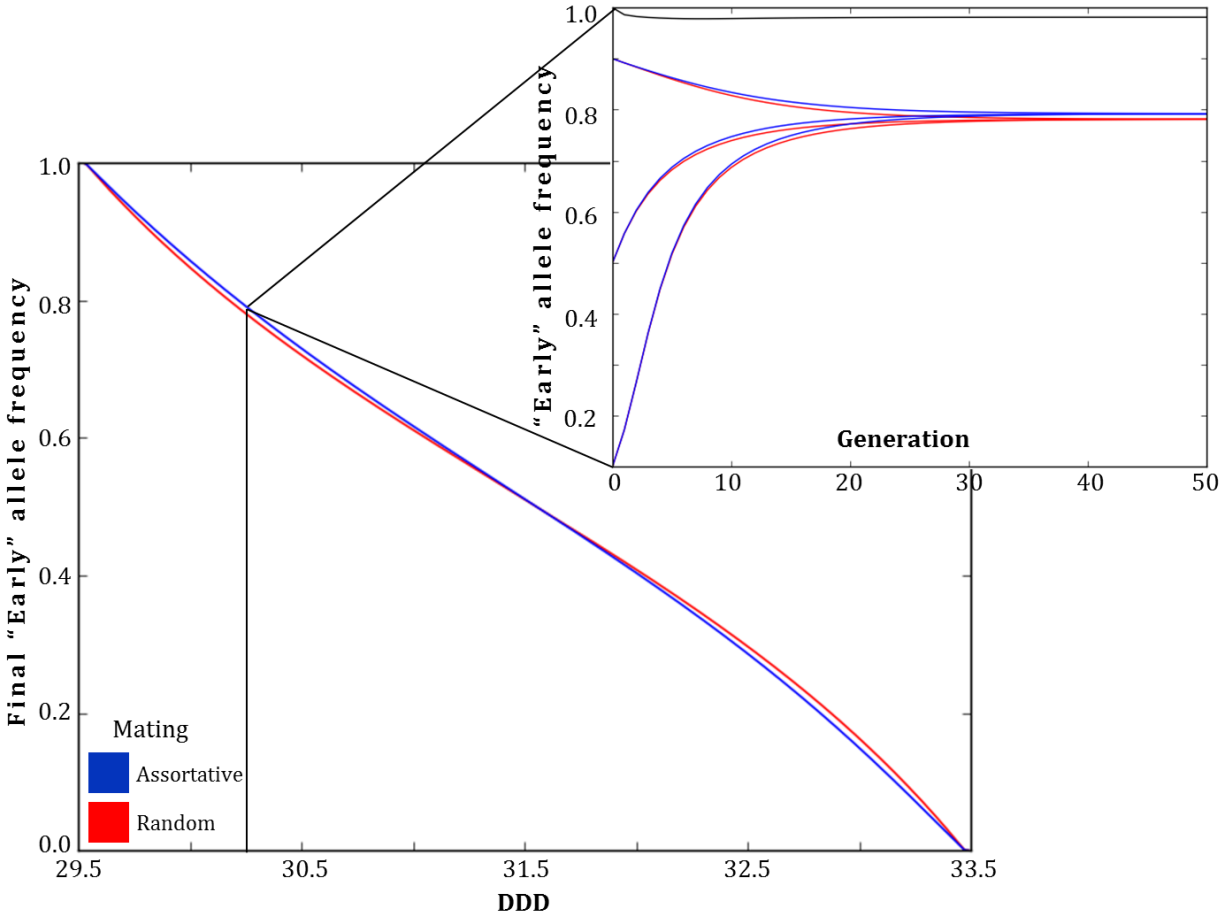
$$n_t = \frac{\beta m_0}{2} e^{rt} (F_{AA} + F_{AB} + F_{BB}) \quad (13)$$

Total seeds are then converted to genotype frequencies as input for the next generation:

$$Z'_{ij} = \frac{S_{ij}}{S_{AA}+S_{AB}+S_{BB}} \quad (14)$$

The difference between AM and random mating models depends on the extent of separation between the flowering time distributions of alternative genotypes. For reasonable parameters values, such as those of Figure 1.1, polymorphism is still stably maintained and the width and position of the region of polymorphism in environmental space is largely unchanged. AM produces a noticeable effect only when we inflate the difference between homozygote means and/or decrease the residual variance (σ^2). In both cases, the equilibrium early allele frequency changes slightly at values for DDD within the region of polymorphism (Figure 1.3). In shorter growing seasons the early allele is more frequent at equilibrium with AM, and in longer growing seasons the late allele is more frequent at equilibrium with AM. Assortative mating should also produce a shortage of heterozygotes relative to what would be expected under Hardy-Weinberg (H-W) equilibrium (Wright 1921; Crow and Kimura 1970). AM does slightly reduce the frequency of heterozygotes (relative to H-W), but by no more than 2% under the parameters we investigated (see black line in Figure 1.3 panel inset, where equilibrium $z_{AB}/2pq = 0.9813$).

Figure 1.3- Comparing equilibrium early allele frequency with random or assortative mating for a range of values for DDD. Panel inset shows allele frequency through time at DDD = 30.25, with variable starting allele frequency. Black line in panel inset is $z_{AB}/2pq$ (the observed frequency of heterozygotes divided by the expected frequency under Hardy-Weinberg) when initial p = 0.1. Model parameters are as follows: AAm = 25.846281, ABm = 29.787871, BBm = 33.729461, σ = 3.85, r = 0.5 (β and m_0 are omitted from this model). The difference in homozygote genotype means is 10-fold that of Figure 1.1 panel A.



Discussion

Antagonistic pleiotropy (AP) is substantially vindicated as a potential mechanism for maintaining polymorphism through a model based explicitly on the flowering phenology of annual plants. Fitness-component specific dominance, previously considered a restrictive condition, emerges naturally from our model, even with strictly additive gene action on the rate of development. Dominance in survival and reproduction is generated by the ecology of the system. While the details of the model are motivated by results from *Mimulus guttatus*, the underlying phenomenon may be much more broadly applicable. Many species confront a 'hard' end to the life cycle that produces conflicting pressures on different fitness components, such as annual plants, organisms inhabiting ephemeral habitats such as vernal pools, and univoltine insects (Mulroy and Rundel 1977; Simovich and Hathaway 1997; Johansson and Rowe 1999).

The model posits that alleles that delay flowering afford a plant greater fecundity if it survives to flower. Drought concludes the growing season in many species; most obviously for desert plants adapted to brief periods after rain (Sharitz and McCormick 1973; Mulroy and Rundel 1977; Kemp 1983), but also for annuals growing in 'Mediterranean climates' present in Europe, Africa, Australia, and sections of North and South America (diCastri and Mooney 1973). In these populations, flowering phenology determines whether an individual survives to reproduce before desiccation. Numerous experiments on alpine populations of *Mimulus guttatus* indicate a genetic correlation between flowering phenology, viability, and fecundity (Kelly 2003a; Kelly and Arathi 2003; Kelly 2008; Mojica and Kelly 2010; Mojica et al. 2012a; Monnahan and Kelly 2015). Directional selection on flower size, which is correlated with reproductive capacity, produces conflicting responses on DTF and fecundity (Kelly 2008; Mojica and Kelly 2010).

Selection for smaller flowers favors alleles that accelerate progression to flowering at the expense of reduced reproductive capacity through both male (pollen) and female (ovules) function. Selection for larger flowers favors alleles that increase allocation towards vegetative growth (size of first few leaf pairs) at the expense of delayed progression to flowering; these plants have much higher reproductive capacity (Table 2 of Kelly (2008)). While these responses reflect the pleiotropic effects of many loci, the tradeoff between developmental speed and size at flowering has also been demonstrated at the scale of individual loci in both laboratory (Scoville et al. 2009) and field experiments (Mojica et al. 2012a; Monnahan and Kelly 2015).

Genetic correlations between flowering phenology, viability, and fecundity have been demonstrated in many other angiosperm species. In *Arabidopsis thaliana*, flowering time QTL exhibit pleiotropic effects on survival and fecundity in both laboratory and field environments (Ward et al. 2012; Fournier-Level et al. 2013). The *Arabidopsis* gene *FRIGIDA* exhibits AP with fast growth alleles conferring drought escape, while later flowering alternatives exhibit "dehydration avoidance" (increased water use efficiency) to reproduce (Lovell et al. 2013)(see also Juenger et al. (2005) and Mckay et al. (2003)). At other flowering time loci, fast alleles reduce resistance to rabbit herbivory, at least under some environmental conditions (Weinig et al. 2003). In *Boechera stricta*, the gene *nFT* exhibits AP; the "Colorado allele" delays flowering but increases leaf number at time of flowering relative to the "Montana allele" (Anderson et al. 2011; Anderson et al. 2013; Anderson et al. 2014; Lee et al. 2014). In a wild lettuce relative, *Lactuca serriola*, seed set is positively correlated with amount of time spent in the vegetative growth (rosette) stage, and survival to reproduction is related to timing of germination (Marks and Prince 1981). In *Geranium carolinianum*, fecundity is positively correlated with adult-stage plant

weight, rosette area, and number of leaves, several measures of vegetative growth (Roach 1986). In *Rhododendron aureum*, a perennial shrub that flowers following snow melt, plants that flower later set more fruit, in part due to better alignment with pollinator availability (Kudo 1993). In summary, flowering phenology, viability, and fecundity are routinely correlated in natural populations.

Emergent overdominance: AP and overdominance are often described as alternative models of balancing selection, but this can be misleading. In a constant environment, AP only maintains stable polymorphism if it generates a kind of overdominance: the overall fitness of the heterozygotes must exceed that of alternative homozygotes (Rose 1982). Gene action is typically described in terms of its effects on the fitness components where heterozygotes have intermediate values. Overdominance emerges only when integrating the numerous pleiotropic effects of a locus into total fitness. Emergent overdominance is responsible for stable polymorphism of early and late flowering time alleles in our model. It occurs even when gene action on flowering time is strictly additive (Figure 1.2). While this addresses a major theoretical objection to AP, it comes with an important empirical caveat. This sort of overdominance is not easily observed under constant laboratory conditions. If one measured the plants of our model grown in the greenhouse (such that all plants flower), there would be no evidence of overdominance for DTF or fecundity. The heterozygote mean for each trait would be intermediate between the two homozygotes, because that is how the model is written. Instead, overdominance is generated by the field-specific selection regime, when survival to flower is not guaranteed.

The dependence on field-specific selection is important to remember when considering the predicted effects of AP on the variance components of fitness (Rose 1982; Charlesworth and Hughes 2000). If AP contributes substantially to variation in fitness, the dominance variance, V_D, should be large (Falconer and Mackay 1996), at least for overall fitness. This prediction would apply to our model, but only if the genetic variance in fitness is estimated and partitioned on plants measured under field conditions. Such an experiment would need to include the invisible fraction, individuals that die before the key trait of DTF is expressed (Grafen 1988; Bennington and McGraw 1995; Sinervo and McAdam 2008; Mojica and Kelly 2010). Interestingly, DTF does exhibit substantial dominance variation for *M. guttatus* plants even when grown under greenhouse conditions suggesting dominance effects even without field-specific selection (Kelly and Arathi 2003).

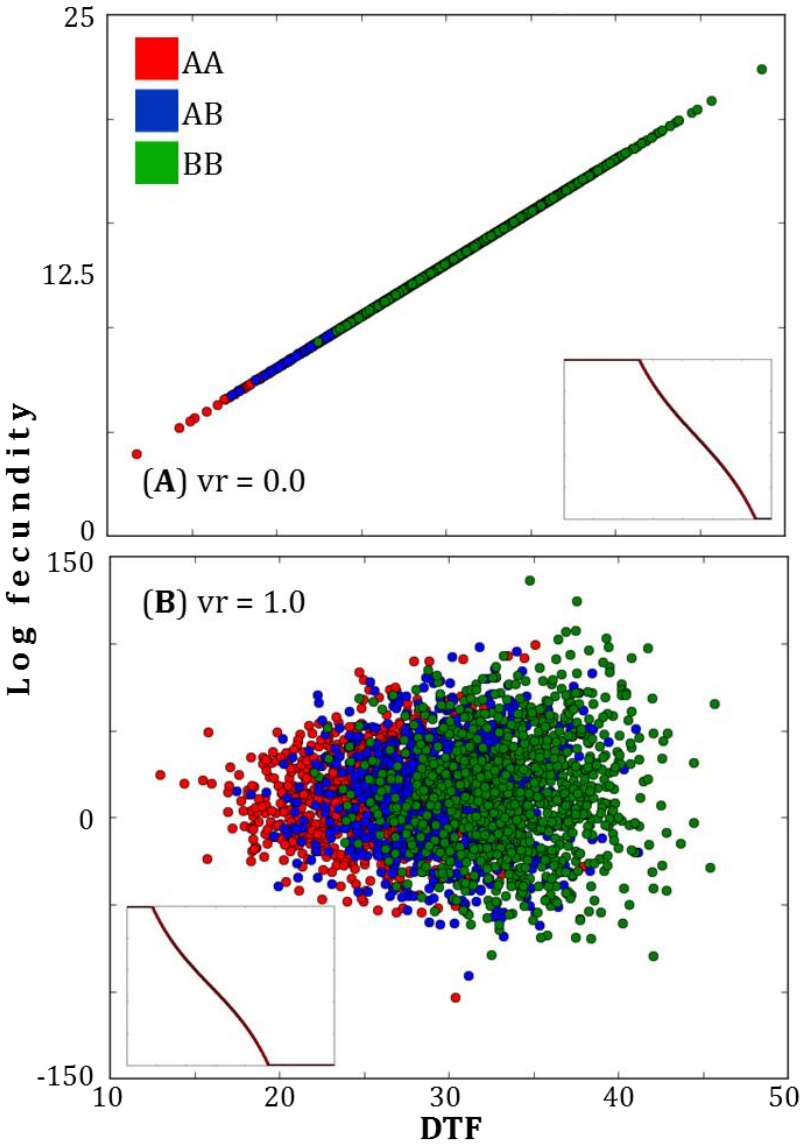
Environmental variation: In our model, emergent overdominance allows protected polymorphism in a constant environment (Supplemental Figure 1.2). Many theoretical studies have generated balancing selection through environmental stochasticity in the form of either spatial heterogeneity or temporal fluctuations (Levene 1953; Dempster 1955; Haldane and Jayakar 1963b; Gulisija and Kim 2015). Interestingly, we find that temporal fluctuations do not seem to increase or decrease the size of the window for polymorphism, but can shift its location towards longer growing seasons (Supplemental Table 1.1). Second, we investigated the effect of spatial heterogeneity by allowing the growth rate to vary between individuals of the same genotype, depending on an organism's micro-environment. In nature, this could be the result of individuals that happen to land close to a water source or in a patch of nitrogen-rich soil, while others might germinate in a shaded, dry patch of depleted soil. This additional variation does not

hinder polymorphism, but does eliminate any observable correlation between DTF and fecundity at the whole plant level (Figure 1.4).

The nonsignificant correlation between DTF and fecundity shown in Figure 1.4 is noteworthy because a routine objection to the resource allocation tradeoff model is the lack of negative correlations in fitness components. While genetic tradeoffs have been observed in birds, mammals, and insects (Roff 1990; Charmantier et al. 2006; Nussey et al. 2008), laboratory-grown *M. guttatus* collected from annual populations in California exhibit conflicting evidence for the tradeoff between rate of progression to flower and reproductive output. Neighboring populations of this species can exhibit both positive and negative correlations between DTF and flower size (Carr and Fenster 1994; Fenster and Carr 1997).

Variation in growth rate across the range of a population invokes an important assumption of the model: exponential growth between germination and flowering. Several previous studies have measured elements of plant growth such as height and total leaf biomass and concluded that growth over the whole life of a plant grown in the greenhouse is more logistic in shape (Farris and Lechowicz 1990; Overman and Scholtz 2013; Weraduwage et al. 2015). A realistic assumption, then, is that flowering should occur in nature during the exponential phase of growth. However, this need not be the case and it is not critical for the maintenance of variation under our model. We found that polymorphism is also protected with linear or logistic growth (Supplemental Table 1.2). With linear growth, the region widens slightly, and shifts to longer growing season lengths. It is also unaffected by changes in the growth rate, unlike the exponential growth model.

Figure 1.4- Fecundity of 1000 individuals of each genotype with DTF and r drawn from normal distributions. Variance in r differs between A (vr = 0.0) and B (vr = 1.0) panels. Insets show the final "early" allele frequency in a range of DDD between 27 and 34. Note that the large panels' x-axes extend beyond the largest value for DDD that sustains polymorphism. This demonstrates the case where every individual flowers. All other parameter values are the same as in Figure 1.1 panel A additive inheritance (red line).



A second general explanation for positive fitness correlations cites "general vigor," a selection component that several groups have attempted to factor out by measuring fitness in different environments (Futuyma and Philippi 1987; Jaenike 1989; Fry 1993). Individuals that are simply better adapted to their environments on a genomic level will outperform individuals that are poorly adapted, regardless of genotype at flowering time alleles. Lack of a fitness tradeoff at the organismal level, be it due to luck or vigor, can render allele-level tradeoffs difficult to observe. Mutational load can also mitigate allelic tradeoffs (Spassky et al. 1965; Kimura and Maruyama 1966b; Austen et al. 2017). Particularly in plant populations with some degree of inbreeding, the varying expression of deleterious mutations segregating within a population can alter the correlation between different fitness components. Individuals with a heavy genome-wide load will be less fit across all measured aspects of fitness, resulting in a potentially positive correlation thereof (Charlesworth and Charlesworth 1999; Whitlock and Bourguet 2000). Lastly, nonsignificant or positive correlations in fitness components can also result from variation in resource acquisition traits, instead of variation in resource allocation (Futuyma 1998). For example, an allele that improves carbon fixation efficiency could theoretically produce a positive correlation between survivorship and fecundity.

Model caveats and extensions: A sudden shift in total energy allocation from vegetative growth to reproductive effort at anthesis has been assumed in previous models (King and Roughgarden 1983; Weis et al. 2014). Weis et al. also assumed, as do we here, no loss of vegetative biomass from herbivory or senescence between germination and anthesis, removing the need for such loss terms in the model. Based on field observations from *Mimulus guttatus*, we have assumed truncation selection with a hard cutoff because surviving individuals typically produce only one

or two flowers prior to the end of the growing season (Monnahan and Kelly 2015). However, many annual plants continue to flower between first anthesis and death, accumulating seed set over a period of time. This potential for accrual of seed set is diminished as a plant waits longer to flower (Schemske 1977; O'Neil 1997). Weis et al. (2014) considered this when developing a model that instead accounts for individuals that make it to flower before death, but do not have enough time to set seed. Termed the "tail of zeros," these individuals have high DTF and no fecundity, against the positive correlation assumed for the population. Our model does not oppose this phenomenon; it groups those individuals with others that did not flower in time. The truncated growing season excludes any individuals that did not flower soon enough to set seed.

We also modified our model to consider the slightly different type of selection in Weis et al. (2014). These authors allowed plants to accumulate reproductive output after flowering until the end of the growing season. We substituted this fitness function into our model (see Supplemental Appendix *S2* for details), which replaces our hard-edged truncation selection with declining fitness for very late flowering plants, owing to a decrease in the amount of time remaining to accumulate reproductive output prior to the DDD (Supplemental Figure 1.6). Selection still maintains polymorphism (Supplemental Figure 1.6 inset). This extension of the model suggests that its main predictions regarding the maintenance of polymorphism may be robust to changes in the model of growth and mortality.

Conclusion

Evolution by natural selection relies on the presence of standing variation but acts to diminish it. This conundrum drives the need to understand what forces maintain standing variation in nature

(Mitchell-Olds et al. 2007), and has produced many studies investigating the circumstances under which heterozygosity at a locus is preserved through evolutionary time (Hedrick 1976, 2006; Delph and Kelly 2014; Scotti et al. 2016). Despite criticism stemming from previous theoretical models, our model demonstrates that variation in resource allocation can result in fitness tradeoffs in life-history traits, and those tradeoffs can protect polymorphism, maintaining natural variation.

Supplemental Appendix

Appendix S1.1: 2-locus model recursions

Consider frequencies for genotypes at 2 unlinked loci at generation 0:

$$g_{ij,kl\,(0)} \qquad (s1)$$

Such that *ij* denotes genotype at locus 1, *kl* denotes genotype at locus 2, and *i*, *j*, *k*, and *l* represent either the fast or slow allele at each locus. There are 9 unordered genotypes if there are two alleles at each locus (F or S, for fast or slow):

$g_{FF,FF}$	$g_{FF,FS}$	$g_{FF,SS}$
$g_{FS,FF}$	$g_{FS,FS}$	$g_{FS,SS}$
$g_{SS,FF}$	$g_{SS,FS}$	$g_{SS,SS}$

Because average fitness for a genotype is a function of both survival to reproduce and fecundity, we then convert these genotype frequencies into total average contributions to the next generation through the fitness function:

$$g'_{ij,kl(0)} = g_{ij,kl(0)} * \frac{W_{ij,kl}}{\bar{W}} \qquad (s2)$$

$$W_{ij,kl} = \int_0^{DDD} \left(\frac{1}{\sqrt{2\sigma^2\pi}} e^{\frac{-(t-(U+\alpha_1+\alpha_2))^2}{2\sigma^2}} \right) (\beta m_0 e^{rt}) dt \qquad (s3)$$

In equation *s3*, U denotes some population constant DTF for the genetic background, and α_1 and α_2 give the effects on the mean of locus 1 and 2 respectively. Here we are assuming no epistasis between the two loci.

Given random mating and genetic segregation, we then calculate gamete frequencies from post-selection genotype frequencies:

$$FF = g'_{FF,FF} + \frac{1}{2} g'_{FF,FS} + \frac{1}{2} g'_{FS,FF} + \frac{1}{4} g'_{FS,FS}$$

$$FS = g'_{FF,SS} + \frac{1}{2} g'_{FF,FS} + \frac{1}{2} g'_{FS,SS} + \frac{1}{4} g'_{FS,FS}$$

$$SF = g'_{SS,FF} + \frac{1}{2} g'_{FS,FF} + \frac{1}{2} g'_{SS,FS} + \frac{1}{4} g'_{FS,FS}$$

$$SS = g'_{SS,SS} + \frac{1}{2} g'_{FS,SS} + \frac{1}{2} g'_{SS,FS} + \frac{1}{4} g'_{FS,FS}$$

Here, we have assumed that the two loci are unlinked. Finally, we calculate next generation genotype frequencies from the gametes contributed in the current generation:

$g_{FF,FF}(1)=FF^2$ $g_{FF,FS}(1)=2*FF*FS$ $g_{FF,SS}(1)=FS^2$

$g_{FS,FF}(1)=2*SF*FF$ $g_{FS,FS}(1)=2*FF*SS+2*FS*SF$ $g_{FS,SS}(1)=2*FS*SS$

$g_{SS,FF}(1)=SF^2$ $g_{SS,FS}(1)=2*SF*SS$ $g_{SS,SS}(1)=SS^2$

Appendix S1.2: Weis model extension

Weis et al. (2014) developed a model that accounts for the possibility of seed set accumulation between flowering and the end of the growing season. Under this model, total reproductive output for an individual becomes:

$$w = \beta m_0 \sum_{t=DTF}^{DDD} r e^{r*DTF} \quad (s4)$$

Equation 4 becomes:

$$W_{ij} = \int_0^{DDD} \left(\frac{1}{\sqrt{2\sigma^2\pi}} e^{\frac{-\left(t-\mu_{ij}\right)^2}{2\sigma^2}} \right) (\beta m_0 r * (e^{rt}) * (DDD - t) dt \quad (s5)$$

Equations 5 and 6 become

$$\int_0^{DDD} \left(e^{rt - \frac{(t-\mu_{AB})^2}{2\sigma^2}} * (DDD - t) \right) dt > \int_0^{DDD} \left(e^{rt - \frac{(t-\mu_{AA})^2}{2\sigma^2}} \right) * (DDD - t) dt \quad (s6)$$

And

$$\int_0^{DDD} \left(e^{rt - \frac{(t-\mu_{AB})^2}{2\sigma^2}} \right) * (DDD - t) dt > \int_0^{DDD} \left(e^{rt - \frac{(t-\mu_{BB})^2}{2\sigma^2}} \right) * (DDD - t) dt \quad (s7)$$

Supplemental Figures and Tables

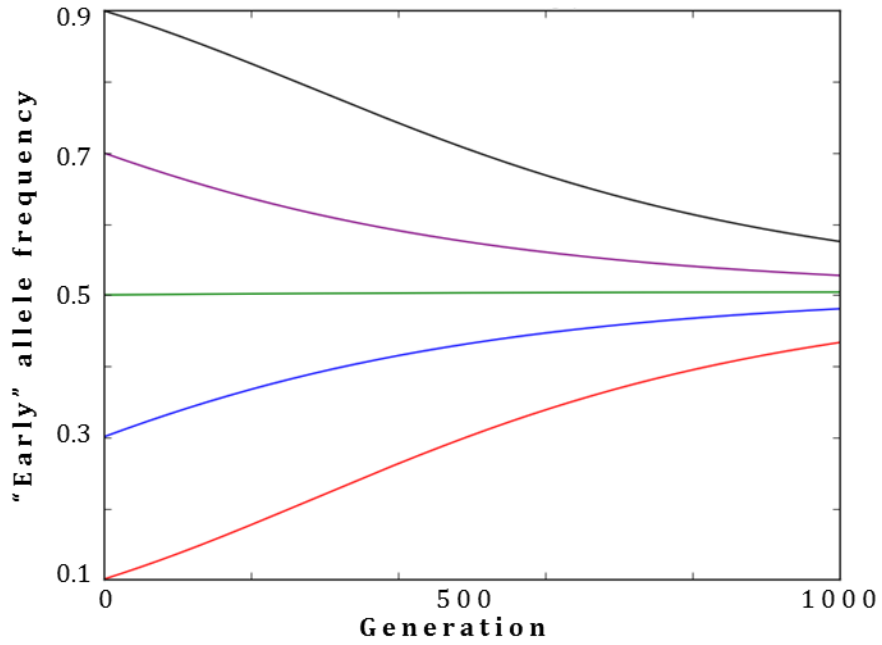


Figure S1.1- The trajectory of the "early" allele frequency through time at DDD = 31.4. Each color demonstrates a different starting allele frequency at generation 0. Either allele increases when rare (best seen in the black and red lines). At different values for DDD, the equilibrium frequency differs, but is always stable. Parameter values are equivalent to Figure 1.1 panel additive inheritance (red line).

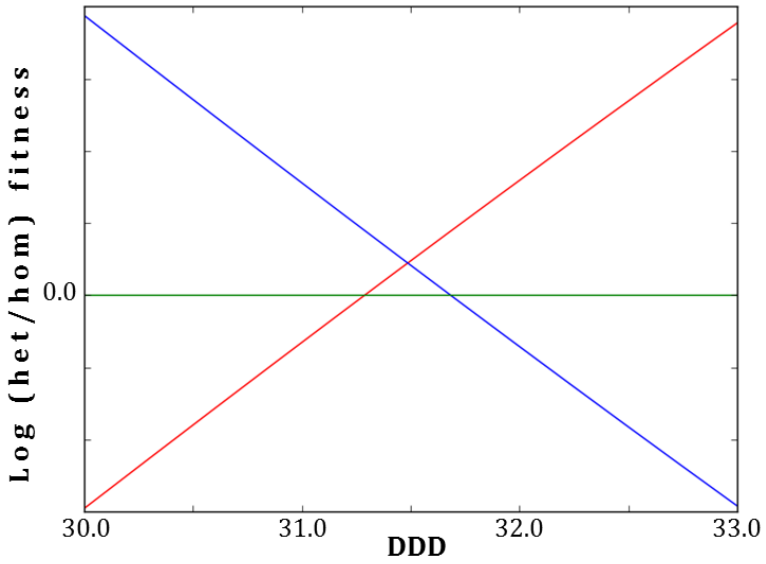


Figure S1.2- Fitness differences between each homozygous genotype (AA or BB) and the heterozygous genotype (AB). Overdominance occurs when the fitness of the het exceeds either homozygote. So:

$$F(AA) < F(AB)$$

$$F(BB) < F(AB)$$

Then:

$$Log\ (F(AB) / F(AA)) > 0\ (blue\ line)$$

$$Log\ (F(AB) / F(BB)) > 0\ (red\ line)$$

In the figure, the two above equations are true when the blue and red lines exceed the green zero line. In this triangular region, overdominance will protect polymorphism. Parameter values are equivalent to Figure 1.1 panel additive inheritance (red line).

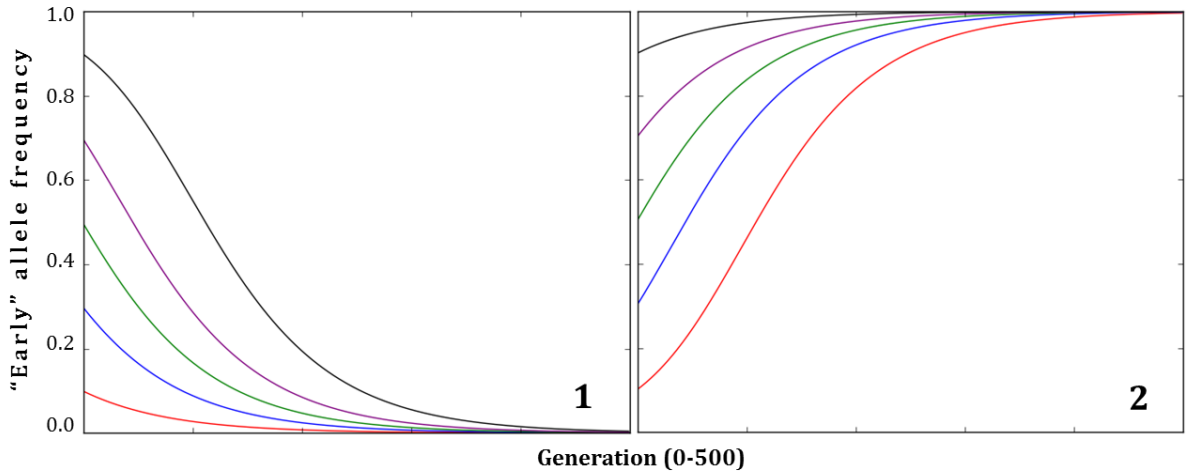
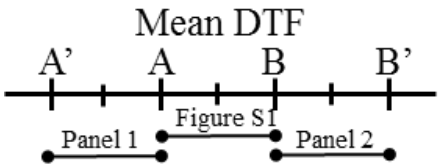


Figure S1.3- Frequency of the "earlier" of two alleles through time. At DDD = 31.4, stable polymorphism is predicted with 2 alleles (A and B) and additive inheritance (Figure 1.1). In panel 1, an "earlier" allele (A') was introduced, and in panel 2, a "later" allele (B') was introduced.



Parameter values are as follows: A'A'm = 28.605394, AA'm = 28.999553, AAm = 29.393712, BBm = 30.18203, BB'm = 30.576189, B'B'm = 30.970348. All other parameters are identical to Figure 1.1 panel A additive inheritance (red line).

A fixes and A' is lost (panel 1), B fixes and B' is lost (panel 2). In a particular environment, the population will walk toward some local optimum for the population mean DTF. A mutation that brings the population away from that optimum will be lost.

Because DTF is affected by many loci across the genome, the large mutational target provides opportunity for mutations to arise that move a population towards a maxima. Also, spatial

heterogeneity, population admixture, and temporal environmental fluctuations mean that selection might vary across a landscape, or on long time scales. Fitness maxima will move, but the population will move toward the new maxima.

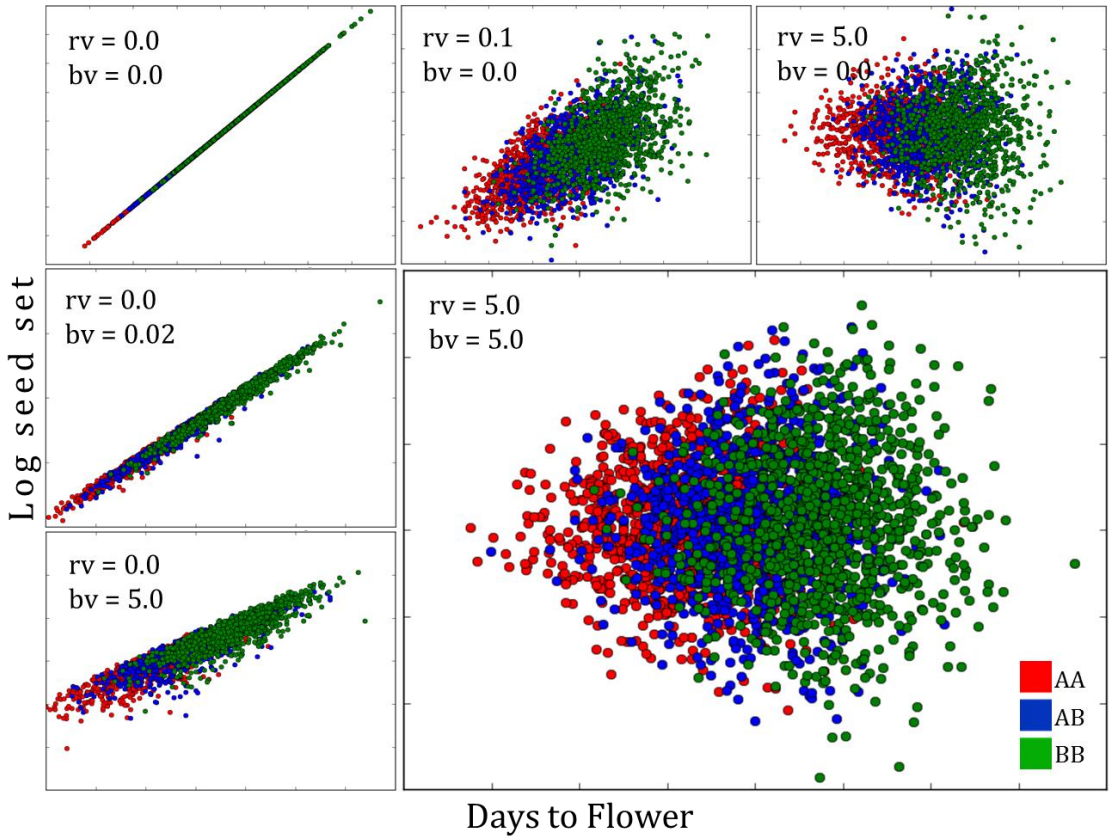


Figure S1.4- Spatial environmental heterogeneity. Growth rate (r) and the product bm_0 are both variable across a landscape. For each of 1000 individuals per genotype, DTF is drawn from a normal distribution defined by each genotype's mean and variance. Then, r is drawn from a normal distribution with mean = 0.5 and standard deviation rv. Finally, b is drawn from a normal distribution with mean = 0.0723244012 and standard deviation bv. Because seed set cannot be negative, if the randomly drawn value for b is less than 0, it is drawn again. The figure above shows the relationship between DTF and log seed set for different values for bv and rv. As either increases, the positive correlation between DTF and fecundity becomes less strong. All other parameters are identical to Figure 1.1 panel A additive inheritance (red line).*

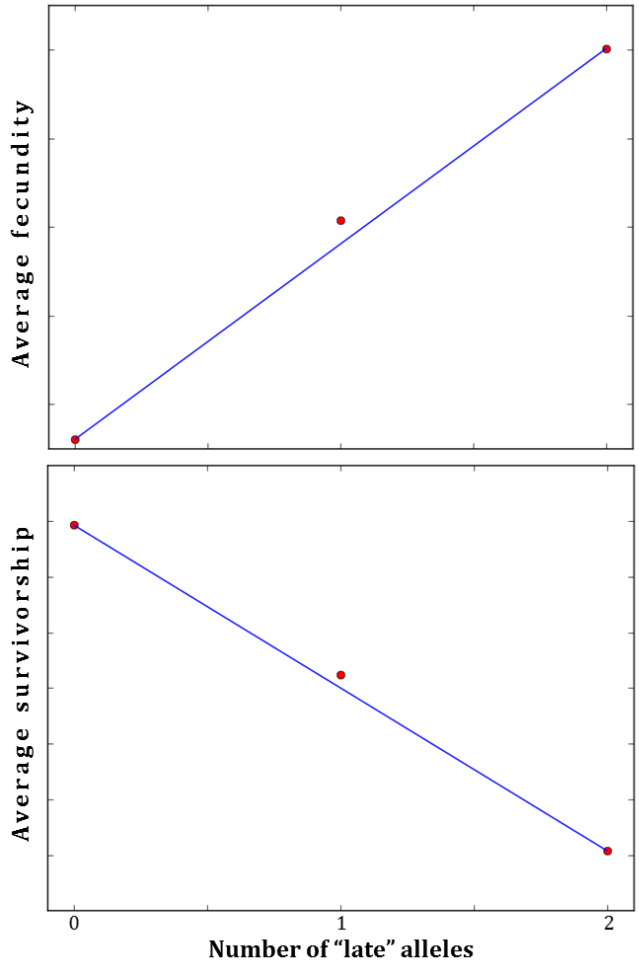


Figure S1.5- Average survivorship and fecundity of each genotype given by the number of "late" alleles, 0 (AA), 1 (AB), 2 (BB). Here, DDD = 31.4 and inheritance is strictly additive. The blue line is the average between the two homozygotes. Both components of the heterozygote fitness exceed the average between the homozygotes. This demonstrates that overdominance occurs without imposing conditional dominance on each trait separately, through the ecology of the system. Genotype means are identical to Figure 1.3, all other parameters are identical to Figure 1.1 panel A additive inheritance (red line).

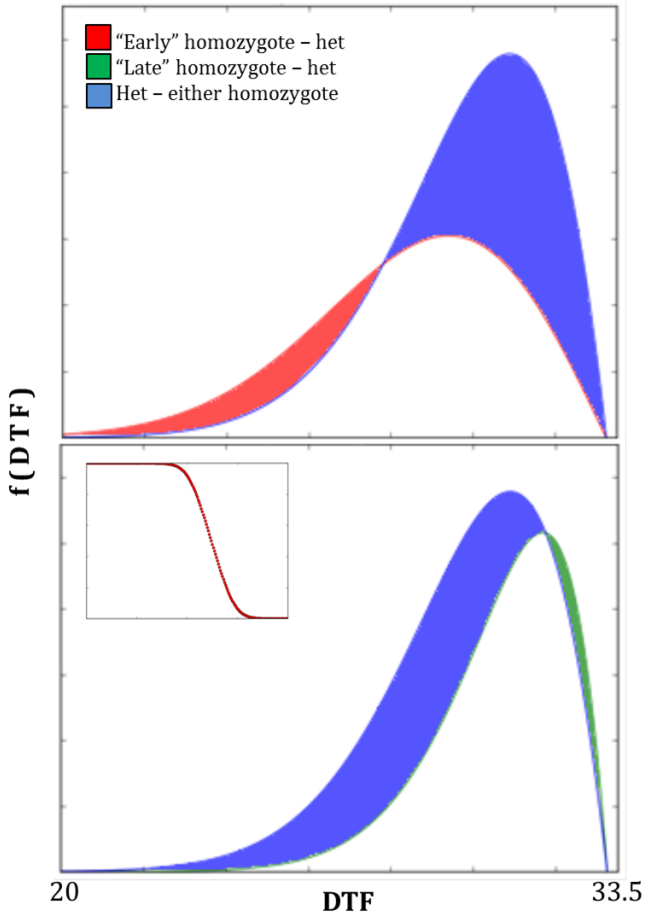


Figure S1.6- Incorporation of the Weis model (See Supplemental Appendix S2 for details). As in Figure 1.2, the curves above demonstrate that equations (5) and (6) are satisfied when DDD = 33.25, as it is here. Polymorphism is still maintained, as seen in the figure inset, which is the final "early" allele frequency between DDD of 32 and 34. Genotype means are identical to Figure 1.3, all other parameters are identical to Figure 1.1 panel A additive inheritance (red line).

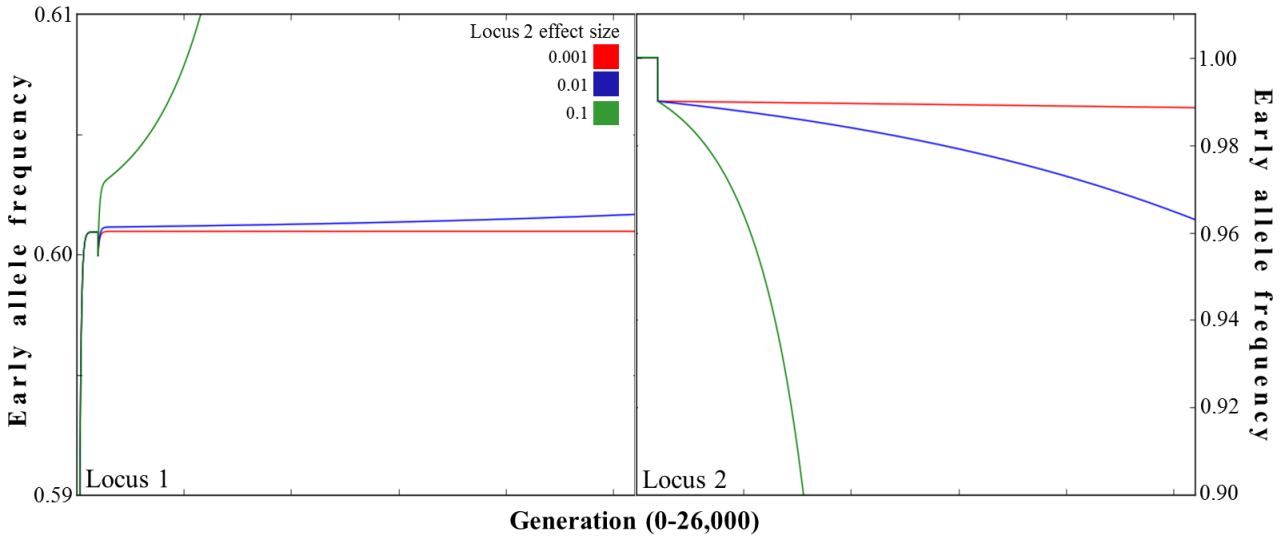


Figure S1.7- 2-locus extension. The first locus is allowed to reach equilibrium frequency before a late allele is introduced at the second locus at an initial frequency of 0.01. The effect of the second locus is additive (no epistasis) and the magnitude varies (red, blue, and green lines). DDD = 31.6, the average DTF of the fast homozygote at the first locus is 29.0, and the effect of the slow allele at the first locus is 1.0. All other parameters are identical to Figure 1.1 panel A additive inheritance (red line).

For ease of viewing, graphs are zoomed in to show a particular range of frequencies. For locus 1, the green line equilibrates around 0.8 (not shown). For locus 2, each late allele (regardless of effect size) fixes eventually.

Table S1.1: Environmental stochasticity

DDD variance	Autocorrelation	Width	Midpoint
0.0	0.0	3.93	31.505
10.0	0.0	3.98	31.67
10.0	0.9	3.96	32.25
10.0	-0.9	3.96	31.65

Calculated width and midpoint of the region of polymorphism with high generation to generation variance in the environment, including with autocorrelation. First, two values for DDD are calculated: final "early" allele frequency of 1, and of 0 (fixation and loss). Width is calculated as the difference between those two values, and midpoint is calculated as the average of the two. For DDD variance = 10, width and midpoint values are averaged over 100 independent runs. High variance in the environment shifts the midpoint of the region to longer growing seasons, but the width of the region does not increase. High positive autocorrelation and high environmental variance shifts the region toward even longer growing seasons, without increasing the width. All other parameters are identical to Figure 1.1 panel A additive inheritance (red line).

Environmental stochasticity is often assumed as a mechanism for balancing selection, as variation within a population is predicted to allow the population to move towards shifting fitness maxima (Levene 1953; Dempster 1955; Haldane and Jayakar 1963b; Gulisija and Kim

2015). *Our model suggests that temporal variability in the environment is not required, and it does not increase the region of environmental parameter space that can protect polymorphism.*

Table S1.2: Exponential vs. Linear growth

Growth Rate	Exponential Width	Exponential Mid	Linear Width	Linear Mid	Logistic Width	Logistic Mid
0.5	0.39	31.485	0.41	36.165	0.49	32.245
0.1	0.39	34.195	0.41	36.165	0.42	35.43
0.9	0.39	30.825	0.41	36.165	0.52	31.33

Width and midpoint of the region of polymorphism, calculated as described in Table S1.1, and as a function of growth rate (r) and shape of growth curve (exponential, linear, or logistic). Values shown are without temporal environmental variability. All other parameters are identical to Figure 1.1 panel A additive inheritance (red line).

Exponential growth, as mostly discussed in the main text, is given by the second half of the integrand in equation 4:

$(\beta m_0 e^{rx})$

For linear growth, this equation is replaced with:

$(\beta m_0 rx)$

And for logistic growth, it is replaced with:

$\left(\frac{\beta m_0}{1 + e^{rx}}\right)$

None of the alternative growth equations eliminates the potential for maintenance of polymorphism.

Chapter 2: Severe inbreeding depression is predicted by the "rare allele load" in *Mimulus guttatus*

Abstract

Most flowering plants are hermaphroditic and experience strong pressures to evolve self-pollination (automatic selection, reproductive assurance). Inbreeding depression (ID) can oppose selection for selfing, but it remains unclear if ID is typically strong enough to maintain outcrossing. To measure the full cost of sustained inbreeding on fitness, and its genomic basis, we planted highly homozygous, fully genome-sequenced inbred lines of yellow monkeyflower (*Mimulus guttatus)* in the field next to outbred plants from crosses between the same lines. The cost of full homozygosity is severe: 65% for survival, 86% for lifetime seed production. Accounting for the unmeasured effect of lethal and sterile mutations, we estimate that the average fitness of fully inbred genotypes is only 3-4% that of outbred competitors. The genome sequence data for these lines provides no indication of simple overdominance, but the number of rare alleles carried by a line is a significant negative predictor of fitness measurements. These findings are consistent with a deleterious allele model for ID. High variance in rare allele load among lines and the genomic distribution of rare alleles both suggest that migration might be an important source of deleterious alleles to local populations.

Introduction

Over 160 years since Darwin (1876, 1877) identified the problem, it remains a paradox that hermaphroditic species maintain outcrossing despite strong and relentless selection for self-fertilization (Fisher 1941; Lloyd 1979). Over 90% of flowering plants are hermaphroditic (Renner and Ricklefs 1995) and most are substantially or predominantly outcrossing (Goodwillie 2005; Igic et al. 2006). Plants have evolved complex mechanisms to prevent self-fertilization, such as molecular self-incompatibility (Takayama and Isogai 2005), herkogamy including flexistyly and heterostyly (Ganders 1979; Li et al. 2001a; Opedal 2018), and dichogamy (Bertin and Newman 1993). Classical theory predicts that a population should maintain outcrossing if inbreeding depression (ID) is strong enough, specifically that $\delta > 0.5$ where δ equals one minus the fitness of selfed relative to outcrossed progeny (Kimura 1959; Lande and Schemske 1985; Charlesworth and Charlesworth 1987). The "$\delta > 0.5$ rule" has motivated experimental estimation of ID in many species. Winn et al. (2011) recently reviewed estimates from plants and found that the mean of δ for lifetime fitness was slightly greater than 0.5 for both highly outcrossing and mixed mating species.

Interpretation of δ estimates near 0.5 is problematic because, for a number of reasons, the $\delta > 0.5$ rule underestimates the necessary strength of ID to halt selfing. Lloyd (1979) showed that "delayed selfing," where a plant self-fertilizes ovules after the opportunity to outcross has passed, can evolve even with very high ID. The reproductive assurance provided by selfing is also advantageous for the colonization of new habitats (Baker 1955) and for range expansion (Grossenbacher et al. 2015). Delayed selfing can purge deleterious mutations from a population, increasing the likelihood that "competing selfing" (self- and cross-fertilization compete for the

same ovules (Lloyd 1979)) might be favored. Even without a history of purging deleterious alleles, competing selfing can evolve in certain situations when $\delta >> 0.5$. If a selfing mutation fortuitously fixes within a lineage with low mutational load, this lineage can expand to exclude outcrossing genotypes from a population (Lande and Schemske 1985; Holsinger 1988; Uyenoyama et al. 1993). The selfing mutation can "find" the right lineage through a combination of chance, if it occurs within a plant carrying fewer deleterious mutations, and subsequent purging of deleterious alleles (Kelly and Tourtellot 2006).

Countering these arguments, ID might just be much stronger than is widely appreciated. Most experimental estimates of ID are based on first generation selfed progeny assayed under benevolent conditions (Winn et al. 2011). First generation selfs (inbreeding coefficient $F = 0.5$) are the typical inbred individual in a predominantly outcrossing population, excepting bi-parental inbreeding (Uyenoyama 1986). However, if the selfing rate increases within a population, reproduction by inbred adults will necessarily produce increasingly inbred progeny (F ranging from 0.75 to 1.0). Fitness declines monotonically with F (Morton et al. 1956b; Charlesworth and Charlesworth 1987), perhaps often in an accelerating fashion (Kimura and Maruyama 1966a; Kondrashov 1988; Charlesworth et al. 1991).

In this paper, we demonstrate the dramatic effect of sustained selfing on fitness evaluated under natural conditions. We compare inbred lines to F1 crosses between lines of yellow monkeyflower (*Mimulus guttatus*). The lines were derived from randomly sampled, field collected individuals and allele frequencies in the lines match estimates from direct field collections (Troth et al. 2018). Because these lines are fully genome sequenced, the fitness

estimates address the genetic basis of ID. The two main genetic theories for ID are the "dominance hypothesis" (inbreeding reveals rare recessive or partially recessive deleterious alleles that are at low frequency in the population) and the "overdominance hypothesis" (inbreeding reduces heterozygosity at loci for which heterozygotes have the highest fitness) – see Charlesworth and Willis (2009) for a review of these models. We find no evidence for overdominance at individual SNPs (Single Nucleotide Polymorphisms), but there is significant negative correlation between the number of rare alleles in the genome for a given line ("rare allele load") and fitness measurements. This surprising result is consistent with the dominance hypothesis, insofar as the rare allele load is correlated with the deleterious mutation load.

Methods

Study system, and line development

Mimulus guttatus (Phrymaceae, syn. *Erythranthe guttata*) grows in North America, west of the Rocky Mountains, from northern Mexico to Alaska. Populations are annual or short-lived perennials, reliant on bees for pollination. Estimated selfing rates vary among *M. guttatus* populations, and between years within a population, but most populations are predominantly outcrossing (Ritland and Ganders 1987; Awadalla and Ritland 1997; Sweigart et al. 1999). Here, we investigate the Iron Mountain (IM) population of *M. guttatus* (Oregon, U.S.A.; 44.402217 N, –122.153317 W), an annual population with an outcrossing rate of over 90% (Willis 1993b).

Each of the inbred lines used in the present study was initiated from a single seed sampled from IM, each seed from a distinct maternal plant. A total of 1200 lineages from one collection of wild plants were started in 1995 (those with a prefix "IM"), but after six generations of single

seed descent (selfing with random selection of a single seedling per family for the next generation), only 300 remained (Willis 1999b; Kelly 2003b). The purpose of this experiment was to allow lethal and sterile mutations to fix within lines in proportion to their frequency in the natural population. After extinction of these lineages, the resulting "purged" population ($F > 0.98$) carried only sub-lethal and mildly deleterious mutations. Of course, plants can fail to reproduce for non-genetic reasons, but outbred IM plants have nearly 100% survival under greenhouse conditions. Also, Kelly (2003b) attempted to re-grow seed from the 4th and 5th generation lines that had failed by generation 6, but very few of these lineages were resurrected. The survival of 300 lineages yields our estimate of 75% (900 of 1200) genetic death in line formation. Since 2003, we have periodically germinated and selfed these lines and they are now 6-12 generations inbred. A second collection of inbred lines from the Zia-1 base population are distinguished by the prefix "Z." Each of the Z lines is derived from a single IM seed (Kelly 2008). However, single-seed descent for the Z lines was performed with cold stratification of seed (1 week) before germinating in the greenhouse. The IM lines were formed without cold treatment. In total, 187 lines have been whole-genome sequenced and confirmed to be highly homozygous (Troth et al. 2018). For the field component of the present study, we selected 37 IM lines established to be wholly unrelated by the kinship matrix of Troth et al. (2018). For the greenhouse study, we planted 165 of the sequenced lines (116 IM and 49 Z).

Field experimental design

We generated both self-fertilized progeny and outcrossed progeny by growing 3-4 plants from each line to maturity in the U. Kansas greenhouses. We performed two different types of crosses to obtain outcrossed progeny: fertilization with pollen from a single non-self IM line (random

mate-pair crosses), and fertilization with a mixture of pollen from 7 other non-self IM lines (group crosses). The latter were done to test if producing more diverse progeny increases the average fitness of progeny. We germinated seed from each cross/self in the University of Oregon greenhouses, with seed to soil on May 7, 2018. On May 21-23, 2018, we transplanted 1,176 greenhouse-germinated seedlings into absorbent peat/wood fiber pots (Jiffy Strip, Blue Ridge Greenhouses), one seedling per pot (548 selfed, 304 group outcrossed, 324 single outcrossed). One day after transplanting, we settled pot strips into the soil/moss matrix of the Browder Ridge Trailhead site (Oregon, USA; 44.373238 N, –122.130675 W) in two cohorts, one day apart. Browder Ridge is our "transplant site," geographically close and ecologically similar to IM (Mojica et al. 2012b). We arranged the transplants such that, in each flat of eight plants, there was a row of four outcrossed plants next to a row of four selfed plants, all from the same maternal line when possible. This was meant to minimize the random effect of spatial variation across the field plot. The timing of transplant ensured that wild individuals were at the same developmental stage as our experimental transplants (cotyledon or 2 leaf stage). On July 21, 2018, we harvested experimental plants to estimate fitness. All but five of the transplants were fully desiccated, and only one still had an open flower. We noted which individuals had survived to flower and collected all fruits. We scored all individuals for survival to flower (0/1). For all survivors, we determined the number of flowers, number of fruits, seed set per fruit, and total seed set.

Greenhouse follow-up

We grew 165 of the whole-genome sequenced IM lines (on January 11, 2019) under the same conditions in which the lines were initiated. Z lines were stratified at 4°C for one week prior to

being transferred to the U. Kansas greenhouse, while IM lines were not stratified. We had previously established an interaction between stratification treatment and line type (Z vs. IM) on germination success in the greenhouse (Figure S2.1), so we have only grown lines in their initiation conditions for this experiment. We transplanted germinants to 2.25" pots after 14 days in the greenhouse, randomizing them between flats on the greenhouse bench. Two weeks after transplanting, we cut each plant at the hypocotyl/root junction and collected the above-ground mass into coin envelopes. We dried the tissue in an oven at 300°F for one hour and weighed/recorded the dry mass, accurate to a tenth of one milligram.

Analysis

For the field data, we first tested the distinct outcross treatments to determine if progeny of group crosses were different from single matings. There was no evidence of difference for any fitness component (Figure S2.2). Thus, for subsequent whole-plant analyses, we combine all outcrossed progeny of a maternal line into a single category. We cannot combine group and single crosses for genomic analyses because the progeny genome sequence cannot be inferred for the group crosses (specific father unknown). Sample sizes are not sufficient for a meaningful SNP-level testing, which we confirm by applying a genome wide association analysis to the field data (Supplemental Appendix 1). To calculate the rare allele load of each line, we determined allele frequencies in the full (unfiltered) variant call file obtained by Troth et al. (2018). We suppressed one line (IM764) from the frequency calculations because it is excessively divergent across multiple chromosomes. Supplemental Table 2.1 reports all 5,018,997 SNPs where the minor allele is <= 5% in the inbred lines ("rare" for the purposes of analysis). We scored each line for number of loci in this set at which the line carries the rare allele. The rare allele load is

the count of rare alleles divided by the total scored sites for each line. For each family consisting of F1 progeny from reciprocal single-crosses planted in the field (17 in total), we also calculated the genome-wide heterozygosity as a fraction of scored loci.

Statistics

We performed all statistical calculations and model fits using R (R Core Team, 2013). We tested for the effect of cross type, rare allele load, or heterozygosity on field survival (categorized as a binomial response with a logit link function) using generalized linear mixed-effects models fit with the *glmer* function in the *lme4* package (Bates et al. 2007), which uses restricted maximum likelihood. The mixed-effects models included maternal line or family as a random effect and interactions when necessary. This model was compared to a generalized linear model without random effects using the built-in R function *glm*.

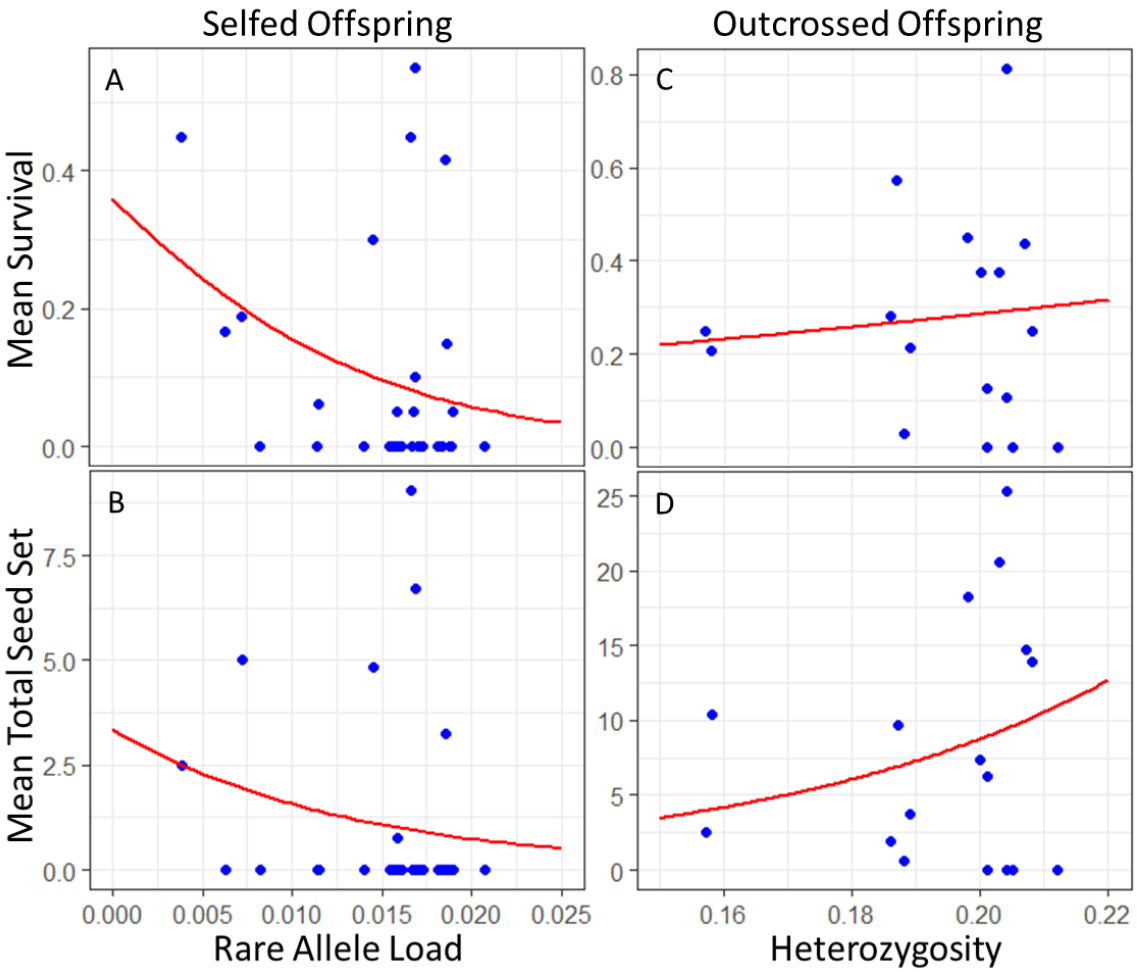
Total fitness in the field, measured by seed set, is overdispersed relative to the Poisson distribution (Figure S2.3). For this reason, we used the *reaster* function from the R package *aster* to fit an exponential family regression model (Geyer et al. 2007; Geyer et al. 2013). Aster models test for an effect on total fitness as a cumulative trait composed of different stages of life history by allowing each stage to have a different response type. *Reaster* fits an aster model with random effects. We fit a model that included either cross type, rare allele load, or heterozygosity as fixed effects and maternal line (and its interaction with the fixed effect) as a random effect. We structured the life history model into three stages: survival to flower (binomial), any seed set (binomial), number of seeds (zero-truncated Poisson) and compared these models to the corresponding model without random effects using *aster*.

For plants grown in the greenhouse, the natural log of above ground dry mass yields normal residuals (Figure S2.4), so we performed multiple linear regression on the average ln dry mass for every line. We used the built-in R function *lm* to test for an effect of rare allele load on line means of ln(mass) using line collection (IM vs Z) as an additional factor.

Results

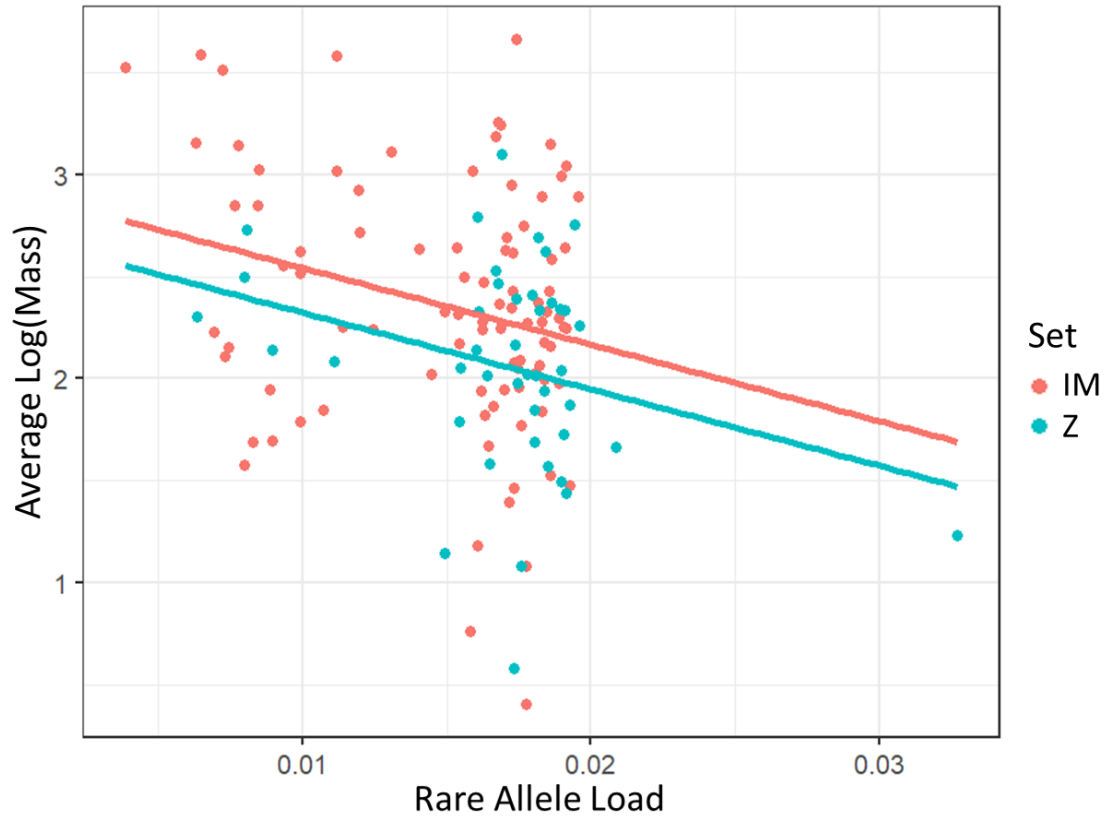
Inbreeding depression- We find a 7-fold decrease in the total fitness of selfed individuals compared to outcrossed individuals in the field (7.6 vs 1.1, $\delta = 0.86$, $z = 4.63$ for out vs. self, $p = 3.75e\text{-}6$). This high level of ID is in part caused by an almost 3-fold decrease in survival to flowering (28.5% vs. 9.85%, $\delta = 0.65$, $z = -6.044$, $p = 1.5e\text{-}9$). Among survivors, mean outcrossed seed set is 26.8 seeds versus 11 seeds for inbred plants ($\delta = 0.59$). Statistics reported here are for the full model that includes both maternal line and line by cross interaction as random effects (See Table S2.2 for all model fits). We find a significant effect of line ($z = 3.32$, $p = 0.000454$) and line by type of cross interaction ($z = 3.26$, $p = 0.0056$) on total fitness in the model *reaster* model that includes both as random effects (see Figure S2.5 for a visual representation of the interaction). The variance in average phenotype among families was higher for outcrossed families than inbred families for both survival (0.050 vs 0.026) and total seed (105.01 vs 5.32).

Figure 2.1: Effect of rare allele load (RAL) of inbred lines (A, B) and heterozygosity (H) of F1s (C, D) on survival and total seed in the field experiment. Curves are predicted from simple model fits in glm. Predictions are given by: (A) Survival = (e^(-0.577-110.6* RAL))/(1+e^(-0.577-110.6* RAL)); (B) Total Fitness = e^(1.206-75.44* RAL); (C) Survival = (e^(-2.33+7.105*H))/(1+e^(-2.33+1.105*H)); (D) Total Fitness = e^(-1.529+18.506*H).



Genomic predictors of inbred and outbred fitness- The 31 lines included in the field experiment with selfed progeny have a rare allele load between 0.38% and 2.1% (mean 1.5%). Among selfed progeny, rare allele load is a highly significant predictor of survival (Figure 2.1A; $z = -3.64$, $p = 0.00027$), but not lifetime seeds (Figure 2.1B; $z = -1.45$, $p = 0.15$). If maternal line is included as a random effect, tests become non-significant (Table S2.3) because line ID and rare allele load are strongly confounded (co-linear predictors). The lines with lower rare allele load have higher survival (left portion of Figure 2.1A), but the limited replication at this end of the scale limits inference. We performed the greenhouse experiment (results below) on a much larger collection of lines to more clearly distinguish the effect of rare allele load. This experiment reveals a highly significant effect of rare allele load on above-ground biomass at day 28 (Figure 2.2; $F = 13.44$, $p = 0.00036$). Line set has a marginal effect (IM vs Z: $F = 3.98$, $p = 0.048$). One line (Z12) is an outlier for rare allele load (0.033), but after dropping that point, rare allele load remains highly significant ($F = 10.45$, $p = 0.0016$). Dropping set as a factor changes the estimated effect of rare allele load minimally (Table S2.4).

Figure 2.2: Regression of rare allele load on line averages of ln(mass) for greenhouse experiment. Model included line set (IM vs Z) as a factor



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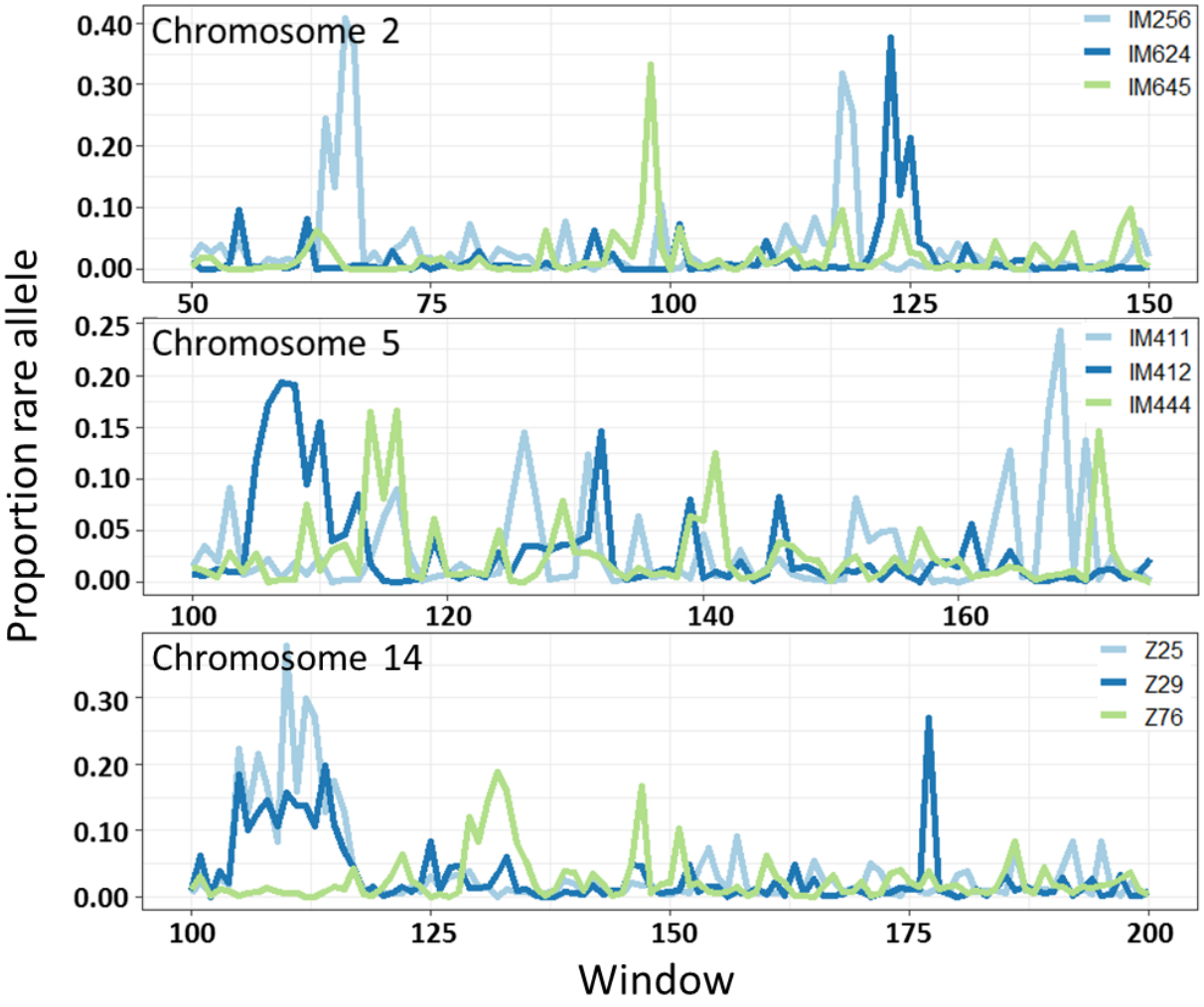
The outcross progeny from crosses between single lines (F1s) have known whole genome sequences. The overall heterozygosity of these plants varied from 15.7% to 21.2%. Heterozygosity is not a significant predictor of survival either as a single predictor or with line included as a random factor (Figure 2.1C; Table S2.5). It has an apparently positive effect on lifetime seeds (Figure 2.1D), but this effect is marginally non-significant ($p = 0.075$) without line and entirely non-significant ($p = 0.46$) when line is included as a random factor.

For each line, we scored the rare allele load within 100kb windows across the genome. Rare alleles are elevated within local "patches" across the in a line specific manner (Figure 2.3). For example, line Z25 is homozygous for the rare allele at 10-35% of variable sites for a 1Mb window on chromosome 14 (Figure 2.3). Only a small subset of the lines are elevated within any window (otherwise the alleles would not be rare). We categorized SNPs as synonymous, non-synonymous, or non-coding using SnpEff (Cingolani et al. 2012), but found counts of each highly correlated. Lines/windows with high rare allele counts for one mutation type had high counts for the others.

Figure 2.3: Rare allele proportion is non-uniformly distributed across the genome and varies among lines. Each panel pictured represents a portion of a different chromosome. Each window is 100kb windows. The specific lines and chromosomes are a random sample of the full dataset.



Discussion

Severity of ID- Hermaphroditic populations should evolve self-fertilization unless inbreeding depression (ID) is sufficiently strong (Kimura 1959; Charlesworth and Charlesworth 1987). The simplest prediction is that δ, 1– (inbred offspring fitness/outbred offspring fitness), must be greater than 0.5 to maintain outcrossing. This rule is burdened with many caveats (Lloyd 1979; Uyenoyama et al. 1993; Johnston et al. 2009), but most of these exceptions favor selfing and thus increase the necessary severity of ID to maintain outcrossing. In this experiment, we find that inbreeding to (nearly) full homozygosity has an enormous fitness cost. In the field, the lifetime seed production of inbred plants was only 14% that of their outbred competitors ($\delta = 0.86$). If we include the estimated effect of lethal and sterile mutations, the cumulative δ increases to 0.965 (see below). This value is much higher than previously obtained from species with similar (5-15%) selfing rates (Winn et al. (2011)), which range from 0.21 in *Campanula americana* (Galloway et al. 2003) up to 0.53 in *Yucca filamentosa* (Huth and Pellmyr 2000) . However, ID in these studies was measured using greenhouse-grown first generation selfed progeny from field collected plants. Here, we evaluate the fitness consequences of high homozygosity and find the cost is great enough to maintain predominant outcrossing.

The field δ (0.86) is an underestimate because it does not include lethal or sterile mutations. Such mutations segregate in the natural population but not in the lines of our experiment. Creation of the lines via single seed descent revealed recessive lethal/sterile mutations as homozygotes, and as consequence of their effects, about 3/4 of the lines perished over generation 5 of selfing. In fact, purging of the lethal/sterile mutations was the motivation for creating these lines (Willis 1999a, b). The large fitness reduction observed in the field is due to the remaining

variation: minor effect deleterious alleles as well as any balanced polymorphisms contributing to ID. Combining both mutation types, we estimate the relative fitness of homozygous plants as (0.25)(0.14) = 0.035. The resulting δ (0.965) is large but at least roughly consistent with previous estimates from *M. guttatus*, which range from δ = 0.69 (IM population in Oregon, Willis, 1993b) to 0.70-0.73 (S and T populations in California, Carr and Dudash, 1995). While lower, these prior estimates are based on first-generation selfs. The fitness decline caused by inbreeding is predicted to double progressing from first-generation selfs to full homozygosity (Morton et al. 1956b; Charlesworth and Charlesworth 1987).

Two caveats require attention. First, adaptation to the laboratory/greenhouse environment likely occurred during the process of forming the inbred lines. Homozygous genotypes cannot adapt (except by de novo mutation), but during the process of creating the inbred lines, differential germination of seeds in the greenhouse could have favored some genotypes over others. For the field study, we germinated plants in the greenhouse and then transplanted seedlings into the field, but germination relevant loci could have pleiotropic effects on field performance. While potentially real, our experimental design is insulated from this potential bias. The outbred plants in the experiments are derived from crosses between the inbred lines and thus carry the same (putatively) lab-adapted alleles in the same frequency. Any shift in allele frequencies between lines and the field population is shared equally by inbred and outbred plants, which differ only in heterozygosity. Also, given that germination is a component of fitness (not considered by our experiments), ID may actually be greater than we estimate.

A more serious concern is that we measured fitness as lifetime seed production. We did not measure outcross siring success, which is half of adult fitness in the predominantly outcrossing IM population. However, several previous experiments in *M. guttatus* indicate a severe effect of inbreeding on male reproductive capacity. The number of viable pollen grains produced by a plant declines more substantially than other fitness components, with several populations showing an ***accelerating*** decline of pollen viability with increasing homozygosity (Willis 1993a; Carr and Dudash 1997; Carr et al. 1997; Kelly 2005). Thus, as with germination, our neglect of male fitness suggests that our already severe estimate of ID is likely an underestimate.

Cause of ID- Genome sequencing provides new opportunities to evaluate mechanisms for ID and heterosis. Evidence for dominance and overdominance varies significantly among species and even among crosses and phenotypes within species, as do complications such as epistasis (Yu et al. 1997; Li et al. 2001b; Luo et al. 2001; Springer and Stupar 2007; Schnable and Springer 2013). In principle, sequencing allows direct evaluation of the models by attributing fitness effects to individual loci. Unfortunately, genome wide association studies (GWAS) struggle to estimate the effects of rare alleles (Myles et al. 2009; Josephs et al. 2017), which are the cause of ID under the dominance model. Allele frequencies should be intermediate with overdominance, and thus it is noteworthy that we found no evidence of heterozygote superiority at individual SNPs (Supplemental Appendix 1). However, we concede the field experiment is underpowered to detect SNPs with slight overdominance, and the small variance in overall F_1 heterozygosity impedes testing for an aggregate effect of (putative) overdominant loci (Figure 2.1C,D).

Genome-wide distillations of sequence data (e.g. Figure 2.1A,B) have proven useful for investigating the deleterious mutation load. For example, Genomic Evolutionary Rate Profiling (GERP) uses phylogenetic conservation across highly divergent taxa to identify sites that are constrained (Cooper et al. 2005). Scoring non-conserved alleles at such sites as putatively deleterious, Yang et al. (2017) found a negative correlation between GERP score for a SNP and frequency in a collection of inbred maize lines, as well as a positive correlation between GERP score and the magnitude of effect on grain yield, which is analogous to fitness in an agricultural environment. In this paper, we identify all loci with extreme allele frequency (minor allele less than 5%) and score each line for how frequently they carry the rare allele across such loci (the rare allele load). It is noteworthy that this simple statistic is a significant predictor of fitness (Figures 2.1-2.2) given that the great majority of rare alleles might be neutral or nearly neutral (Kimura and Ohta 1971).

The first remarkable feature of rare allele load is the extent that it varies among lines. If minor alleles were randomly assigned to lines (linkage equilibrium), the variance in rare allele load would be approximately equal to the mean divided by the number of SNPs, i.e. the Poisson expectation (Schultz and Willis 1995). The actual variance (1.499E-05) is over 4000 times greater than Poisson (predicted variance of 3.51E-09 given an average rare allele frequency of 0.0154 and 4,379,000 SNPs scored (on average) per line). The inflation is due to positive linkage disequilibrium (LD); rare alleles tend to co-occur within localized genomic windows of each line (Figure 2.3). The rare allele load is a standardized sum of 0/1 values across SNPs. Its variance is the sum of single locus variances (binomial) plus twice the sum of covariances across

all pairs of loci. Even if the great majority of rare alleles are neutral, or nearly so, the rare allele load may be an indicator of deleterious mutation load due to the positive LD.

Rare allele load is an apparently strong predictor of survival to flower in the field (Figure 2.1A). This result is statistically ambiguous owing to limited replication of lines with low rare allele load, but the greenhouse experiment confirmed an effect using much greater replication of lines (Figure 2.2). The fact that the effect is evident in both field and greenhouse is notable given that the genomic distribution of rare alleles per line (Figure 2.3) suggests that migration of pollen and/or seed from other populations into IM might be an important source of deleterious alleles. Localized segments of rare alleles appear like "introgressed segments" in an experimental inter-cross population (Tanksley 1983; Patterson et al. 2004). Migration-selection balance could reproduce the pattern of Figure 2.3 if the rare allele patches are the remnants of immigrant genomes. Of course, if local environmental conditions make rare alleles deleterious, why is the relationship evident in the novel greenhouse environment? Unconditionally deleterious mutations are likely a major component of ID under both field and greenhouse conditions, but we measured growth rate, not fitness, in the greenhouse. To this point, it is relevant that IM has a short growing season that exerts selection for fast growth and rapid progression to flower (Mojica et al. 2012b). If the migrant alleles confer slower growth very early in life (in terms of above ground biomass), they could generate the relationship in Figure 2.2. This hypothesis warrants further study.

Supplemental Appendix 1

To test individual SNPs for effects on field fitness, we used the mixed-linear model (MLM) function in TASSEL (Bradbury et al. 2007). This did not identify any SNPs with an association with either field survival or total fitness at a genome-wide significance threshold with 7,231,363 tests. We also did not find any SNPs with a significant dominance effect. We compared our most significant SNPs, a set of 59 sites with $p < 10e\text{-}4$, to the significant SNPs affecting a number of fitness related phenotypes in separate greenhouse and field studies from Troth et al. (2018), hereby called the Troth Greenhouse and Troth Field sets. The significance threshold for the two Troth sets for this comparison is $p < 10e\text{-}5$. We find no overlapping single SNPs; ours are on average 367,205bp from the nearest Troth Greenhouse SNP (range of 6,515-1,265,234bp), and 276,128bp from the nearest Troth Field SNP (range of 785-871,916bp).

We checked the genomic location of any of our 59 SNPs that was within 10kb of either Troth set, to check in the SNPs were in the same gene, even if the two analyses had not discovered the exact same SNP. One site from the Troth Greenhouse set and one from our set are within the same gene on scaffold 6, a MYB transcription factor called F02036. A putative homolog based on sequence similarity is PHOSPHATE RESPONSE1 (PHR1), a gene with a conserved function in regulating the response to phosphate deficiency across diverse taxa including *Arabidopsis thaliana, Oryza sativa,* and *Chlamydomonas reinhardtii* (Rubio et al. 2001; Nilsson et al. 2007; Meina et al. 2015; Liu et al. 2017).

Supplemental Figures

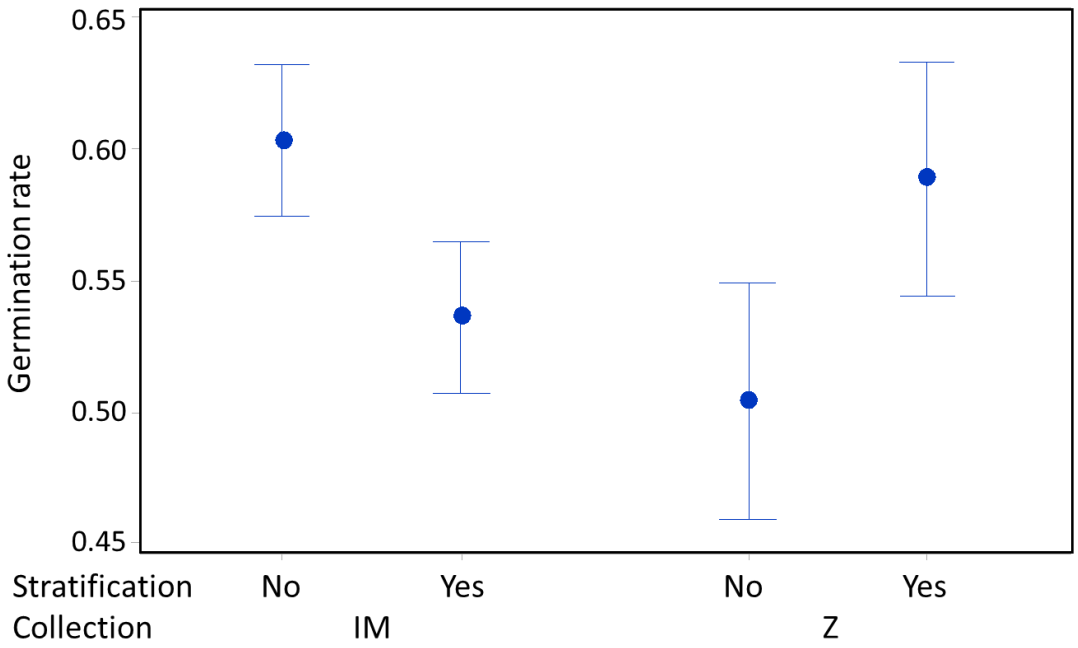


*Figure S2.1**:** Interaction between line set (IM vs Z) and stratification treatment on greenhouse germination rate. IM lines were generated without stratification and Z lines were generated with stratification. This justifies our choice to only grow lines in their generation condition for the present study.*

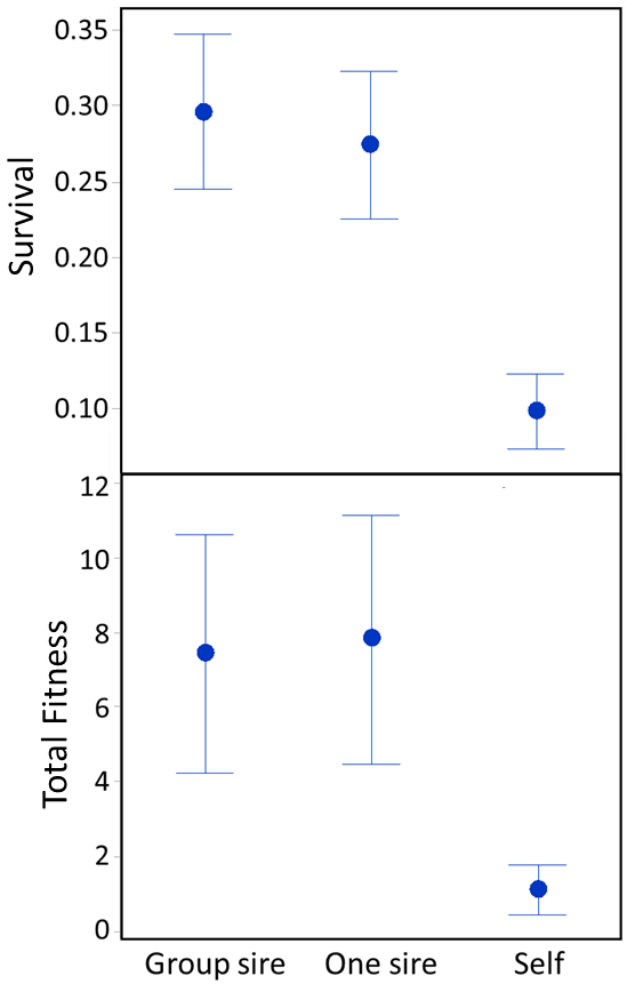


Figure S2.2: We find no difference in either survival or total fitness in the field between outcrossed progeny from single vs group sires. These categories were combined into a single category for subsequent analysis.

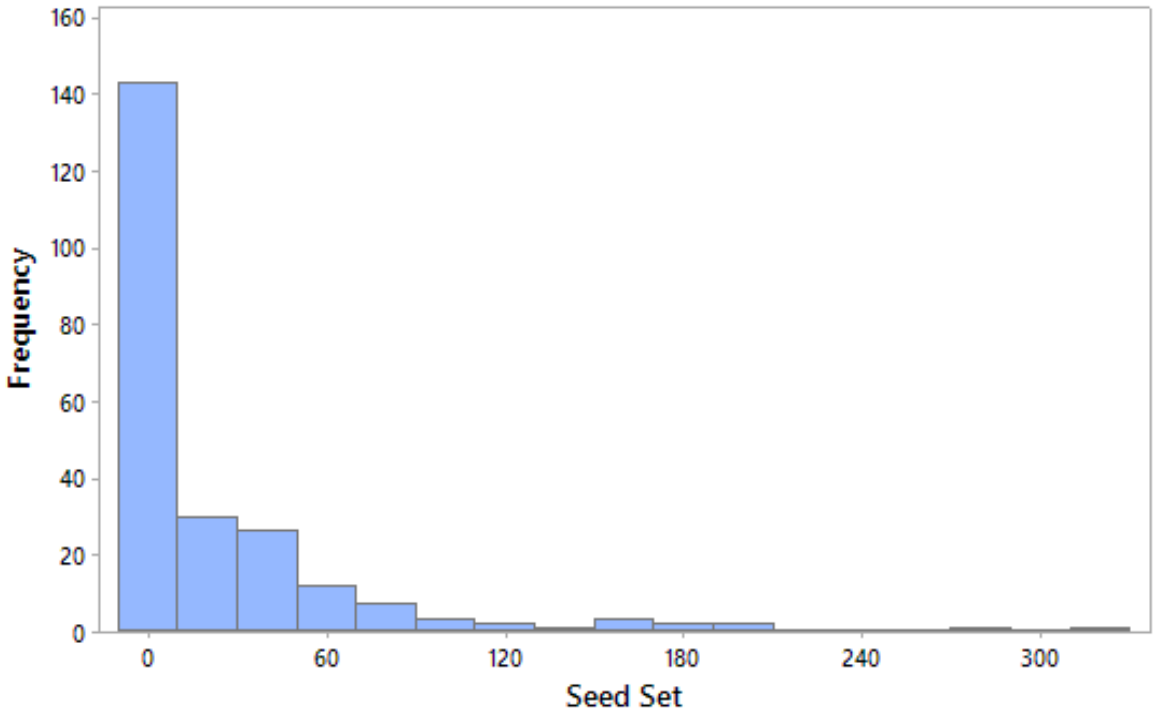


Figure S2.3: Histogram of seed set in the field. Overdispersion necessitates the use of Aster models to test for effects on total fitness.

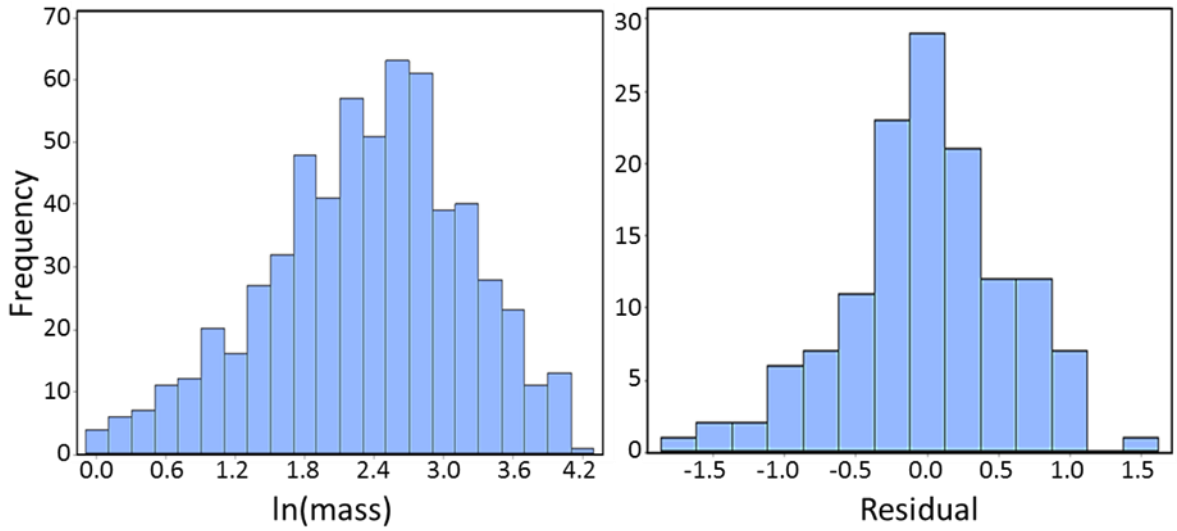


Figure S2.4: Histogram of ln(mass) in greenhouse grown plants, and residual values for the multiple linear regression of average mass and rare allele load (Figure 2.2). Normality of residuals justifies the use of multiple linear regression. Mass was measured in milligrams.

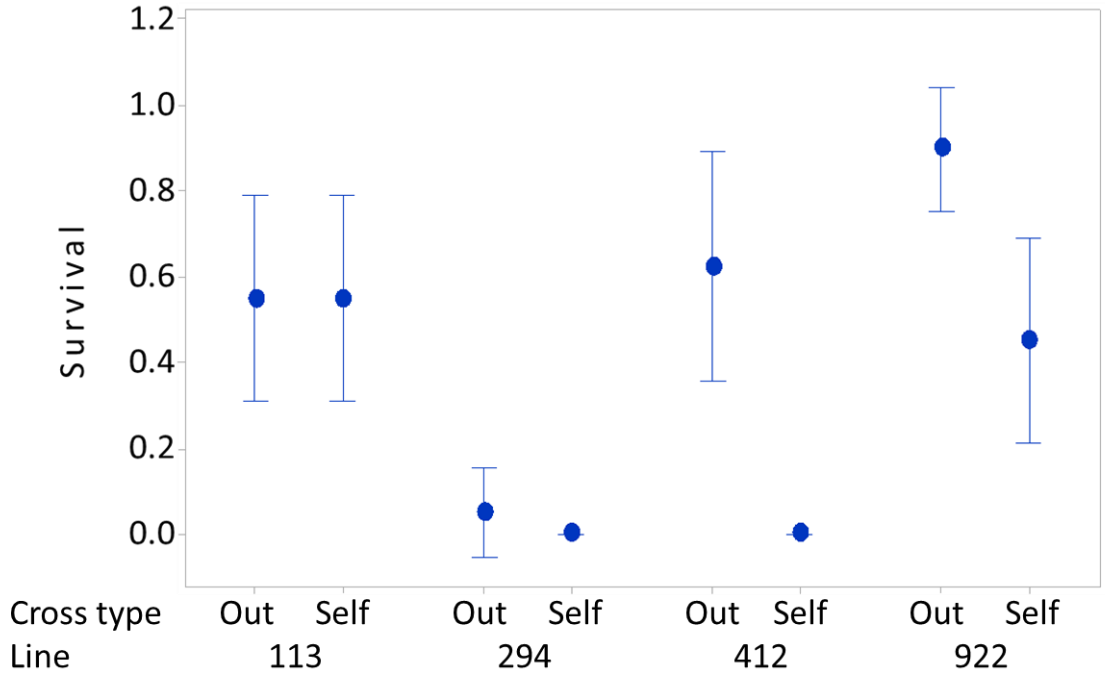


Figure S2.5: Examples of the differences in effect of cross type between lines on field survival.

Supplemental Tables

Table S2.1 (separate file): Table of 5,018,997 variants for which one allele is considered rare (at a frequency of <= 5% in the inbred lines).

Phenotype	**Program**	**Model**	**Z statistic (cross)**	**p-value (cross)**	**Z statistic (Line)**	**p-value (line)**
Survival	*glm*	Surv ~ Cross	-7.684 (self)	1.54e-14		
Survival	*glmer*	Surv ~ Cross + (Line)	-8.369 (self)	< 2e-16		
Survival	*glmer*	Surv ~ Cross + (Line) + (Line:Cross)	-6.044 (self)	1.50e-09		
Total fitness	*aster*	Fit ~ Cross	6.686 (out)	2.3e-11		
Total fitness	*reaster*	Fit ~ Cross + (Line)	7.049 (out)	1.8e-12	6.126	4.51e-10
Total fitness	*reaster*	Fit ~ Cross + (Line) + (Line:Cross)	4.625 (out)	3.75e-06	3.318 (3.257)†	0.000454 (0.000563)†

Table S2.2: Model fits for the effect of self vs. outcross on field fitness. Random effects are given in parentheses, and interactions are indicated with a colon. Reported statistics and p-values are for the fixed effect, type of cross; the category for the estimated effect follows the z-statistic in parentheses. P-values for random effects are one-tailed and only calculated for total fitness because glm does not calculate p-values for random effects. †Statistics in parentheses are for line:cross interaction term.

Phenotype	**Program**	**Model**	**Z Statistic**	**p-value**
Survival	*glm*	Surv ~ FracRare	-3.643	0.000269
Survival	*glmer*	Surv ~ FracRare + (Line)	-0.767	0.443
Total Fitness	*aster*	Fit ~ FracRare	-1.445	0.148
Total Fitness	*reaster*	Fit ~ FracRare + (Line)	-1.162 (3.982)	0.245 (3.42e-05)

Table S2.3: Model test for the effect of rare allele load on field fitness in the single outcrossed families. Line is included as a random effect, and the statistics for the effect of line on total fitness are given in parentheses (p values for family are one-tailed). Glmer does not calculate p-values for random effects.

Data	**Model**	**F statistic (Fracrare)**	**p-value (Fracrare)**	**F statistic (set)**	**p-value (set)**
Full	Ln(mass) ~ FracRare	13.243	0.00039		
Full	Ln(mass) ~ FracRare + set	13.5455	0.00034	4.0099	0.047
W/out Z12	Ln(mass) ~ FracRare	10.3	0.00167		
W/out Z12	Ln(mass) ~ FracRare + set	10.5275	0.00149	3.8947	0.051

Table S2.4: Model fits for effect of rare allele load on ln(mass) in the greenhouse implemented in the built-in R function lm. Results are shown for the full data set as well as with the outlier (line Z12) removed to demonstrate that it is not entirely responsible for the regression.

Phenotype	**Program**	**Model**	**Z Statistic**	**p-value**
Survival	*glm*	Surv ~ FracHet	0.832	0.405
Survival	*glmer*	Surv ~ FracHet + (Family)	0.054	0.957
Total Fitness	*aster*	Fit ~ FracHet	1.779	0.0752
Total Fitness	*reaster*	Fit ~ FracHet + (Family)	0.745 (3.604)	0.457 (0.000157)

Table S2.5: Model fits for the effect of proportion heterozygous loci on field fitness in the single outcrossed families. Family is included as a random effect, and the statistics for the effect of family on total fitness are given in parentheses (p values for family are one-tailed). Glmer does not calculate p-values for random effects.

Chapter 3: Dissection of a flower size QTL in Mimulus guttatus reveals a complex path from genotype to phenotype

Introduction

An important goal for predicting how populations with differences in genetic composition will be affected by changes in the environment is understanding how genotype influences phenotype. In the case of easily observed macroscopic phenotypes, the mapping from genotype to phenotype is often as simple as a single loss of function mutation that results in a large phenotypic change. Examples of this include flower color shifts (Smith and Rausher 2011; Wessinger and Rausher 2014), domestication traits in crops (Doebley et al. 2006), and disease causing mutations in humans (Feinstein and Wilson 2005; Samocha et al. 2014). However, for more subtle changes in quantitatively variable traits, the relationship between genotype and phenotype can be more complicated. Complex traits can be massively polygenic, wherein a mutation at any one locus will likely have diminishing effects as more genes are involved in determining the trait (Fisher 1918; Barton et al. 2016). Additionally, variation can be due to regulatory changes, in which case mutations may result in quantitative changes in gene expression that have cascading effects on observable phenotypes (Carroll 2000). These complications have led to the development of methods for identifying genetic polymorphisms with quantitative contributions to phenotypic variation, such as genome-wide association studies (GWAS) or quantitative trait locus (QTL) mapping.

Recent studies provide evidence that most regions of the genome, if dissected into small enough pieces, contain variation that affects any number of traits. For example, in *C. elegans*, Bernstein et al. (2018) tested for QTL within a 1.4 Mb region of the genome by using a series of Nearly Isogenic Lines (NILs) to break the region up into 15 pieces. They found that 11 of the 15 smaller pieces contained QTL, many of them linked to other QTL with antagonistic effects. Even in

humans, recent estimates predict that most 100kb windows of the genome should contain a variant influencing height (Boyle et al. 2017). These findings indicate that GWAS or QTL mapping studies are likely underestimating the number of contributors to variation in quantitative traits, and possibly failing to detect closely-linked antagonistic loci.

Flower size in the yellow monkeyflower, *Mimulus guttatus*, is a model trait for studying quantitative trait variation, life-history fitness tradeoffs, and the genetic basis of population-level variation. Additionally, *M. guttatus* has a well-annotated reference genome and a collection of fully sequenced inbred lines derived from a single natural population, Iron Mountain (IM), from Oregon (Troth et al. 2018). These genetic resources make monkeyflower a good system to study how complex phenotypes are genetically determined.

Previous QTL mapping efforts identified many places across the genome that affect flower size, or correlated life-history traits like time to flower. These QTL were discovered by first artificially selecting on flower size in both directions for six generations, and then crossing the large and small flowered plants (Kelly 2008; Lee 2009). The resulting F1s were then backcrossed for four generations to their donor genomes: either IM62 or IM767, both highly homozygous inbred lines derived from single seeds taken from the Iron Mountain population. In each generation, plants were recurrently selected for flower size, in order to identify QTL with consistent effect on flower size (Scoville et al. 2011a). NILs were then genotyped by capillary electrophoresis at a set of length polymorphisms to identify regions that remained polymorphic in more lines than expected (indicative of selection on flower size retaining heterozygosity). This

process identified several QTL; one such large effect QTL on chromosome 8 (hereby QTL8), has been fine-mapped to a 25kb region (Lee 2009; Scoville et al. 2011a).

This QTL includes all or part of 5 genes: Migut.H00454, a transducin/WD-40 domain-containing gene; Migut.H00455, a predicted reductase or dehydrogenase; Migut.H00456, a SAP domain-containing gene; Migut.H00457, a serine/threonine protein kinase; and Migut.H00458, a predicted phosphoglycerate mutase. None of the genes have well-characterized orthologs in closely-related model plants, so there is not an obvious flower size candidate gene among them.

In this study, we dissect the previously identified flower size QTL8 to find variants that influence flower size, utilizing the natural genetic variation captured in the sequenced Iron Mountain population inbred lines. We first whole-genome sequence the QTL8 NILs in order to identify the subset of variants in the IM population segregating in the QTL. Because the whole QTL region, and all polymorphisms therein, is completely confounded in the NILs, we then use qRT-PCR and a set of IM inbred lines to narrow the QTL region to a few loci, each containing several polymorphisms in high LD, both single nucleotide (SNPs) and indels. We use these loci to choose a larger set of lines in which the putative causal regions in the QTL are broken up. We use 3'RNA sequencing to focus on the effect of each locus on gene expression and life-history traits. We find three loci in the QTL that affect expression of one or more of the five genes. Through allele-specific expression assays, we also find that the alternative allele at a single SNP upstream of gene 455 that affects flower size decreases expression of the gene in cis. Because stable germline transformation is unavailable, we then use knockouts of homologous genes in *Arabidopsis thaliana* to look for an effect of extreme expression difference. We find minor

evidence for conservation of the effect of transcription of genes 455 and 457 on life-history phenotypes using T-DNA insertion knockouts of the orthologs in *Arabidopsis*.

Methods

Study System- Mimulus guttatus (Phrymaceae, syn. *Erythranthe guttata*), the yellow monkeyflower, grows west of the Rocky Mountains in North America from northern Mexico to Alaska. One annual population, the Iron Mountain (IM) population (Oregon, U.S.A.; 44.402217 N, –122.153317 W) has been studied extensively with regard to the genetic basis of variation in floral and life-history phenotypes, like flower size and time to flower. In 1995, Willis initiated a collection of inbred lines, each from a single seed from a distinct maternal plant sampled from IM (Willis 1999c). The lines continue to be propagated by single seed descent and are now between 6-12 generations inbred. In 2018, 187 of the IM lines were whole-genome sequenced (Troth et al. 2018).

QTL8 Nearly Isogenic Lines

We grew plants from seeds remaining from an intermediate stage in the QTL8 NIL clean-up and fine-mapping process in the University of Kansas greenhouse individually in 2.25” pots, with weekly fertilization treatments. They are best described as a cousin of the original plants used in Scoville et al. (2011). As such, we expect to have two genotypes, a small-flowered QTL8 genotype and a big-flowered QTL8 genotype, both in an IM62 background (on which the *M. guttatus* reference genome is based) (Hellsten et al. 2013). We collected leaf and meristem tissue for DNA extraction as described below, but extracted DNA using a CTAB buffer protocol optimized for *Mimulus* (Holeski et al. 2014). We made whole-genome sequencing libraries using

the Nextera DNA Library Prep kit (Illumina) per provided protocol. We sequenced the resulting libraries across two lanes of Illumina HiSeq RR-PE100bp. We mapped reads to the hard masked V2 reference genome with the bwa mem function, sorted and indexed reads with Samtools, and called variants with the UnifiedGenotyper tool in GATK (Li et al. 2009; McKenna et al. 2010; Li 2013).

Identification of an eQTL using Iron Mountain Inbred Lines (IM ILs)

We initially selected 25 of the IM ILs that differed in genotype at many sites in the QTL8 region to perform quantitative reverse-transcriptase PCR (qRT-PCR) to look for an association between flower size phenotype and expression of the 5 genes in the QTL. We planted between 49 and 119 seeds from each line individually in wells of 96-well greenhouse flats. Each flat contained seeds of 7 or 14 different lines, and each line was replicated across multiple flats with a different set of lines in each. This was done to randomize the effect of germination conditions on phenotype (both expression and flower size). We measured days to germination for each individual, and randomly tagged 9 plants per line for tissue collection. At least 14 seedlings germinated for each line (average 66.57), and we transplanted them to 2.25" pots 12-16 days after germination.

For plants that were not tagged for tissue collection, we measured day of first flower, flower size (both corolla width and pistil length), and plant height on day of first flower. We averaged these phenotypes within a line to compare average phenotype to average gene expression.

For tagged plants, we collected bud tissue from the first flowering node. Because the lines differ in developmental timing, branching phenotype, size of bud, length of time between budding and

anthesis, etc. we chose a recognizable and consistent developmental stage to collect tissue, when buds at the second flowering node were visible. We collected 3 plants' buds into each of 3 replicate tubes, for a more consistent average expression in each replicate. Buds were collected randomly into each tube so that tubes weren't weighted with regard to flowering time phenotype. We collected tissue into liquid nitrogen and ground it finely with a plastic micropestle before storage at -80°C. We extracted RNA using the Qiagen RNeasy Plant Mini Kit (Hilden, Germany) per the provided protocol. We removed DNA contamination using the Turbo DNA-free Kit (Invitrogen, Carlsbad CA, USA) and converted RNA to cDNA using the RevertAid First Strang cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham MA, USA).

For qRT-PCR on the cDNA libraries, we designed primers for each of the 5 QTL8 genes and the housekeeping gene using Primer3 (Untergasser et al. 2012) (Table S3.1). We chose glyceraldehyde 3-phosphate dehydrogenase (GAPDH, gene ID Migut.I00488) as a housekeeping gene because of its demonstrated consistency in expression across tissue types and genotypes in M. guttatus using RNAseq (Stanton et al. 2017). We chose primer sequences in regions that were invariable between the 30 lines using the whole-genome sequence data so that they would amplify across all genotypes. Amplicons were between 183bp and 304bp and spanned large introns so that remaining genomic DNA contamination would be unlikely to inflate expression estimates. We performed qRT-PCR reactions using Fast SYBR Green Master Mix, and a StepOnePlus Real-Time PCR System (both Thermo Fisher Scientific, Waltham MA, USA), using the reaction conditions in Table S3.2.

We also performed a dilution series with a standard sample for each primer set in order to calculate primer efficiency (reported in Table S3.1). We estimated expression levels using the $2^{-\Delta\Delta Ct}$ method (using the primer efficiency instead of 2) by first calculating the expression difference between each gene and GAPDH, and then standardizing each sample to a reference sample that was included in each qRT-PCR plate (Schmittgen and Livak 2008). Estimated expression was averaged for each line. We used a linear regression model implemented in Minitab to test for an association of expression and each trait.

To test for an effect of genotype at each polymorphism in QTL8, we used the MLM function in TASSEL (Bradbury et al. 2007). This function incorporates a relatedness matrix to account for some lines being more similar across the genome than others. We tested genotype as a predictor of all phenotypes, both expression and life-history traits. For loci that showed an effect, we used Hapmap in TASSEL to look for linkage between individual SNPs and indels.

3'Sequencing

Using the Hapmap identified blocks of polymorphisms in high LD within a locus (Figure 3.1), we chose an additional 47 lines that differed at the four loci in as many different combinations as were present for 3' RNA-sequencing (3'seq) and included 25 of the lines from the qRT-PCR experiment. We grew plants, collected late-stage bud tissue and extracted RNA in the same manner as above, but did not DNase treat the new samples, as it is not recommended for Lexogen Quantseq (discussed in manufacturer's protocol). We also planted additional non-collected plants for measuring average flower size and time to flower phenotypes for each line. We generated sequencing libraries using the QuantSeq 3'mRNA-Seq Library Prep Kit for

Illumina (Lexogen, Vienna, Austria) per protocol, and we sequenced on a NextSeq flow cell with HO-SR75bp reads (Illumina, San Diego CA, USA). We used the QuantSeq analysis pipeline (BlueBee), which is recommended by Lexogen for analyzing 3'seq data. BlueBee included quality control, read trimming, and mapping to the *M. guttatus* reference genome, and the output was a table of read counts for each annotated monkeyflower gene. We calculated expression of each QTL8 genes as the number of reads mapping to each gene divided by the total reads mapped, since this is 3'seq data where calculating something like RPKM is not necessary. We estimated associations between genotype and phenotype (either expression or floral trait) using an ANOVA in Minitab.

Allele-specific expression

To assess whether the apparent effect of genotype at Loci 2 and 4 within QTL8 on expression of gene 455 is a cis- or trans-effect, we measured allele-specific expression. We first identified pairs of lines that differed at a locus and also at one of two (or both) SNPs in the second exon of the coding sequence of 455 at positions 2774708 and 2774790. This yielded 3 pairs of lines for each locus. We crossed each pair reciprocally and planted reciprocal progeny separately in 5.25"x3.5" pots, transplanting them to individual 2.25" pots when they had two true leaf pairs. We collected tissue for RNA from late stage buds in the same manner as described above for each of the three earliest flowering plants on each side of each cross. We also collected leaf and meristem tissue for DNA extraction from the same plants. Tissue for DNA was stored at -20°C until it was ground in liquid nitrogen using 3.2mm chrome-steel beads (Biospec Products, Bartlesville OK, USA, catalog number 11079132c) and a modified reciprocating saw (Alexander et al. 2007). We extracted RNA, DNase treated the RNA, and converted it to cDNA as described above. We

extracted DNA from the leaf tissue using the Qiagen DNeasy Plant mini kit with some modifications to the supplied protocol: we used twice as much of buffers AP1 and P3 (because more *M. guttatus* tissue is required for the same yield), and we respun the eluted DNA through the column a second time to increase yield.

We designed primers to flank the 2 SNPs in gene 455 using Primer 3 (sequences in Table S3.3), making sure that they didn't span an intron so that the gDNA and cDNA amplicons would be identical. We added MSEI restriction enzyme cut sites on to each primer and amplified the 177bp sequence in all samples using LongAmp *Taq* DNA Polymerase (New England Biolabs, Ipswich MA, USA) and PCR conditions listed in Table S3.4. We made sequencing libraries of 2-3 technical replicates of each of 6 biological replicates per family using a Multiplexed Shotgun Genotyping method (Andolfatto et al. 2011) utilizing the added MSEI cut sites, and omitting the size selection step. We sequenced these libraries on an Illumina MiSeq-V3 PE75bp. We mapped the reads to the *M. guttatus* V2 reference genome using the mem function in bwa (Li 2013). We used mpileup in Samtools and Varscan to call variants (Koboldt et al. 2009; Li et al. 2009). We used the percent of reads with the alt allele (averaged between technical replicates for each sample) for an ANOVA in Minitab to test for differences between DNA and RNA samples. RNA reads were tested against DNA to account for possible PCR bias.

Arabidopsis synteny

A simple BLAST (ncbi) established that the *Arabidopsis thaliana* genes with the highest sequence identity with QTL genes 454, 455, 457, and 458 are syntenic. They are present in the same order in a 31.5kb region on Chromosome 3. To confirm orthology, we built gene trees

using other sequence similar genes in monkeyflower, Arabidopsis, maize (*Zea Mays*), rice (*Oryza sativa*), potato *(Solanum tuberosum*), tomato (*Solanum lycopersicum)*, and soybean *(Glycine max)* (Figure S3.1). We used MAFFT to do the alignment and the "simple phylogeny" tool in the ClustalW2 package to make the tree (both through EMBL-EBI). We used the following options to make the tree: Tree format = NEXUS, Distance correction = on, Exclude gaps = on, Clustering method = neighbor joining, PIM = off. We visualized the tree using Figtree (Rambaut 2012). The putative homolog of QTL gene 456 is elsewhere in the Arabidopsis genome.

We ordered tDNA insertion knockout mutants of each of the four syntenic orthologs from the ABRC (line IDs in Table S3.5) (Sessions et al. 2002; Alonso et al. 2003). We grew the Arabidopsis knockouts in the University of Kansas greenhouse individually in 2.25" pots, as well as the ecotype in which each of the knockouts was generated for comparison (Col-0 or Col-3). We measured time to first flower, rosette width at time of first flower, height at time of first flower, and average silique length of the first 10 siliques on the primary inflorescence. Silique length is known to correlate with seed number (Roux et al. 2004), so we used that phenotype as a proxy for fitness. We used an ANOVA in Minitab to analyze the effect of knockout on phenotype.

Results

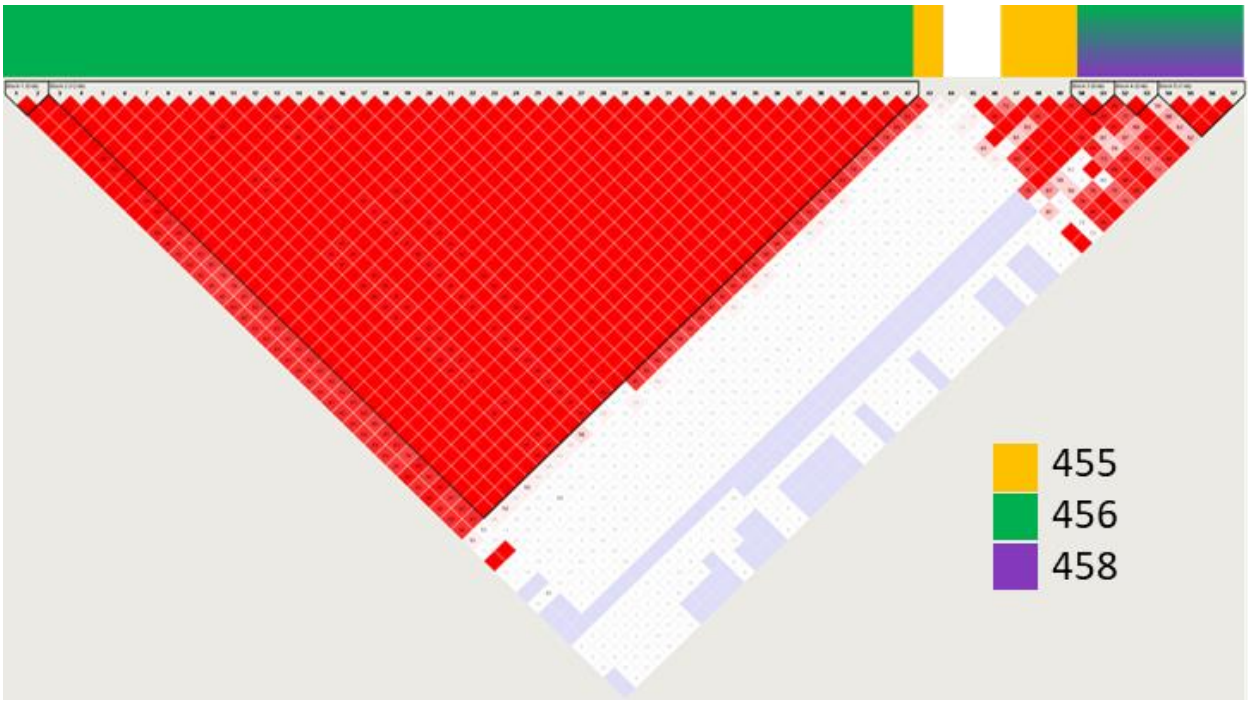
NILs- By whole-genome sequencing the QTL8 NIL cousins, we verified that the QTL region is segregating between them. Additionally, we found several other regions throughout the genome

of residual segregating polymorphism that had not been eliminated in the backcrossing. We can confirm that the polymorphisms that make up the QTL are a subset of the variation in the Iron Mountain population (as evidenced by the IM inbred lines). In trying to find causal loci, however, the NILs are an inferior resource to the IM lines, because the QTL is completely confounded. There are no other combinations of different polymorphisms, only all reference allele or all alternative allele across the whole QTL. In other words, for any two SNPs in the QTL, the reference allele at one only exists with the reference allele at the other. So, we turn to the inbred lines to break up the region, using expression as an intermediate phenotype predicted to link genotype and life-history phenotype.

IM Inbred Line qRT-PCR

Estimating gene expression with qRT-PCR on the initial set of 25 IM inbred lines, we find no significant effects of expression level on any measured life-history phenotypes. However, by fitting a mixed linear model (MLM) in TASSEL, we do find some individual polymorphisms within QTL8 with an effect on either expression of one of the five QTL genes, a life-history phenotype, or both. Within the larger collection of 187 sequenced lines from Troth et al. (2018), these polymorphisms are linked in four loci with high LD (Figure 3.1). From a completely confounded QTL, we managed to break up the region into smaller pieces, with just a small selection of the variation available in the IM lines. We then used these loci to choose additional lines with as many different combinations of reference and alternative alleles to do the more thorough 3’seq experiment.

Figure 3.1- Blocks of polymorphisms in high LD in QTL8. Linkage was established with the full collection of 187 lines. Colored bars on top indicate in which gene the polymorphisms in the locus affected expression.



3'seq results on QTL8-specific genes-

With the better resolution of 3'seq and the additional lines, we now have the power to detect effects of expression of QTL8 genes on life-history traits, and also loci with a putatively causal effect on both expression and traits. With lines that vary as much as possible among the potentially important loci within the QTL, we uncover various effects of both expression and genotype on traits, as well as genotype on expression (Tables 3.1-3.3, Figure 3.2). Using expression alone as predictors of phenotype, one of each of the five genes affects at least one measured trait (Table 3.1). However, when genotype at each locus is controlled for in an ANCOVA, some of those effects become nonsignificant (Table 3.4).

Notably, the alt allele at Locus 2, which is a single SNP at position 2770858, decreases gene 455 expression and corolla width (Tables 3.2 and 3.3), which is consistent with the positive regression between 455 expression and days to germination, even when genotype is included as a factor (Table 3.4). This is also consistent with the results from the qRT-PCR experiment: Locus 2 was identified because the alt allele had a negative effect on 455 expression, that was just barely nonsignificant when correcting for multiple testing. Locus 2 is the only factor with a significant effect on corolla width in the ANCOVA, for which QTL8 was originally mapped. While there are many potential effects of gene expression on life-history traits, the QTL was mapped due to a genetic variant, and only Locus 2 shows a significant effect on any trait when gene expression is controlled.

Table 3.1- Multiple regression of gene expression on life-history phenotypes. Expression levels of all genes were considered simultaneously. Grow-up was included in the model as a random factor. P-values are given for each test, an F-statistic is given in parentheses for each significant test, with the direction of correlation given by an arrow.

Expression	Days to Germination	Days to Flower	Flowering Time (DTF-DTG)	Corolla Width	Pistil Length	Height
454	**0.038 (4.47) ↓**	0.073	0.256	0.064	**0.014 (6.41) ↓**	**0.010 (6.96) ↓**
455	**0.005 (8.66) ↑**	0.487	0.654	0.176	0.803	0.400
456	**0.044 (4.24) ↑**	0.071	0.322	0.225	0.939	0.053
457	0.301	0.062	0.062	**0.018 (5.92) ↑**	0.081	0.113
458	0.388	**0.014 (6.44) ↑**	**0.018 (5.86) ↑**	0.472	0.345	0.445

Table 3.2- ANOVA p-values for the effect of genotype at each locus of SNPs on life-history phenotypes. All loci were considered simultaneously. Grow-up was included in the model as a random factor. P-values are given for each test, an F-statistic is given in parentheses for each significant test, with the effect of the alt allele given by the arrow.

Locus	Days to Germination	Days to Flower	Flowering Time (DTF-DTG)	Corolla Width	Pistil Length	Height
1	**0.018 (6.03) ↓**	0.796	0.425	0.556	0.871	0.183
2	0.413	0.497	0.468	**0.009 (7.52) ↓**	0.106	0.364
3	0.268	0.833	0.694	0.529	0.769	0.172
4	0.224	0.361	0.124	0.656	0.287	0.247

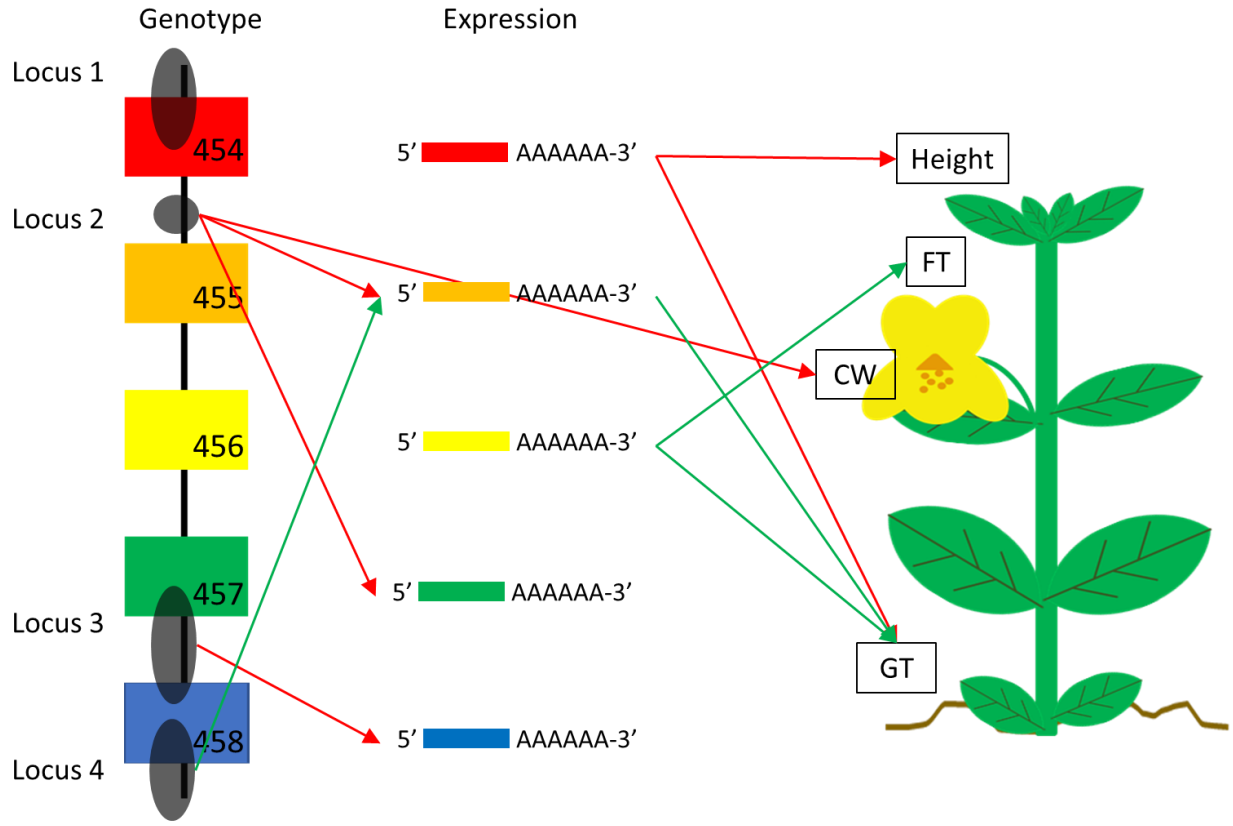
Table 3.3- ANOVA p-values for the effect of genotype at each locus of SNPs on expression. All loci were considered simultaneously. Grow-up was included in the model as a random factor. P-values are given for each test, an F-statistic is given in parentheses for each significant test, with the effect of the alt allele given by the arrow.

Locus	454 Expression	455 Expression	456 Expression	457 Expression	458 Expression
1	0.463	0.449	0.248	0.289	0.829
2	0.576	**0.010 (7.25) ↓**	0.938	**0.036 (4.69) ↓**	0.457
3	0.737	0.225	0.067	0.855	**0.050 (4.07) ↓**
4	0.766	**0.013 (6.76) ↑**	0.582	0.973	0.465

Table 3.4- ANCOVA p-values for the effect of either genotype at each locus or expression of each gene on life-history phenotypes. Grow-up was included in the model as a random factor. P-values are given for each test, an F-statistic is given in parentheses for each significant test, with either the direction of correlation or the effect of the alt allele given by the arrow.

Predictor	Days to Germination	Days to Flower	Flowering Time (DTF-DTG)	Corolla Width	Pistil Length	Height
454exp	**0.007 (8.15) ↓**	0.086	0.375	0.204	0.129	**0.018 (6.16) ↓**
455exp	**0.003 (10.15) ↑**	0.153	0.644	0.340	0.766	0.541
456exp	**0.039 (4.59) ↑**	**0.021 (5.83) ↑**	0.093	0.431	0.787	0.219
457exp	0.191	0.493	0.233	0.903	0.931	0.468
458exp	0.458	0.535	0.374	0.325	0.867	0.802
Locus 1	0.125	0.661	0.290	0.661	0.979	0.194
Locus 2	0.154	0.239	0.372	**0.010 (7.52) ↓**	0.120	0.620
Locus 3	0.301	0.908	0.713	0.737	0.729	0.464
Locus 4	0.697	0.267	0.186	0.869	0.260	0.399

Figure 3.2- Various effects of genotype or expression. Genotype image is not to scale, but relative position of loci is accurate. Phenotypes are Height, Flowering Time (FT), Corolla Width (CW), and Germination Time (GT). Only effects that were significant in the ANCOVA analysis are shown. Green arrows indicate a positive correlation or positive effect of the alt allele, red arrows indicate a negative correlation or negative effect of the alt allele.



Because gene 455 expression is antagonistically affected by two different Loci (2 and 4) and retains an effect on a trait (germination time) even when genotype is controlled (Table 3.4), it became a good target for asking how genotype within the QTL can influence gene regulation.

Allele-specific expression-

We generated F1 progeny between inbred lines that differed at either Locus 2, Locus 4, or both to test for cis-regulatory effects of the alt allele on expression of gene 455. We find no effect of parent on expression in any cross, indicating no parental bias in expression. We do find lower expression of 455 from the parent with the alt allele (evidence of cis-regulation) at Locus 2, but only in two of the three different crosses ($p = 0.006$ and 0.009 for cross 851x709 and Z247x709 respectively), indicating influence of the genetic background (Figure 3.3A, Table 3.5). This is consistent with the above result that the alt allele at Locus 2 negatively affects 455 expression.

For F1s heterozygous at Locus 4, we see no evidence of cis-regulation in the two crosses that vary only at Locus 4 (420x923 and 62xZ503). The only F1 family that does show a decrease in 455 expression, 851x709, is also heterozygous at Locus 2 (Figure 3.3B). The supports a trans-regulatory effect of Locus 4 on expression of gene 455.

Figure 3.3- Frequency of reads mapping to the amplified region that contained the alt allele at either the first or second SNP in gene 455. Crosses in panel A are heterozygous at Locus 2 and crosses in panel B are heterozygous at Locus 4. In all cases the alt allele within gene 455 is on the same chromosome as the alt allele at either locus (from the same parent). Read frequencies shown for genomic DNA are for comparison and to account for PCR bias.

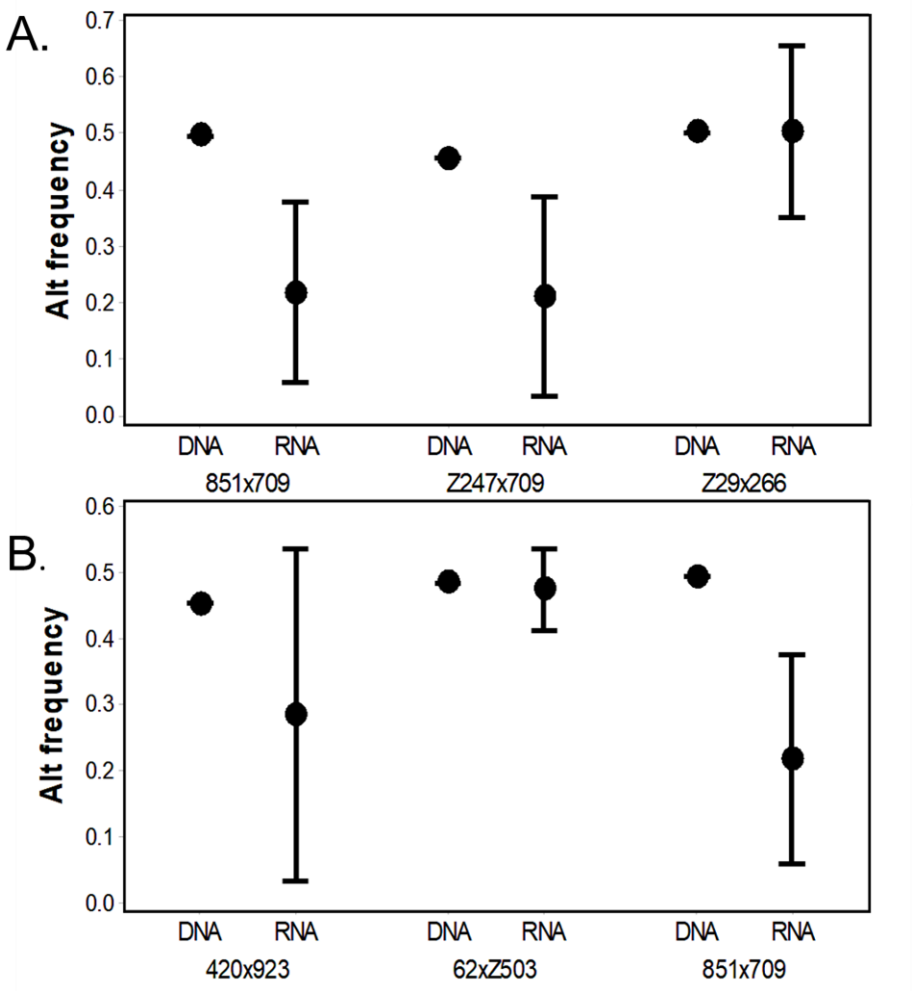


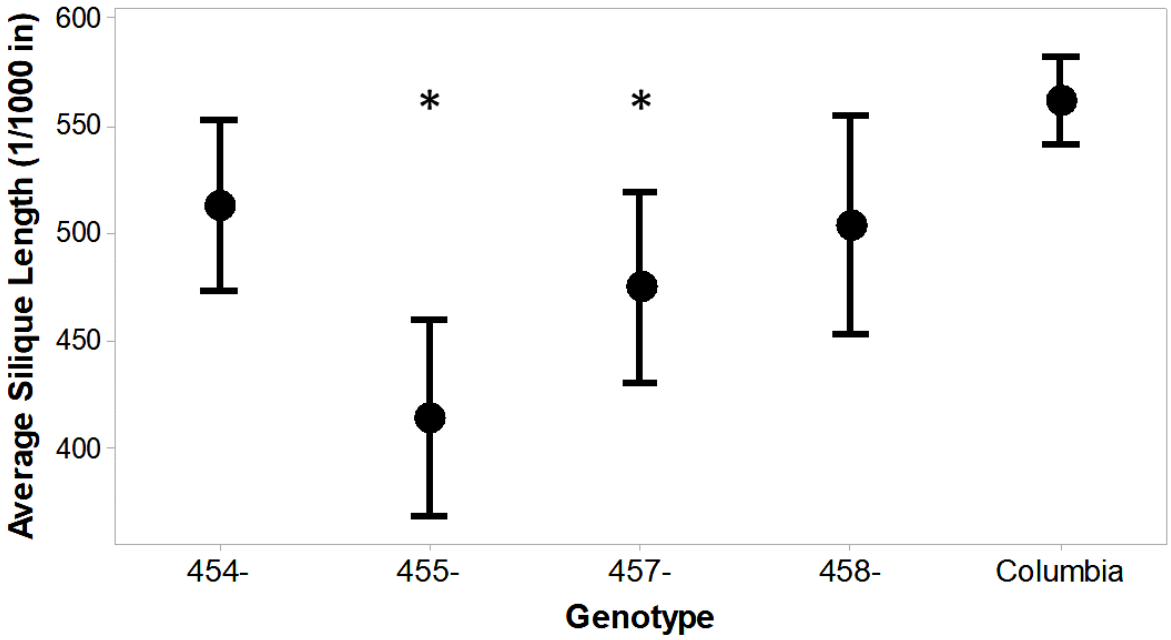
Table 3.5- Results of a one-sample t-test comparing the alt-allele expression of gene 455 to a null hypothesis mean of 0.5 (no allele-specific expression). Significant tests are bolded.

Cross	Locus	T-value	P-value
851x709	**Both**	**-4.55**	**0.006**
Z247x709	**2**	**-4.19**	**0.009**
Z29x266	2	0.07	0.949
420x923	4	-2.21	0.078
62xZ503	4	-1.03	0.349

Arabidopsis orthologs-

In *Arabidopsis thaliana*, knocking out some of the putative orthologs of *M. guttatus* QTL8 genes has some significant effects on life-history and fitness related phenotypes. The knockout of gene AT3G50560, the ortholog (see supplemental gene trees) of Migut.H00455, significantly increases rosette width at time of first flower ($F = 9.32$, $p = 0.003$) and significantly decreases average silique length ($F = 36.75$, $p < 0.000$) (Figure 3.4). The knockout of gene AT3G50530, the putative ortholog of Migut.H00457, significantly decreases average silique length ($F = 7.01$, $p = 0.009$) (Figure 3.4). All tests were done as pairwise comparisons between a single knockout and its specific background ecotype. No other knockouts/phenotype combinations tested showed significant effects compared to the background genotypes (full statistics in Table S3.6). Silique length is our proxy phenotype for fitness, and the most analogous measured trait we have in *Arabidopsis* to flower size in *Mimulus*, since both are correlated with seed set. Regarding the effect of knocking out either gene 455 or 457 orthologs, not only does it decrease average silique length, but changes the distribution (Figure S3.2).

Figure 3.4- Effects of knocking out QTL8 homologs in Arabidopsis on average silique length. Although knockouts were in two different backgrounds (Col-0 and Col-3), the two background ecotypes did not significantly differ for silique length (one-way ANOVA, p = 0.084, F = 3.05), so the pictured comparison is to the average of both backgrounds. Statistics in Table S3.4 are testing each knockout against its own background in a pairwise fashion. Asterisks indicate significant tests.



Discussion

Methods for determining the genetic mechanisms governing variation in a trait of interest, like GWAS and QTL-mapping, are sometimes limited in resolution. For drastic, whole-organism level single-gene trait effects, or in model systems with well annotated genomes, candidate genes are sometimes easy to guess and test (the "low-hanging fruit" approach). In many such cases, the causal locus has been identified as a loss of function mutation and confirmed to be sufficient to cause the phenotypic change (Doebley et al. 2006). However, for more subtle phenotypic changes, such as quantitative variation within a population, issues of polygenic inheritance and a prevalence of regulatory modifications can complicate the approach to identifying causal variation. In this study, we demonstrate that the path from genotype to phenotype is complicated for quantitative traits, and it can be mediated by gene expression phenotypes.

We began this experiment with the results of a painstaking effort to fine-map QTL8, which resulted in narrowing down the region to 25kb. In other species with better established functional genetic resources, producing the knockouts of each of the five genes in the QTL may have been a reasonable next step to identifying causal variants. However, this technique would assume responsibility of a loss-of-function mutation. Instead, we have chosen to use gene expression as an intermediate phenotype, knowing that the polygenic nature of quantitative traits often results in causal regulatory changes. From a single QTL discovered for its effect on a single trait, we reveal an explosion of complexity by using natural variation to deconvolute the region, and to exploit a varied combination of genetic polymorphisms within the QTL.

In the qRT-PCR experiment, we started with the entirety of QTL8 including all five genes. With the limited set of randomly chosen lines, we lacked the power and genetic diversity at important loci to detect a correlation between gene expression and life-history traits. However, by using the information that some genetic polymorphisms seemed to affect both expression and life-history traits, we were able to identify 54 polymorphisms (both SNPs and indels), clustered together in four loci with high LD. These loci provided the criteria for choosing the additional lines on which to perform the more thorough and precise 3'seq experiment. Within the 187 sequenced lines, we identified a set that differed at the four loci in as many ways as possible, with as many combinations of alternative and reference alleles as existed.

By focusing in on a limited set of genetic variants, it becomes quite clear that there are many varied interactions between genotype, expression, and trait within even this small genomic region. On their own, every gene's expression affects at least one phenotype (Table 3.1). Many of those effects are negated when genotype is controlled (Table 3.4). Less widespread are the effects of genotype on traits, with only Locus 2's effect on corolla width remaining a significant predictor. Locus 2, which is only a single SNP, is only 3,162 bp upstream of the transcription start site of gene 455, well within the reasonable range for a transcriptional regulation sequence in plants (Weber et al. 2016). It was this proximity that prompted the allele-specific expression assay. Since 455 expression is also apparently affected by Locus 4, which is more than 15kb downstream of gene 455, we also tested its effects. Due to the differences in proximity, we expected Locus 2 to have a cis-regulatory effect and Locus 4 to have a trans-regulatory effect, which is what we found (Figure 3.3).

Consistent with the findings of Bernstein et al. (2018) in *C. elegans*, we also uncovered examples of antagonistic effectors located within our QTL. Loci 2 and 4 affect expression of gene 455, but in opposing, and almost equal, ways (coefficient for Locus 2 = $-3x10^{-6}$, coefficient for Locus 4 = $4 x10^{-6}$). Also, although the original QTL was mapped for flower size and not germination time, we found that expression of genes 454, 455, and 456 all affect germination time, also in opposing ways, even when controlling for genotype (Table 3.4).

In the absence of functional genetic confirmation, it can be potentially useful to look for conservation of function in other systems. Synteny is sometimes conserved across deep taxonomic time scales, but it is unclear if gene function is as likely to be conserved. In *Arabidopsis thaliana*, putative orthologs of genes in our flower size QTL also seem to affect the fitness-related trait silique length.

It is also important to note that we did verify that the loci we identified varied between the two QTL8 genotypes in the NILs used for fine-mapping. If that had not been the case, we would have identified additional naturally segregating variants in the same region affecting the same traits, but absent in the mapping population.

The results of this work demonstrate one method for using natural variation to sequentially dissect a QTL into pieces to identify regions within that affect a trait of interest. It also highlights the degree to which complex traits are polygenic, and the resulting complications in mapping genotype to phenotype. Perhaps most interestingly, using this approach we were able to identify that Locus 2 is a potential quantitative trait nucleotide (QTN), the effect of which is perhaps

mediated through expression of gene 455. This would be a good candidate for functional genetic work, if a technique for reliable stable germline transformation in *Mimulus guttatus* is developed.

Supplementals

Table S3.1- Primer sequences for qRT-PCR on IM ILs. The dilution series for H00457 did not work properly, so we used 2 as the prime efficiency in calculations.

Gene	Forward	Reverse	Amplicon length	Primer efficiency
GAPDH	TTGAAGGGAATCTTGGGCTA	CATTTGACGTACCATAAACGAGTA	242bp	1.77
H00454	TTGGCCAAGGATCAATCTCG	TTCGAGCATCTGTTTGGCAT	273pb	1.77
H00455	TGCCTTGCTAGAGAGTTCCA	GTCCATTACACCATCCCCAC	183bp	1.8
H00456	GGCCGCAACCTCTATAGAAT	CGGAACTTTGACCAGGACTG	304bp	1.82
H00457	AAGAAAGGGGAGCTCAAAGG	TTATCTAAAAGCTCGCCTCCTT	218bp	2*
H00458	ATACCAGCGTTCCACATCAG	CTCACATCACCCCACGATTT	213bp	1.71

Table S3.2- PCR conditions for qRT-PCR

Step	Temperature	Number of Cycles
Holding Stage	50°C	2 minutes
	95°C	2 minutes
Cycling Stage (40 cycles)	95°C	15 seconds
	55°C	15 seconds
	72°C	30 seconds
Melt Curve Stage	95°C	15 seconds
	55°C	1 minute
	95°C	15 seconds

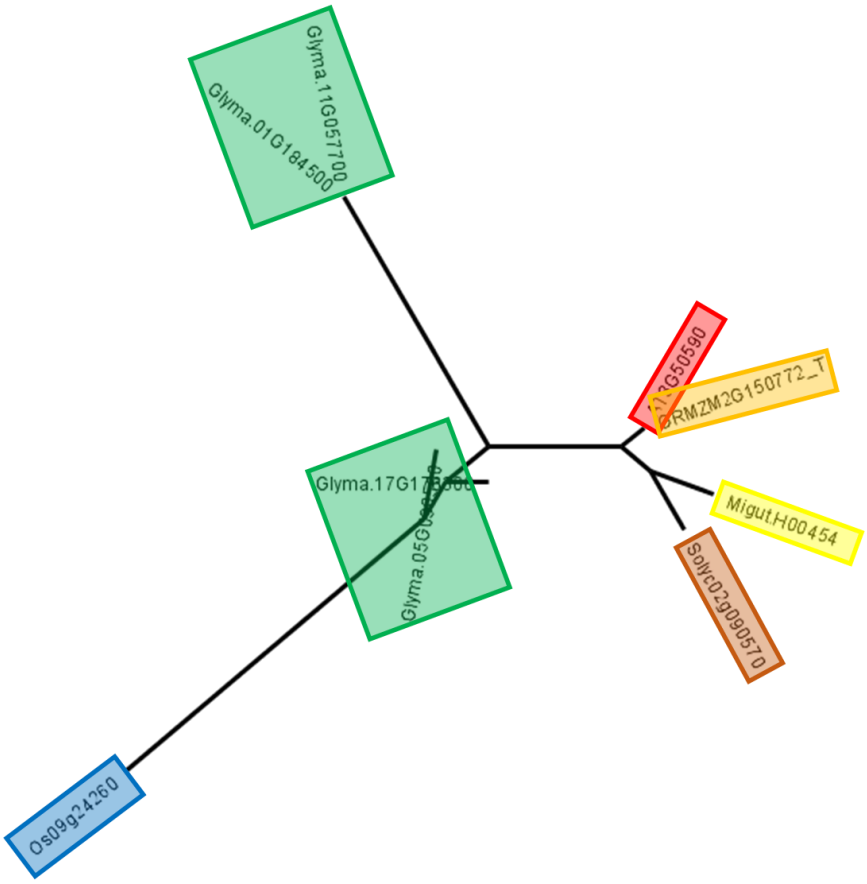
Table S3.3- Primers for allele-specific expression, sequence in blue is the added MSEI cut site, and sequence in gray is added random nucleotides to facilitate binding by the restriction enzyme.

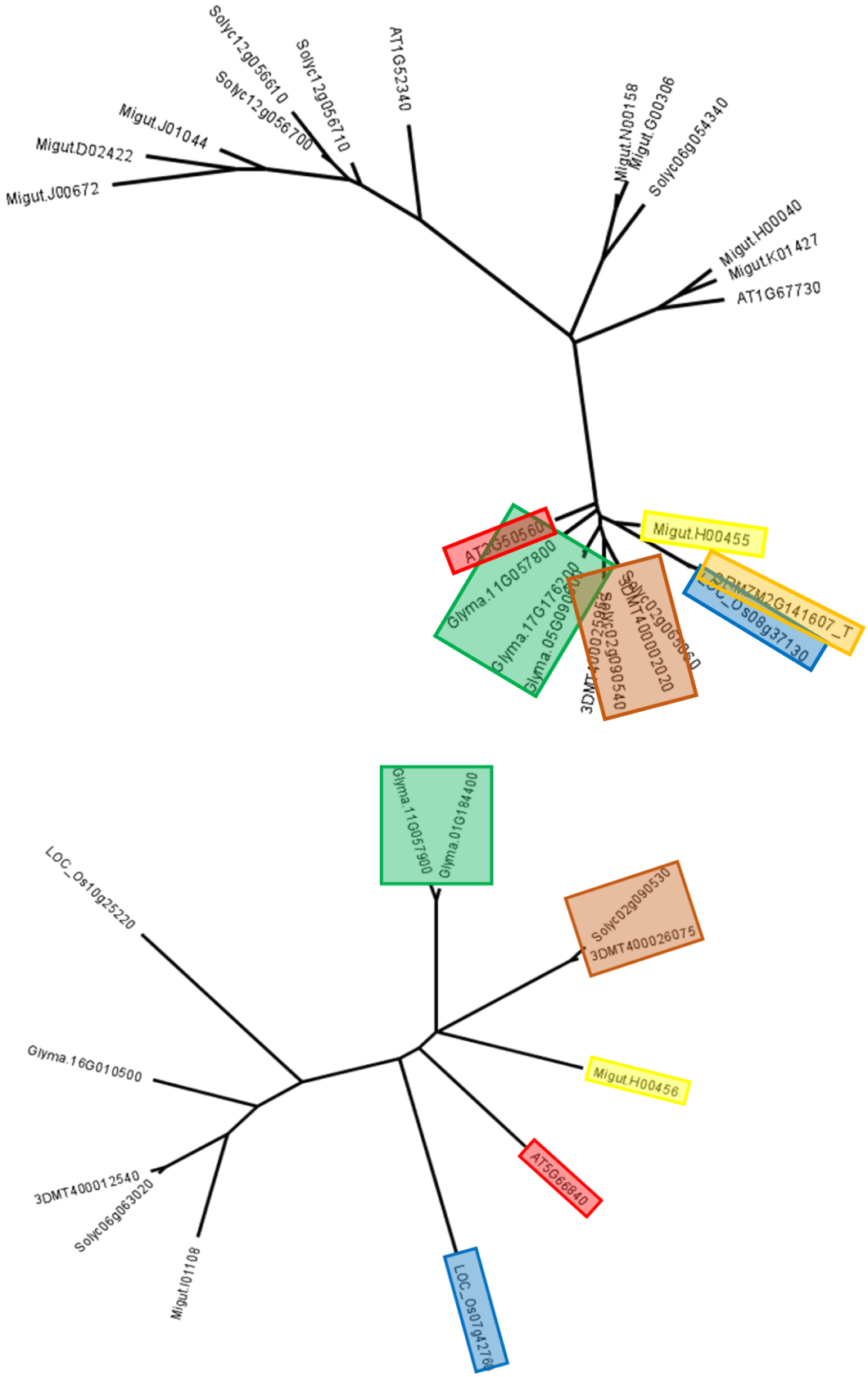
Forward primer	Reverse primer	Amplicon
CGATTTAAGGATCGACTGCTCGGAATC	TAGCTTAAAAACGGCGAGGGATTTCT	177bp

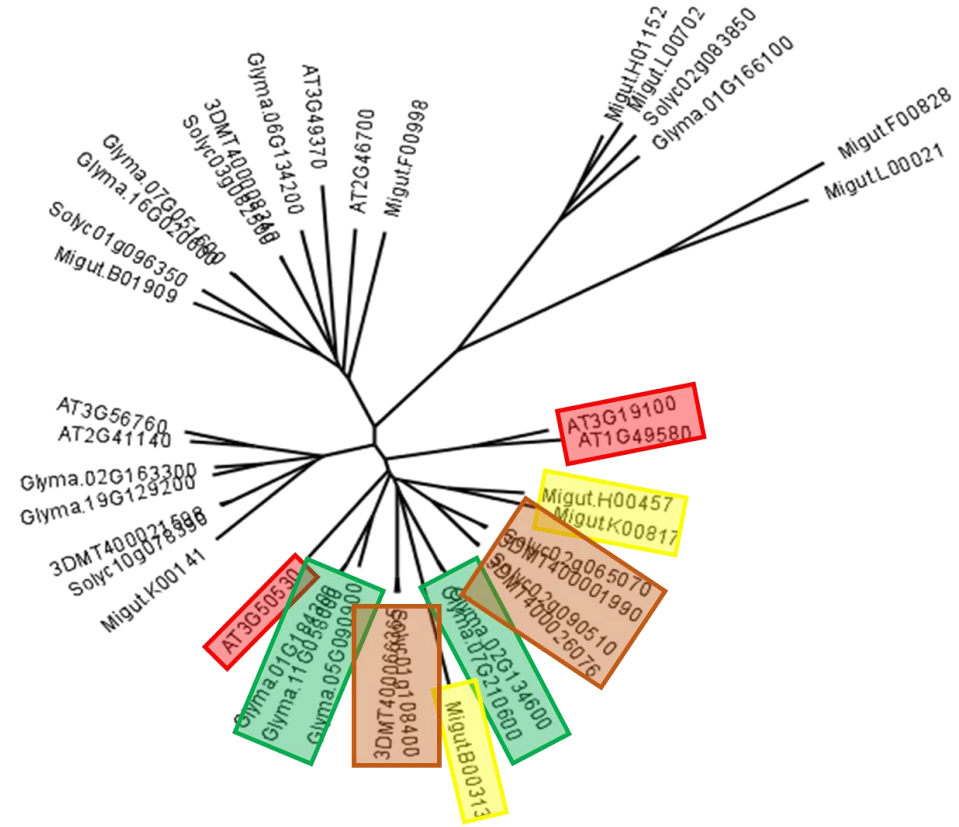
Table S3.4- PCR conditions for Allele-specific expression

Step	Temperature	Time
Denaturation	94°C	30 seconds
30 cycles	94°C 60°C 65°C	30 seconds 30 seconds 10 seconds
Final Extension	65°C	10 minutes

Figure S3.1- Gene trees to demonstrate Arabidopsis synteny




Solyc12g056610
Solyc12g056700
Solyc12g056710
AT1G52340
Migut.J01044
Migut.D02422
Migut.J00672
Migut.N00158
Migut.G00306
Solyc06g054340
Migut.H00040
Migut.K01427
AT1G67730
AT2G50560
Glyma.11G057800
Glyma.17G176200
Glyma.05G090200
Migut.H00455
Solyc02g090540
3DMT400025980
3DMT400002020
LOC_Os08g37130
Glyma.11G057900
Glyma.01G184400
LOC_Os10g25220
Solyc02g090530
3DMT400026075
Glyma.16G010500
Migut.H00456
3DMT400012540
Solyc06g063020
Migut.I01108
AT5G66840
LOC_Os07g42760


Migut.H01152
Migut.L00702
Solyc02g083850
Glyma.01G166100
Migut.F00828
Migut.L00021
AT3G49370
AT2G46700
Migut.F00998
Glyma.06G134200
Solyc01g096350
Migut.B01909
AT3G56760
AT2G41140
Glyma.02G163300
Glyma.19G129200
Migut.K00141
AT3G19100
AT1G49580
Migut.H00457
Migut.K00817
AT3G50530
Glyma.02G134600
Glyma.07G210600
Migut.B00313

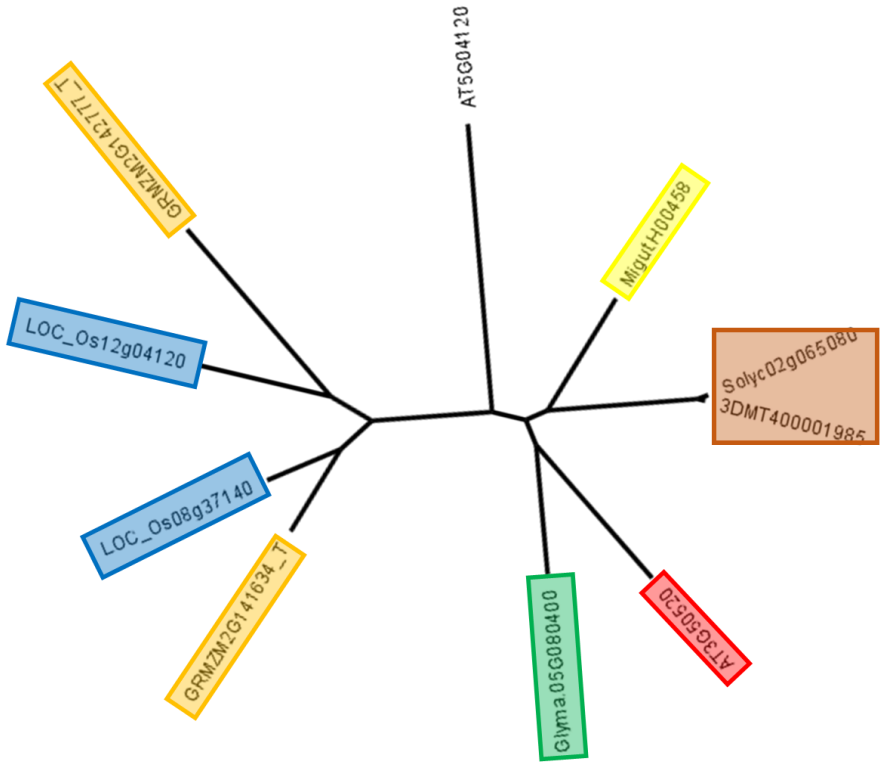

AT5G04120
GRMZM2G142777_T
LOC_Os12g04120
LOC_Os08g37140
GRMZM2G141634_T
Migut.H00458
Solyc02g065080
3DMT400001985
Glyma.05G080400
AT3G50530

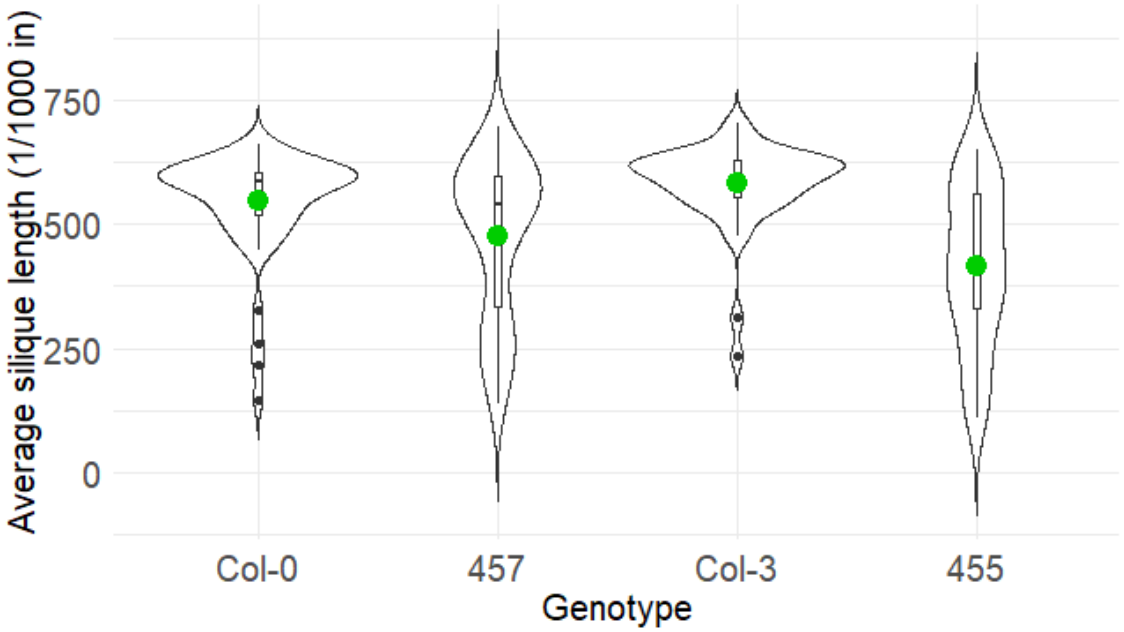
Table S3.5- Arabidopsis stock lines grown for synteny

Stock	Knockout	Background
CS875145	AT3G50530 (457 homolog)	Columbia-0
CS846930	AT3G50590 (454 homolog)	Columbia-0
CS28171	None	Columbia-3
SALK_000997C	AT3G50520 (458 homolog)	Columbia-0
CS819551	AT3G50560 (455 homolog)	Columbia-3
CS22681	None	Columbia-0

Table S3.6- p-values for one-way ANOVAs for each trait measured in the Arabidopsis synteny experiment. F-statistics are given in parentheses for significant tests, and the direction of effect is indicated by the arrow.

Trait	454 KO	455 KO	457 KO	458 KO
DTF	0.455	0.096	0.556	0.235
RW	0.322	**0.003 (9.32)** ↑	0.531	0.765
Height	0.183	0.397	0.695	0.591
SL	0.184	**0.000 (36.75)** ↓	**0.009 (7.01)** ↓	0.127

Figure S3.2- Violin plots of the effect of knocking out either the Arabidopsis thaliana homolog of gene 457 or gene 455 on average silique length.



References

Alexander, P. J., G. Rajanikanth, C. D. Bacon, and C. D. Bailey. 2007. Recovery of plant DNA using a reciprocating saw and silica-based columns. Molecular Ecology Notes 7:5-9.

Alonso, J. M., A. N. Stepanova, T. J. Leisse, C. J. Kim, H. Chen, P. Shinn, D. K. Stevenson, J. Zimmerman, P. Barajas, and R. Cheuk. 2003. Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science 301:653-657.

Anderson, J. T., C. R. Lee, and T. Mitchell-Olds. 2011. Life history of QTLs and natural selection on flowering time in *Boechera stricta*, a perennial relative of *Arabidopsis*. Evolution 65:771-787.

Anderson, J. T., C. R. Lee, and T. Mitchell-Olds. 2014. Strong selection genome-wide enhances fitness trade-offs across environments and episodes of selection. Evolution 68:16-31.

Anderson, J. T., C. R. Lee, C. A. Rushworth, R. I. Colautti, and T. Mitchell-Olds. 2013. Genetic trade-offs and conditional neutrality contribute to local adaptation. Mol. Ecol. 22:699-708.

Andolfatto, P., D. Davison, D. Erezyilmaz, T. T. Hu, J. Mast, T. Sunayama-Morita, and D. L. Stern. 2011. Multiplexed shotgun genotyping for rapid and efficient genetic mapping. Genome research 21:610-617.

Arathi, H., A. Rasch, C. Cox, and J. K. Kelly. 2002. Autogamy and floral longevity in Mimulus guttatus. International Journal of Plant Sciences 163:567-573.

Austen, E. J., L. Rowe, J. R. Stinchcombe, and J. R. Forrest. 2017. Explaining the apparent paradox of persistent selection for early flowering. New Phytologist.

Awadalla, P. and K. Ritland. 1997. Microsatellite variation and evolution in the Mimulus guttatus species complex with contrasting mating systems. Molecular Biology and Evolution 14:1023-1034.

Baker, H. G. 1955. Self-compatibility and establishment after "long-distance" dispersal. Evolution 9:347-349.

Barton, N. H., A. M. Etheridge, and A. Véber. 2016. The infinitesimal model. bioRxiv:039768.

Bates, D., D. Sarkar, M. D. Bates, and L. Matrix. 2007. The lme4 package. R package version 2:74.

Bennington, C. C. and J. B. McGraw. 1995. Phenotypic selection in an artificial population of *Impatiens pallida*: the importance of the invisible fraction. Evolution:317-324.

Berlocher, S. H. and J. L. Feder. 2002. Sympatric speciation in phytophagous insects: moving beyond controversy? Annual review of entomology 47:773-815.

Bernstein, M. R., S. Zdraljevic, E. C. Andersen, and M. V. Rockman. 2018. Tightly-linked antagonistic-effect loci underlie polygenic demographic variation in C. elegans. BioRxiv:428466.

Bertin, R. I. and C. M. Newman. 1993. Dichogamy in angiosperms. The Botanical Review 59:112-152.

Boyle, E. A., Y. I. Li, and J. K. Pritchard. 2017. An expanded view of complex traits: from polygenic to omnigenic. Cell 169:1177-1186.

Bradbury, P. J., Z. Zhang, D. E. Kroon, T. M. Casstevens, Y. Ramdoss, and E. S. Buckler. 2007. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633-2635.

Carr, D. E. and M. R. Dudash. 1995. Inbreeding depression under a competitive regime in Mimulus guttatus: consequences for potential male and female function. Heredity 75:437.

Carr, D. E. and M. R. Dudash. 1997. The effects of five generations of enforced selfing on potential male and female function in Mimulus guttatus. Evolution 51:1797-1807.

Carr, D. E. and C. B. Fenster. 1994. Levels of genetic variation and covariation for *Mimulus* (*Scrophulariaceae*) floral traits. Heredity 72:606-618.

Carr, D. E., C. B. Fenster, and M. R. Dudash. 1997. The relationship between mating-system characters and inbreeding depression in Mimulus guttatus. Evolution 51:363-372.

Carroll, S. B. 2000. Endless forms: the evolution of gene regulation and morphological diversity. Cell 101:577-580.

Carter, A. J. and A. Q. Nguyen. 2011. Antagonistic pleiotropy as a widespread mechanism for the maintenance of polymorphic disease alleles. BMC Medical Genetics 12:160.

Caspari, E. 1950. On the selective value of the alleles Rt and rt in *Ephestia kuhniella*. The American Naturalist 84:367-380.

Charlesworth, B. and D. Charlesworth. 1999. The genetic basis of inbreeding depression. Genetical Research 74:329-340.

Charlesworth, B. and K. A. Hughes. 2000. The maintenance of genetic variation in life-history traits. Pp. 369-392 *in* C. B. K. R.S. Singh, ed. Evolutionary genetics: from molecules to morphology. Cambridge University Press, Cambridge, U.K.

Charlesworth, B., M. T. Morgan, and D. Charlesworth. 1991. Multilocus Models Of Inbreeding Depression With Synergistic Selection and Partial Self-Fertilization. Genetical Research 57:177-194.

Charlesworth, D. and B. Charlesworth. 1987. Inbreeding Depression and Its Evolutionary Consequences. Annual Review Of Ecology and Systematics 18:237-268.

Charlesworth, D. and J. H. Willis. 2009. The genetics of inbreeding depression. Nature reviews genetics 10:783.

Charmantier, A., C. Perrins, R. H. McCleery, and B. C. Sheldon. 2006. Quantitative genetics of age at reproduction in wild swans: support for antagonistic pleiotropy models of senescence. Proc. Natl. Acad. Sci. 103:6587-6592.

Cingolani, P., A. Platts, L. L. Wang, M. Coon, T. Nguyen, L. Wang, S. J. Land, X. Lu, and D. M. Ruden. 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly 6:80-92.

Cole, L. C. 1954. The population consequences of life history phenomena. Q. Rev. Biol. 29:103-137.

Conner, J. K. and D. L. Hartl. 2004. A Primer of Ecological Genetics. Sinauer Associates Incorporated.

Cooper, G. M., E. A. Stone, G. Asimenos, E. D. Green, S. Batzoglou, and A. Sidow. 2005. Distribution and intensity of constraint in mammalian genomic sequence. Genome research 15:901-913.

Crow, J. F. and M. Kimura. 1970. An introduction to population genetics theory. An introduction to population genetics theory.

Curtsinger, J. W., P. M. Service, and T. Prout. 1994. Antagonistic pleiotropy, reversal of dominance, and genetic polymorphism. The American Naturalist 144:210-228.

Darwin, C. R. 1876. The effects of cross- and self-fertilisation in the vegetable kingdom. John Murray, London.

Darwin, C. R. 1877. The different forms of flowers on plants of the same species. John Murray, London.

Delph, L. F. and J. K. Kelly. 2014. On the importance of balancing selection in plants. New Phytologist 201:45-56.

Dempster, E. R. 1955. Maintenance of genetic heterogeneity. Biological Laboratory.

diCastri, F. and H. A. Mooney. 1973. Mediterranean type ecosystems: origin and structure. Springer-Verlag, New York.

Dobzhansky, T. 1950. Origin of heterosis through natural selection in populations of Drosophila pseudoobscura. Genetics 35:288-302.

Dobzhansky, T. 1955. A review of some fundamental concepts and problems of population genetics. Pp. 1-15. Cold Spring Harbor Symposia on Quantitative Biology. Citeseer.

Dobzhansky, T., A. Hunter, O. Pavlovsky, B. Spassky, and B. Wallace. 1963. Genetics of natural populations. XXXI. Genetics of an isolated marginal population of Drosophila pseudoobscura. Genetics 48:91.

Dobzhansky, T. and B. Spassky. 1953. Genetics of natural populations. XXI. Concealed variability in two sympatric species of Drosophila. Genetics 38:471.

Dobzhansky, T. and B. Spassky. 1954. Genetics of natural populations. XXII. A comparison of the concealed variability in Drosophila prosaltans with that in other species. Genetics 39:472.

Doebley, J. F., B. S. Gaut, and B. D. Smith. 2006. The molecular genetics of crop domestication. Cell 127:1309-1321.

Dudash, M. R. and K. Ritland. 1991. Multiple paternity and self-fertilization in relation to floral age in Mimulus guttatus (Scrophulariaceae). American Journal of Botany:1746-1753.

Ehrlén, J. and Z. Münzbergová. 2009. Timing of flowering: opposed selection on different fitness components and trait covariation. The American Naturalist 173:819-830.

Ennos, R. and R. Dodson. 1987. Pollen success, functional gender and assortative mating in an experimental plant population. Heredity 58:119-126.

Ennos, R. and K. Swales. 1991. Genetic variation in a fungal pathogen: response to host defensive chemicals. Evolution:190-204.

Falconer, D. and T. Mackay. 1996. Introduction to quantitative genetics, Essex, England.

Farris, M. A. and M. J. Lechowicz. 1990. Functional interactions among traits that determine reproductive success in a native annual plant. Ecology 71:548-557.

Feinstein, S. C. and L. Wilson. 2005. Inability of tau to properly regulate neuronal microtubule dynamics: a loss-of-function mechanism by which tau might mediate neuronal cell death. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 1739:268-279.

Fenster, C. and D. Carr. 1997. Genetics of sex allocation in *Mimulus* (*Scrophulariaceae*). J. Evol. Biol. 10:641-661.

Fenster, C. B. and K. Ritland. 1994. Evidence for natural selection on mating system in *Mimulus* (*Scrophulariaceae*). Int. J. Plant Sci. 155:588-596.

Fisher, R. A. 1918. 009: The Correlation Between Relatives on the Supposition of Mendelian Inheritance.

Fisher, R. A. 1941. AVERAGE EXCESS AND AVERAGE EFFECT OF A GENE SUBSTITUTION. Annals of Eugenics 11:53-63.

Forrest, J. R. 2014. Plant size, sexual selection, and the evolution of protandry in dioecious plants. The American Naturalist 184:338-351.

Fournier-Level, A., A. M. Wilczek, M. D. Cooper, J. L. Roe, J. Anderson, D. Eaton, B. T. Moyers, R. H. Petipas, R. N. Schaeffer, and B. Pieper. 2013. Paths to selection on life

history loci in different natural environments across the native range of *Arabidopsis thaliana*. Mol. Ecol. 22:3552-3566.
Fry, J. D. 1993. The" general vigor" problem: can antagonistic pleiotropy be detected when genetic covariances are positive? Evolution 47:327-333.
Futuyma, D. J. 1998. Evolutionary Biology, Third Edition. Sinauer Associates, Inc., Sunderland, Massachusetts.
Futuyma, D. J. and T. E. Philippi. 1987. Genetic variation and covariation in responses to host plants by *Alsophila pometaria* (*Lepidoptera*: *Geometridae*). Evolution:269-279.
Galloway, L., J. Etterson, and J. Hamrick. 2003. Outcrossing rate and inbreeding depression in the herbaceous autotetraploid, Campanula americana. Heredity 90:308.
Ganders, F. R. 1979. The biology of heterostyly. New Zealand Journal of Botany 17:607-635.
Geyer, C. J., C. E. Ridley, R. G. Latta, J. R. Etterson, and R. G. Shaw. 2013. Local adaptation and genetic effects on fitness: calculations for exponential family models with random effects. The Annals of Applied Statistics 7:1778-1795.
Geyer, C. J., S. Wagenius, and R. G. Shaw. 2007. Aster models for life history analysis. Biometrika 94:415-426.
Goodwillie, C. 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. Annu. Rev. Ecol. Evol. Syst. 36:47-79.
Grafen, A. 1988. On the Uses of Data on Lifetime Reproductive Success. Pp. 454-471 *in* T. H. Clutton-Brock, ed. Reproductive Success. University of Chicago Press.
Greenberg, R. and J. F. Crow. 1960. A comparison of the effect of lethal and detrimental chromosomes from Drosophila populations. Genetics 45:1153.
Grossenbacher, D., R. Briscoe Runquist, E. E. Goldberg, and Y. Brandvain. 2015. Geographic range size is predicted by plant mating system. Ecology letters 18:706-713.
Gulisija, D. and Y. Kim. 2015. Emergence of long-term balanced polymorphism under cyclic selection of spatially variable magnitude. Evolution 69:979-992.
Hahn, M. W. and L. Nakhleh. 2016. Irrational exuberance for resolved species trees. Evolution 70:7-17.
Haldane, J. and S. Jayakar. 1963a. Polymorphism due to selection of varying direction. Journal of Genetics 58:237-242.
Haldane, J. and S. Jayakar. 1963b. Polymorphism due to selection of varying direction. J. Genet. 58:237-242.
Hall, M. C. and J. H. Willis. 2006. Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. Evolution 60:2466-2477.
Hartl, D. L. and A. G. Clark. 1997. Principles of Population Genetics. Sinauer associates Sunderland.
Hedrick, P. W. 1976. Genetic variation in a heterogeneous environment. II. Temporal heterogeneity and directional selection. Genetics 84:145-157.
Hedrick, P. W. 1999. Antagonistic pleiotropy and genetic polymorphism: a perspective. Heredity 82:126-133.
Hedrick, P. W. 2006. Genetic polymorphism in heterogeneous environments: the age of genomics. Annu. Rev. Ecol. Evol. Syst. 37:67-93.
Hedrick, P. W. 2011. Genetics of Populations. Jones & Bartlett Learning.
Hellsten, U., K. M. Wright, J. Jenkins, S. Shu, Y. Yuan, S. R. Wessler, J. Schmutz, J. H. Willis, and D. S. Rokhsar. 2013. Fine-scale variation in meiotic recombination in Mimulus

inferred from population shotgun sequencing. Proceedings of the National Academy of Sciences 110:19478-19482.
Hodges, T. K., K. L. Laskowski, G. L. Squadrito, M. De Luca, and J. Leips. 2013. Defense traits of larval *Drosophila melanogaster* exhibit genetically based trade-offs against different species of parasitoids. Evolution 67:749-760.
Holeski, L. M., P. Monnahan, B. Koseva, N. McCool, R. L. Lindroth, and J. K. Kelly. 2014. A high-resolution genetic map of yellow monkeyflower identifies chemical defense QTLs and recombination rate variation. G3: Genes, Genomes, Genetics 4:813-821.
Holsinger, K. E. 1988. Inbreeding depression doesn't matter: The genetic basis of mating-system evolution. Evolution 42:1235-1244.
Houle, D. 1991. Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. Evolution:630-648.
Huth, C. J. and O. Pellmyr. 2000. Pollen-mediated selective abortion in yuccas and its consequences for the plant–pollinator mutualism. Ecology 81:1100-1107.
Igic, B., L. Bohs, and J. R. Kohn. 2006. Ancient polymorphism reveals unidirectional breeding system shifts. Proceedings of the National Academy of Sciences 103:1359-1363.
Jaenike, J. 1989. Genetic population structure of *Drosophila tripunctata*: patterns of variation and covariation of traits affecting resource use. Evolution:1467-1482.
Johansson, F. and L. Rowe. 1999. Life history and behavioral responses to time constraints in a damselfly. Ecology 80:1242-1252.
Johnston, M. O., E. Porcher, P.-O. Cheptou, C. G. Eckert, E. Elle, M. A. Geber, S. Kalisz, J. K. Kelly, D. A. Moeller, M. Vallejo-Marin, and A. A. Winn. 2009. Correlations among Fertility Components Can Maintain Mixed Mating in Plants. The American Naturalist 173:1-11.
Josephs, E. B., J. R. Stinchcombe, and S. I. Wright. 2017. What can genome-wide association studies tell us about the evolutionary forces maintaining genetic variation for quantitative traits? New Phytologist 214:21-33.
Juenger, T. E., J. K. Mckay, N. Hausmann, J. J. Keurentjes, S. Sen, K. A. Stowe, T. E. Dawson, E. L. Simms, and J. H. Richards. 2005. Identification and characterization of QTL underlying whole-plant physiology in *Arabidopsis thaliana*: δ13C, stomatal conductance and transpiration efficiency. Plant Cell Environ. 28:697-708.
Kelly, J. K. 2003a. Deleterious mutations and the genetic variance of male fitness components in *Mimulus guttatus*. Genetics 164:1071-1085.
Kelly, J. K. 2003b. Deleterious mutations and the genetic variance of male fitness components in Mimulus guttatus. Genetics 164:1071-1085.
Kelly, J. K. 2005. Epistasis in Monkeyflowers. Genetics 171:1917-1931.
Kelly, J. K. 2008. Testing the rare alleles model of quantitative variation by artificial selection. Genetica 132:187-198.
Kelly, J. K. and H. S. Arathi. 2003. Inbreeding and the genetic variance of floral traits in *Mimulus guttatus*. Heredity 90:77-83.
Kelly, J. K. and M. K. Tourtellot. 2006. The genetic analysis of family structured inbreeding depression studies. Heredity 97:346-354.
Kemp, P. R. 1983. Phenological patterns of Chihuahuan Desert plants in relation to the timing of water availability. The Journal of Ecology:427-436.
Kimura, M. 1959. Conflict between self fertilization and outbreeding in plants. Ann. Rep. Natl. Inst. Genet. Japan 9:87-88.

Kimura, M. 1983. The neutral theory of molecular evolution. Cambridge University Press.
Kimura, M. and T. Maruyama. 1966a. The mutation load with epistatic gene interactions in fitness. Genetics 54:1337-1351.
Kimura, M. and T. Maruyama. 1966b. The mutational load with epistatic gene interactions in fitness. Genetics 54:1337.
Kimura, M. and T. Ohta. 1971. Theoretical Aspects of Population Genetics. Princeton University Press.
King, D. and J. Roughgarden. 1983. Energy allocation patterns of the California grassland annuals *Plantago erecta* and *Clarkia rubicunda*. Ecology 64:16-24.
Koboldt, D. C., K. Chen, T. Wylie, D. E. Larson, M. D. McLellan, E. R. Mardis, G. M. Weinstock, R. K. Wilson, and L. Ding. 2009. VarScan: variant detection in massively parallel sequencing of individual and pooled samples. Bioinformatics 25:2283-2285.
Kondrashov, A. S. 1988. Deleterious Mutations and the Evolution Of Sexual Reproduction. Nature 336:435-440.
Kudo, G. 1993. Relationships between flowering time and fruit set of the entomophilous alpine shrub, *Rhododendron aureum* (*Ericaceae*), inhabiting snow patches. Am. J. Bot.:1300-1304.
Lande, R. 1980. The genetic covariance between characters maintained by pleiotropic mutations. Genetics 94:203-215.
Lande, R. and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 39:24-40.
Lee, C. R., J. T. Anderson, and T. Mitchell-Olds. 2014. Unifying genetic canalization, genetic constraint, and genotype-by-environment interaction: QTL by genomic background by environment interaction of flowering time in *Boechera stricta*. PLoS Genetics 10:e1004727.
Lee, Y. W. 2009. Genetics analysis of standing variation for floral morphology and fitness components in a natural population of Mimulus guttatus (common monkeyflower). Duke University.
Leroi, A. M., A. Bartke, G. De Benedictis, C. Franceschi, A. Gartner, E. Gonos, M. E. Feder, T. Kivisild, S. Lee, and N. Kartal-Özer. 2005. What evidence is there for the existence of individual genes with antagonistic pleiotropic effects? Mechanisms of Ageing and Development 126:421-429.
Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. The American Naturalist 87:331-333.
Lewontin, R. C. 1974. The genetic basis of evolutionary change. Columbia University Press New York.
Li, H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv preprint arXiv:1303.3997.
Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin. 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25:2078-2079.
Li, Q.-J., Z.-F. Xu, W. J. Kress, Y.-M. Xia, L. Zhang, X.-B. Deng, J.-Y. Gao, and Z.-L. Bai. 2001a. Pollination: flexible style that encourages outcrossing. Nature 410:432.
Li, Z.-K., L. Luo, H. Mei, D. Wang, Q. Shu, R. Tabien, D. Zhong, C. Ying, J. Stansel, and G. Khush. 2001b. Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. Genetics 158:1737-1753.

Liu, Y., Y. Xie, H. Wang, X. Ma, W. Yao, and H. Wang. 2017. Light and Ethylene Coordinately Regulate the Phosphate Starvation Response through Transcriptional Regulation of PHOSPHATE STARVATION RESPONSE1. The Plant Cell:tpc. 00268.02017.

Lloyd, D. G. 1979. Some Reproductive Factors Affecting the Selection of Self-Fertilization in Plants. The American Naturalist 113:67-79.

Lovell, J. T., T. E. Juenger, S. D. Michaels, J. R. Lasky, A. Platt, J. H. Richards, X. Yu, H. M. Easlon, S. Sen, and J. K. McKay. 2013. Pleiotropy of FRIGIDA enhances the potential for multivariate adaptation. Proc. R. Soc. B 280:20131043.

Luo, L., Z.-K. Li, H. Mei, Q. Shu, R. Tabien, D. Zhong, C. Ying, J. Stansel, G. Khush, and A. Paterson. 2001. Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. II. Grain yield components. Genetics 158:1755-1771.

Lynch, M. and B. Walsh. 1998. Genetics and Analysis of Quantitative Traits. Sinauer Sunderland, MA.

Marks, M. and S. Prince. 1981. Influence of germination date on survival and fecundity in wild lettuce *Lactuca serriola*. Oikos:326-330.

Mckay, J. K., J. H. Richards, and T. Mitchell-Olds. 2003. Genetics of drought adaptation in *Arabidopsis thaliana*: I. Pleiotropy contributes to genetic correlations among ecological traits. Mol. Ecol. 12:1137-1151.

McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, A. Kernytsky, K. Garimella, D. Altshuler, S. Gabriel, and M. Daly. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome research 20:1297-1303.

Meina, G., R. Wenyuan, L. Changying, H. Fangliang, Z. Ming, L. Yingyao, Y. Yanan, D. Xiaomeng, W. Yunrong, and W. Zhongchang. 2015. Integrative comparison of the role of the PHR1 subfamily in phosphate signaling and homeostasis in rice. Plant physiology:pp. 00736.02015.

Mitchell-Olds, T., J. H. Willis, and D. B. Goldstein. 2007. Which evolutionary processes influence natural genetic variation for phenotypic traits? Nature reviews. Genetics 8:845.

Mojica, J. P. and J. K. Kelly. 2010. Viability selection prior to trait expression is an essential component of natural selection. Proc. R. Soc. B 277:2945-2950.

Mojica, J. P., Y. W. Lee, J. H. Willis, and J. K. Kelly. 2012a. Spatially and temporally varying selection on intrapopulation quantitative trait loci for a life history trade-off in *Mimulus guttatus*. Mol. Ecol. 21:3718-3728.

Mojica, J. P., Y. W. Lee, J. H. Willis, and J. K. Kelly. 2012b. Spatially and temporally varying selection on intrapopulation quantitative trait loci for a life history trade-off in Mimulus guttatus. Molecular ecology 21:3718-3728.

Monnahan, P. J. and J. K. Kelly. 2015. Naturally segregating loci exhibit epistasis for fitness. Biology Letters 11.

Morton, N. E., J. F. Crow, and H. J. Muller. 1956a. An estimate of the mutational damage in man from data on consanguineous marriages. Proceedings of the National Academy of Sciences of the United States of America 42:855.

Morton, N. E., J. F. Crow, and H. J. Muller. 1956b. An estimate of the mutational damage in man from data on consanguineous marriages. Proceedings of the National Academy of Sciences USA 42:855-863.

Mukai, T., S. Chigusa, and I. Yoshikawa. 1964. The genetic structure of natural populations of Drosophila melanogaster. II. Overdominance of spontaneous mutant polygenes controlling viability in homozygous genetic background. Genetics 50:711.

Mukai, T., S. Chigusa, and I. Yoshikawa. 1965. The genetic structure of natural populations of Drosophila melanogaster. III. Dominance effect of spontaneous mutant polygenes controlling viability in heterozygous genetic backgrounds. Genetics 52:493.

Muller, H. J. 1950. Our load of mutations. American journal of human genetics 2:111.

Mulroy, T. W. and P. W. Rundel. 1977. Annual plants: adaptations to desert environments. Bioscience 27:109-114.

Myles, S., J. Peiffer, P. J. Brown, E. S. Ersoz, Z. Zhang, D. E. Costich, and E. S. Buckler. 2009. Association Mapping: Critical Considerations Shift from Genotyping to Experimental Design. The Plant Cell 21:2194-2202.

Nilsson, L., R. Müller, and T. H. Nielsen. 2007. Increased expression of the MYB-related transcription factor, PHR1, leads to enhanced phosphate uptake in Arabidopsis thaliana. Plant, cell & environment 30:1499-1512.

Nussey, D. H., A. J. Wilson, A. Morris, J. Pemberton, T. Clutton-Brock, and L. E. Kruuk. 2008. Testing for genetic trade-offs between early-and late-life reproduction in a wild red deer population. Proc. R. Soc. B 275:745-750.

O'Neil, P. 1997. Natural selection on genetically correlated phenological characters in *Lythrum salicaria L.*(*Lythraceae*). Evolution 51:267-274.

Opedal, Ø. H. 2018. Herkogamy, a principal functional trait of plant reproductive biology. International Journal of Plant Sciences 179:677-687.

Overman, A. R. and R. V. Scholtz. 2013. Accumulation of biomass and mineral elements with calendar time by cotton: application of the expanded growth model. PloS One 8:e72810.

Patterson, N., N. Hattangadi, B. Lane, K. E. Lohmueller, D. A. Hafler, J. R. Oksenberg, S. L. Hauser, M. W. Smith, S. J. O'Brien, D. Altshuler, M. J. Daly, and D. Reich. 2004. Methods for high-density admixture mapping of disease genes. American journal of human genetics 74:979-1000.

Rambaut, A. 2012. FigTree v1. 4.

Réale, D., D. Berteaux, A. McAdam, and S. Boutin. 2003. Lifetime selection on heritable life-history traits in a natural population of red squirrels. Evolution 57:2416-2423.

Renner, S. S. and R. E. Ricklefs. 1995. Dioecy and its correlates in the flowering plants. American journal of botany 82:596-606.

Ritland, K. and F. R. Ganders. 1987. Covariation of selfing rates with parental gene fixation indices within populations of Mimulus guttatus. Evolution 41:760-771.

Roach, D. A. 1986. Life history variation in *Geranium carolinianum*. 1. Covariation between characters at different stages of the life cycle. The American Naturalist 128:47-57.

Roff, D. 1990. Antagonistic pleiotropy and the evolution of wing dimorphism in the sand cricket *Gryllusfirmus*. Heredity 65:169-177.

Rose, M. R. 1982. Antagonistic pleiotropy, dominance, and genetic variation. Heredity 48:63.

Rose, M. R. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution:1004-1010.

Roux, F., J. Gasquez, and X. Reboud. 2004. The dominance of the herbicide resistance cost in several Arabidopsis thaliana mutant lines. Genetics 166:449-460.

Rowe, L. and D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. Proc. R. Soc. B 263:1415-1421.

Rubio, V., F. Linhares, R. Solano, A. C. Martín, J. Iglesias, A. Leyva, and J. Paz-Ares. 2001. A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. Genes & development 15:2122-2133.

Samocha, K. E., E. B. Robinson, S. J. Sanders, C. Stevens, A. Sabo, L. M. McGrath, J. A. Kosmicki, K. Rehnström, S. Mallick, and A. Kirby. 2014. A framework for the interpretation of de novo mutation in human disease. Nature genetics 46:944.

Scarcelli, N., J. M. Cheverud, B. A. Schaal, and P. X. Kover. 2007. Antagonistic pleiotropic effects reduce the potential adaptive value of the FRIGIDA locus. Proceedings of the National Academy of Sciences 104:16986-16991.

Schemske, D. W. 1977. Flowering phenology and seed set in *Claytonia virginica* (*Portulacaceae*). Bulletin of the Torrey Botanical Club:254-263.

Schemske, D. W. 1984. Population structure and local selection in *Impatiens pallida* (*Balsaminaceae*), a selfing annual. Evolution:817-832.

Schmittgen, T. D. and K. J. Livak. 2008. Analyzing real-time PCR data by the comparative C T method. Nature protocols 3:1101.

Schnable, P. S. and N. M. Springer. 2013. Progress toward understanding heterosis in crop plants. Annual review of plant biology 64:71-88.

Schultz, S. T. and J. H. Willis. 1995. Individual variation in inbreeding depression: the roles of inbreeding history and mutation. Genetics 141:1209-1223.

Scotti, I., S. C. González-Martínez, K. B. Budde, and H. Lalagüe. 2016. Fifty years of genetic studies: what to make of the large amounts of variation found within populations? Annals of Forest Science 73:69-75.

Scoville, A., Y. W. Lee, J. H. Willis, and J. K. Kelly. 2009. Contribution of chromosomal polymorphisms to the G-matrix of *Mimulus guttatus*. New Phytologist 183 803–815.

Scoville, A. G., Y. W. Lee, J. H. Willis, and J. K. Kelly. 2011a. Explaining the heritability of an ecologically significant trait in terms of individual quantitative trait loci. Biology letters 7:896-898.

Scoville, A. G., Y. W. Lee, J. H. Willis, and J. K. Kelly. 2011b. Explaining the heritability of an ecologically significant trait in terms of individual quantitative trait loci. Biology Letters:rsbl20110409.

Sessions, A., E. Burke, G. Presting, G. Aux, J. McElver, D. Patton, B. Dietrich, P. Ho, J. Bacwaden, and C. Ko. 2002. A high-throughput Arabidopsis reverse genetics system. The Plant Cell 14:2985-2994.

Sgrò, C. M. and L. Partridge. 1999. A Delayed Wave of Death from Reproduction in *Drosophila*. Science 286:2521-2524.

Sharitz, R. R. and J. F. McCormick. 1973. Population dynamics of two competing annual plant species. Ecology 54:723-740.

Simovich, M. A. and S. A. Hathaway. 1997. Diversified bet-hedging as a reproductive strategy of some ephemeral pool anostracans (*Branchiopoda*). J. Crust. Biol. 17:38-44.

Sinervo, B. and A. G. McAdam. 2008. Maturational costs of reproduction due to clutch size and ontogenetic conflict as revealed in the invisible fraction. Proc. R. Soc. B 275:629-638.

Smith, S. D. and M. D. Rausher. 2011. Gene loss and parallel evolution contribute to species difference in flower color. Molecular Biology and Evolution 28:2799-2810.

Spassky, B., T. Dobzhansky, and W. Anderson. 1965. Genetics of natural populations. XXXVI. Epistatic interactions of the components of the genetic load in *Drosophila pseudoobscura*. Genetics 52:653.

Springer, N. M. and R. M. Stupar. 2007. Allelic variation and heterosis in maize: how do two halves make more than a whole? Genome research 17:264-275.

Stanton, K. A., P. P. Edger, J. R. Puzey, T. Kinser, P. Cheng, D. M. Vernon, N. R. Forsthoefel, and A. M. Cooley. 2017. A whole-transcriptome approach to evaluating reference genes for quantitative gene expression studies: a case study in mimulus. G3: Genes, Genomes, Genetics 7:1085-1095.

Stearns, S. C. 1989. Trade-offs in life-history evolution. Functional Ecology 3:259-268.

Sweigart, A., K. Karoly, A. Jones, and J. H. Willis. 1999. The distribution of individual inbreeding coefficients and pairwise relatedness in a population of Mimulus guttatus. Heredity 83:625.

Takayama, S. and A. Isogai. 2005. Self-incompatibility in plants. Annu. Rev. Plant Biol. 56:467-489.

Tanksley, S. D. 1983. Molecular markers in plant breeding. Plant Molecular Biology Reporter 1:3-8.

Team, R. C. 2013. R: A language and environment for statistical computing.

Troth, A., J. R. Puzey, R. S. Kim, J. H. Willis, and J. K. Kelly. 2018. Selective trade-offs maintain alleles underpinning complex trait variation in plants. Science 361:475-478.

Untergasser, A., I. Cutcutache, T. Koressaar, J. Ye, B. C. Faircloth, M. Remm, and S. G. Rozen. 2012. Primer3—new capabilities and interfaces. Nucleic acids research 40:e115-e115.

Uyenoyama, M. K. 1986. Inbreeding and the cost of meiosis: the evolution of selfing in populations practicing biparental inbreeding. Evolution 40:388-404.

Uyenoyama, M. K., K. E. Holsinger, and D. M. Waller. 1993. Ecological and genetic factors directing the evolution of self-fertilization. Oxford surveys in evolutionary biology 9:327-381.

Van Dooren, T. J. 2006. Protected polymorphism and evolutionary stability in pleiotropic models with trait-specific dominance. Evolution 60:1991-2003.

Wallace, B. 1958. The role of heterozygosity in Drosophila populations. Long Island Biological Assn.. Biological Lab., Cold Spring Harbor, NY.

Ward, J. K., D. S. Roy, I. Chatterjee, C. R. Bone, C. J. Springer, and J. K. Kelly. 2012. Identification of a major QTL that alters flowering time at elevated [CO 2] in *Arabidopsis thaliana*. PloS one 7:e49028.

Weber, B., J. Zicola, R. Oka, and M. Stam. 2016. Plant enhancers: a call for discovery. Trends in plant science 21:974-987.

Weinig, C., J. R. Stinchcombe, and J. Schmitt. 2003. QTL architecture of resistance and tolerance traits in *Arabidopsis thaliana* in natural environments. Mol. Ecol. 12:1153-1163.

Weis, A. E., S. M. Wadgymar, M. Sekor, and S. J. Franks. 2014. The shape of selection: using alternative fitness functions to test predictions for selection on flowering time. Evolutionary Ecology 28:885-904.

Wenk, E. H. and D. S. Falster. 2015. Quantifying and understanding reproductive allocation schedules in plants. Ecology and Evolution 5:5521-5538.

Weraduwage, S. M., J. Chen, F. C. Anozie, A. Morales, S. E. Weise, and T. D. Sharkey. 2015. The relationship between leaf area growth and biomass accumulation in *Arabidopsis thaliana*. Frontiers in Plant Science 6.

Wessinger, C. A. and M. D. Rausher. 2014. Predictability and irreversibility of genetic changes associated with flower color evolution in Penstemon barbatus. Evolution 68:1058-1070.

Whitlock, M. C. and D. Bourguet. 2000. Factors affecting the genetic load in *Drosophila*: synergistic epistasis and correlations among fitness components. Evolution 54:1654-1660.

Williams, G. C. 1957. Pleiotropy, Natural Selection, and the Evolution of Senescence. Evolution 11:398-411.

Willis, J. H. 1993a. Effects of different levels of inbreeding on fitness components in Mimulus guttatus. Evolution 47:864-876.

Willis, J. H. 1993b. Partial self fertilization and inbreeding depression in two populations of Mimulus guttatus. Heredity 71:145-154.

Willis, J. H. 1999a. Inbreeding load, average dominance, and the mutation rate for mildly deleterious alleles in Mimulus guttatus. Genetics 153:1885-1898.

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

Willis, J. H. 1999c. The Role of Genes of Large Effect on Inbreeding Depression in Mimulus Guttatus. Evolution 53:1678-1691.

Winn, A. A., E. Elle, S. Kalisz, P. O. Cheptou, C. G. Eckert, C. Goodwillie, M. O. Johnston, D. A. Moeller, R. H. Ree, and R. D. Sargent. 2011. Analysis of inbreeding depression in mixed-mating plants provides evidence for selective interference and stable mixed mating. Evolution: International Journal of Organic Evolution 65:3339-3359.

Wright, S. 1921. Systems of mating. III. Assortative mating based on somatic resemblance. Genetics 6:144-161.

Yang, J., S. Mezmouk, A. Baumgarten, E. S. Buckler, K. E. Guill, M. D. McMullen, R. H. Mumm, and J. Ross-Ibarra. 2017. Incomplete dominance of deleterious alleles contributes substantially to trait variation and heterosis in maize. PLoS genetics 13:e1007019.

Yu, S., J. Li, C. Xu, Y. Tan, Y. Gao, X. Li, Q. Zhang, and M. S. Maroof. 1997. Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proceedings of the National Academy of Sciences 94:9226-9231.