Quantitative trait evolution in *Mimulus guttatus* (yellow monkeyflower)

by

Liza M. Holeski

Submitted to the graduate degree program in Ecology and Evolutionary Biology and the faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Committee members: ----------
Chairperson

Date defended:

The Dissertation Committee for Liza M. Holeski certifies that this is the approved version of the following dissertation:

Quantitative trait evolution in *Mimulus guttatus* (yellow monkeyflower)

Committee members: ----------
Chairperson

Date approved:

Abstract

My dissertation examines several aspects of quantitative trait evolution in Mimulus guttatus. In Chapter 2, I use M. guttatus plants to investigate how response to artificial selection on a trait index differs among replicate populations that differ only in mating system. The results show a divergence in response to selection among mating system types, despite an equivalent selection regime and no direct effect of mating system on fitness. In Chapter 3, I use plants derived from several natural populations to look at geographic differences in genetic and environmental variation in a quantitative trait, trichome density. Constitutive production of trichomes is variable both within and among populations of M. guttatus and there is genetic variation for it both within and among geographically distinct natural populations. Damage on early leaves can induce increased trichome production on later leaves, a plastic response that is likely adaptive. In addition, I show in this chapter that trichome induction can be maternally transmitted by a yet undescribed epigenetic mechanism. There is genetic variation among plants in the capacity for both within and between plant generation induction. In Chapter 4, I examine how trichome density affects plant-insect interactions with an herbivore common to some M. guttatus populations, the meadow spittlebug. While trichomes confer resistance to some forms of herbivory, these experiments show that they do not deter meadow spittlebugs, either in their ability to feed or in their feeding preferences.

Table of Contents:

Introduction

My dissertation examines several aspects of quantitative trait evolution in *Mimulus guttatus*. A quantitative trait is one that is influenced by two or more genes, and may be influenced by the environment. Quantitative trait phenotypes are often continuous, but may also be discrete, as in threshold traits. In general, the field of quantitative genetics attempts to link phenotypic variation to its underlying genetic basis (Lynch and Walsh 1998). Many traits are quantitative rather than Mendelian, and the study of the evolution of quantitative traits has widespread applications including studies of natural populations, crop species and animal breeding, and human genetic disorders and disease.

The *Mimulus* species complex has been a model system for studies of plant evolution since the 1940's (Clausen 1940). The complex as well as the *M. guttatus* species encompass a large range of morphological variation. *M. guttatus* is relatively easy to propagate in the greenhouse, has a short time from germination to seed production, and can be outcrossed or self-fertilized with readily manipulated breeding. All of these factors make it an ideal species for studies of evolutionary ecology and genetics. Studies of *M. guttatus* cover a wide range of topics, including life history and morphology (Campbell 1950; Vickery 1978), systematics (Beardsley and Olmstead 2002), rates and causes of self-fertilization and degree of inbreeding depression (Willis 1993 a, b; Willis 1996; Sweigart 1998; Sweigart et al. 1999; Willis 1999a, b, c; Arathi et al. 2002; Kelly and Willis 2002; Arathi and Kelly 2004), mating system evolution (Fenster and Ritland1994a, b), hybridization and hybrid incompatibility (Fishman and Willis 2001;

Fishman and Willis 2006; Martin and Willis 2007; Sweigart et al. 2007), genetic architecture of floral traits (Fishman et al. 2002), corolla morphology and pollination (Arathi and Kelly 2004; Martin 2004), epistasis (Kelly 2005), and interactions between inbreeding and biotic environmental factors (Carr and Eubanks 2002; Carr et al. 2003; Eubanks et al. 2005a, b; Carr et al. 2006)

In Chapter 2, I use *M. guttatus* plants to investigate how response to artificial selection on a trait index differs among replicate populations that differ only in mating system. The mating system of a population profoundly influences its evolution. Inbreeding alters the balance of evolutionary forces that determine the amount and distribution of genetic variation within a population, potentially altering response to selection. In this chapter, I address the question: If populations differing only in mating system are exposed to the same selection pressures, will they respond in qualitatively different ways? I do this by by imposing selection on an index of two negatively correlated traits (flower size and development rate) within experimental populations that reproduce (a) entirely by outcrossing, (b) entirely by self-fertilizing, or (c) by a mixture of outcrossing and selfing. The results show a divergence in response to selection among mating system types, despite an equivalent selection regime and no direct effect of mating system on fitness. This study thus provides an experimental demonstration of how the interaction of selection, genetic drift, and mating system can produce dramatic short-term changes in trait means, variances, and covariances.

In Chapter 3, I use plants derived from several natural populations to look at geographic differences in genetic and environmental variation in a quantitative trait, trichome density. *Mimulus guttatus* (yellow monkeyflower) frequently produce glandular trichomes, a trait that may resist herbivory. Constitutive production of trichomes is variable both within and among populations of *M. guttatus* and there is genetic variation for it both within and among geographically distinct natural populations. Damage on early leaves can induce increased trichome production on later leaves, a plastic response that is likely adaptive. In addition, I show in this chapter that trichome induction can be maternally transmitted by a yet undescribed epigenetic mechanism. There is genetic variation among plants in the capacity for both *within* and *between* plant generation induction. Despite the clear evolutionary importance of variation in constitutive and induced herbivory-resistance traits, few other studies have noted genetic variation in both within a plant species

In Chapter 4, I examine how trichome density affects plant-insect interactions with an herbivore common to some *M. guttatus* populations, the meadow spittlebug. No-choice and choice experiments using the generalist herbivore, meadow spittlebug (*Philaenus spumarius*) are used to examine how variation in trichome density affects herbivory by meadow spittlebugs and how this herbivory subsequently affects plant fitness. While trichomes confer resistance to some forms of herbivory, these experiments show that they do not deter meadow spittlebugs, either in their ability to feed or in their feeding preferences. This is notable because (1) spittlebug herbivory did significantly reduce

plant fitness, and (2) trichomes have been shown to deter spittlebugs from feeding on other plant species.

Finally, in Chapter 5, I discuss how the experiments in Chapters 2-4 relate to future research directions, in particular a QTL (quantitative trait loci) mapping project and an experiment designed to investigate the mechanistic basis of trangenerational trichome induction.

References:

Arathi, H. S., and J. K. Kelly. 2004. Corolla morphology facilitates both autogamy and bumblebee pollination in *Mimulus guttatus*. International Journal of Plant Sciences 165:1039-1045.

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

Beardsley, P. M., and R. G. Olmstead. 2002. Redefining Phrymaceae: the placement of *Mimulus*, tribe Mimuleae, and *Phryma*. American Journal of Botany 89:1093-1102.

Campbell, G. R. 1950. *Mimulus guttatus* and related species. El Aliso 2:319-335.

Carr, D. E., and M. D. Eubanks. 2002. Inbreeding alters resistance to insect herbivory and host plant quality in *Mimulus guttatus* (Scrophulariaceae). Evolution 56:22-30.

Carr, D. E., J. F. Murphy, and M. D. Eubanks. 2003. The susceptibility and response of inbred and outbred *Mimulus guttatus* to infection by Cucumber mosaic virus. Evolutionary Ecology 17:85-103.

Carr, D. E., J. F. Murphy, and M. D. Eubanks. 2006. Genetic variation and covariation for resistance and tolerance to Cucumber mosaic virus in *Mimulus guttatus* (Phrymaceae): A test for costs and constraints. Heredity 96:29-38.

Clausen, J., D.D. Keck, and W.M. Heisey. 1940. Experimental studies on the nature of species. I. Effects of varied environments on western North American plants. Carnegie Institute of Washington publ. no. 520

Eubanks, M. D., D. E. Carr, and J. F. Murphy. 2005a. Effects of virus infection of *Mimulus guttatus* (Phrymacaea) on host plant quality for meadow spittlebugs, *Philaenus spumarius* (Hemiptera: Cercopidae). Environmental Entomology 34:891-898.

Eubanks, M. D., D. E. Carr, and J. F. Murphy. 2005b. Variation in the response of *Mimulus guttatus* (Scrophulariaceae) to herbivore and virus attack. Evolutionary Ecology 19:15-27.

Fenster, C. B., and K. Ritland. 1994a. Evidence for natural selection on mating system in *Mimulus* (Scrophulariaceae). International Journal of Plant Sciences 155:588-596.

Fenster, C. B., and K. Ritland. 1994b. Quantitative genetics of mating system divergence in the yellow monkeyflower species complex. Heredity 73:422-435.

Fishman, L., A. J. Kelly, and J. H. Willis. 2002. Minor quantitative trait loci underlie floral traits associated with mating system divergence in *Mimulus*. Evolution 56:2138-2155.

Fishman, L., and J. H. Willis. 2001. Evidence for Dobzhansky-Muller incompatibilites contributing to the sterility of hybrids between *Mimulus guttatus* and *M. nasutus*. Evolution 55:1932-1942.

Fishman, L., and J. H. Willis. 2006. A cytonuclear incompatibility causes anther sterility in *Mimulus* hybrids. Evolution 60:1372-1381.

Kelly, J. K. 2005. Epistasis in monkeyflowers. Genetics 171:1917-1931.

Kelly, J. K., and J. H. Willis. 2002. A manipulative experiment to estimate biparental inbreeding in monkeyflowers. International Journal of Plant Sciences 163:575-579.

Martin, N. H. 2004. Flower size preferences of the honeybee (*Apis mellifera*) foraging on *Mimulus guttatus* (Scrophulariaceae). Evolutionary Ecology Research 6:777-782.

Martin, N. H., and J. H. Willis. 2007. Ecological divergence associated with mating system causes nearly complete reproductive isolation between sympatric *Mimulus* species. Evolution 61:68-82.

Sweigart, A. 1998. Microsatellite-Based Estimates on Individual Inbreeding and Relatedness for a Population of the Wildflower, *Mimulus guttatus*. Mathematics and Natural Sciences. Reed University.

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 83:625-632.

Sweigart, A. L., A. R. Mason, and J. H. Willis. 2007. Natural variation for a hybrid incompatibility between two species of *Mimulus*. Evolution 61:141-151.

Vickery, R. K. J. 1978. Case studies in the evolution of species complexes in *Mimulus*. Evolutionary Biology 11:405-507.

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 2 Populations of *Mimulus guttatus*. Heredity 71:145-154.

Willis, J. H. 1996. Measures of phenotypic selection are biased by partial inbreeding. Evolution 50:1501-1511.

Willis, J. H. 1999a. The contribution of male-sterility mutations to inbreeding depression in *Mimulus guttatus*. Heredity 83:337-346.

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

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

Chapter 1. Mating system and the evolution of quantitative traits: An experimental study of *Mimulus guttatus.*

Abstract

The mating system of a population profoundly influences its evolution. Inbreeding alters the balance of evolutionary forces that determine the amount of genetic variation within a population. It redistributes that variation among individuals, altering heritabilities and genetic correlations. Inbreeding even changes the basic relationships between these genetic statistics and response to selection. If populations differing only in mating system are exposed to the same selection pressures, will they respond in qualitatively different ways? Here, we address this question by imposing selection on an index of two negatively correlated traits (flower size and development rate) within experimental populations that reproduce (a) entirely by outcrossing, (b) entirely by self-fertilizing, or (c) by a mixture of outcrossing and selfing. Entirely selfing populations responded mainly by evolving larger flowers while outcrossing populations also evolved more rapid development. Divergence occurred despite an equivalent selection regime and no direct effect of mating system on fitness. The study provides an experimental demonstration of how the interaction of selection, genetic drift, and mating system can produce dramatic short-term changes in trait means, variances, and covariances.

Introduction

For centuries, biologists have been captivated by the diversity of plant mating systems (Darwin 1876; Sprengel 1973; Barrett 2003). Most studies have focused on the evolution of mating systems, on how the balance of evolutionary advantages and costs determines the tendency for plants to self-fertilize versus outcross (Uyenoyama et al. 1993). An important question that has received less empirical study is how differences in mating system impact the evolution of quantitative traits. In fact, the characters that determine mating system, e.g. floral morphology and developmental timing, are usually quantitative. Through their effects on mating system, these traits subsequently influence inbreeding depression and the distribution of genetic diversity within and between populations (Charlesworth and Charlesworth 1987, 1995; Hamrick and Godt 1996; Charlesworth and Wright 2001).

Inbreeding reduces the frequency of heterozygotes relative to homozygotes at polymorphic loci within a population. It thus alters the 'presentation' of genetic variation at the phenotypic level and hence the balance between natural selection and other evolutionary forces (Robertson 1952; Mitchell-Olds and Rutledge 1986; Kelly 1999a). This is most obvious for loci exhibiting genetic dominance, where inbreeding exposes recessive alleles more directly to selection. Deleterious alleles may be more rapidly 'purged' and advantageous alleles more rapidly fixed with partial or complete self fertilization (Caballero and Hill 1992b; Charlesworth 1992; Byers and Waller 1999).

The experiment described here evaluates the most immediate effects of mating system on quantitative trait evolution, i.e. short-term response to selection. The simplest genetic model, a single locus with alleles that act additively, predicts that inbreeding should accelerate response to directional selection. The genetic variance contributed by an additive locus is directly proportional to the inbreeding coefficient of the population (Wright 1951; Falconer and Mackay 1996). If this locus is entirely responsible for trait variation, inbreeding should accelerate response because the segregation of alternative alleles into homozygotes increases the efficiency of selection in changing allele frequencies. With dominance, the genetic variance contributed by a locus will usually increase with inbreeding, but there are cases in which it can decline (Robertson 1952). Also, only a fraction of the genetic variance is available to selection with dominance. This fraction is itself dependent on the mating system, with dominance components becoming increasingly important as the level of inbreeding increases (Harris 1964; Pederson 1969; Jacquard 1974; Cockerham and Matzinger 1985).

When genetic variation is caused by multiple loci, two different kinds of inter-locus associations influence response to selection, linkage disequilibrium and identity disequilibrium. Most forms of selection will generate linkage disequilibria among QTL (quantitative trait loci). Recombination effectively diminishes linkage disequilibrium in randomly mating populations (Bulmer 1980; Turelli and Barton 1994). With inbreeding however, the frequency of doubly heterozygous genotypes is reduced and thus so is the rate that crossing-over generates recombinant gametes. This allows disequilibria to persist and selection can substantially alter the genetic variance, V_G, in the short term

even when allele frequency changes are insubstantial (Hayashi and Ukai 1994; Kelly 1999a). The magnitude and direction of the changes in V_G depend on (1) how selection affects the phenotypic variance, and (2) how different QTL combine to determine the phenotype, i.e. the presence and nature of epistasis. Stabilizing selection, as well as most forms of directional selection, will tend to reduce the phenotypic variance. If QTL combine additively (there is no epistasis), associations among loci will tend to be negative and V_G will be reduced. The magnitude of the reduction depends on the trait heritability, the strength of selection, and the mating system (see Bulmer 1980, pp 155-160 for estimates under random mating; Kelly (1999a), Kelly and Williamson (2000), and Nomura (2005) provide estimates with different levels of selfing). It is more difficult to predict changes in V_G with epistatic interactions among QTL. However, a number of authors have argued that inbreeding allows selection to act more directly on gene combinations, facilitating the evolution of co-adapted gene complexes (Clegg et al. 1972; Allard 1975).

A second kind of inter-locus association, identity disequilibrium, is likely to be important when individuals vary in the extent to which they are inbred (Haldane 1949; Bennett and Binet 1956). Variation among individuals in their respective inbreeding coefficients is inevitable in a mixed mating population (Wright and Cockerham 1985; Kelly 1999a). For traits that exhibit directional dominance, e.g. inbreeding depression, much of the phenotypic variation present in such a population may be due to differences in inbreeding coefficients rather than in the allelic composition of genotypes, as it would be in a randomly mating population (Willis 1996). Under directional selection, allele frequency

evolution is determined primarily by the relationship between phenotype and fitness *among individuals with the same inbreeding coefficient* (Kelly 1999a). Thus, genetic variation due to identity disequilibrium in a mixed mating population is largely 'unavailable' to selection, analogous to how non-additive variation is inaccessible in a randomly mating population.

The preceding discussion focuses on the presentation of genetic variation to selection, but mating system also affects genetic drift, and the interaction between selection and drift. In the absence of selection, inbreeding will tend to reduce the effective population size (N_e) and thus increase the impact of genetic drift. The magnitude of this effect depends on the amount and kind of inbreeding, as well as the variance in family sizes (Caballero and Hill 1992a, and references therein). Selection can further magnify the effect of inbreeding on genetic drift. Caballero and Santiago (1995) show that high selfing rates combined with intense truncation selection can reduce N_e to a tenth of the actual population size. Much of this theory concerns the effect of mating system on the fixation probability of new mutations (e.g. Caballero et al. 1991; Charlesworth 1992; Pollak and Sabran 1992; Caballero and Santiago 1995) and is thus not directly applicable to the results of this study. Here, the immediate response to selection (4 generations in duration) is due to the recruitment of standing variation. However, it is reasonable to hypothesize that the same basic processes will affect selection on standing variation and that inbreeding might accelerate the stochastic divergence of replicate populations. We directly evaluate this hypothesis by comparing the variation in response to selection

among populations within mating system categories: outcrossing, mixed-mating, and selfing.

Evolution of the multi-variate phenotype—In this experiment, we impose selection on an index of two genetically correlated traits, flower size and development rate. Each is a quantitative character and we expect the various factors discussed above to influence the genetic variance in each. However, selection on multiple traits introduces the additional complexity of correlated responses to selection (Lande and Arnold 1983; Kelly 1999b). Like variances, genetic covariances among traits are affected by associations among QTL (linkage and/or identity disequilibria). With multiple traits, inbreeding can alter not only the rate of evolution (whether trait means change more or less rapidly than a comparable randomly mating population), but also the nature of response (which characters evolve and in what direction).

Consider the standard model of multi-trait selection in a randomly mating population (Lande and Arnold 1983): $\Delta\bar{z} = G\beta$, where $\Delta\bar{z}$ is the vector of predicted changes in the mean of each trait, G is the additive genetic variance-covariance matrix, and β is the vector of selection gradients on each trait. Inbreeding clearly alters the elements of G and even the appropriate definition of these elements (Kelly 1999b and see below). However, it can also affect selection parameters in several distinct ways. For example, the combination of mixed-mating and inbreeding depression in fitness can generate false selection gradients on traits with no effect on fitness (Willis 1996a). Inbred individuals may have lower fitness due to the exposure of deleterious alleles in homozygous form,

but also consistently different trait values (for any character that exhibits directional dominance) than outbred individuals. This will yield an apparent association between trait values and fitness, independent of any effect the trait has on fitness. The same basic mechanism can produce genetic correlations between traits even when there is no pleiotropy or linkage disequilibrium (Kelly 1999b).

More generally, the vector of selection gradients is contingent on both the selection regime and the distribution of phenotypic variation: $\beta = P^{-1}S$, where P^{-1} is the inverse of the phenotypic variance/covariance matrix and S is the vector of selection differentials on each trait (Lande and Arnold 1983). If inbreeding alters the means, variances, and covariances of traits, it will shift the location of the phenotypic distribution relative to fitness surface. Gradients reflect the (linear) relationship between trait values and relative fitness *in the vicinity of the current multi-variate mean* (Phillips and Arnold 1989). Gradients will thus change as means change if the fitness function is non-linear, as must usually be the case in nature.

We imposed selection on an index combining flower size and rate of development for several reasons. The first is simply that natural selection typically acts on suites of traits rather than individual characters in isolation. Second, the index allows us to address the question of how mating system might alter response to an 'evolutionary constraint'. The trade-offs that organisms confront, e.g. investment in growth vs. reproduction, manifest themselves at the population level as negative genetic correlations among characters (Arnold 1992). In our study population and growth conditions, corolla width and rate of

development exhibit a negative genetic correlation (Kelly, unpublished results; Appendix A). Rapidly developing plants tend to flower when small. Delayed development allows plants to flower after they have accumulated substantially greater above-ground biomass. In short, larger plants produce larger flowers. It is noteworthy that flower size and development rate also exhibit a negative relationship among populations and species within the *Mimulus guttatus* species complex. Annual populations of *M. guttatus* tend to have more rapidly developing plants with smaller flowers than perennial populations (Holeski, unpublished results). Primarily self-fertilizing species within the complex, such as *Mimulus nasutus* and *M. micranthus*, have greatly reduced flowers and tend to develop more rapidly than *M. guttatus* (Fenster et al. 1995).

Predicting changes in mean phenotypes—Inbreeding can directly change trait means (without selection) by increasing homozygosity within the population. Consider a quantitative character, z, determined by the summed contributions of an arbitrary number of QTL. The mean of z in the population, $\bar{z}$, has two components:

$$\bar{z} = \mu_O + f(\mu_I - \mu_O) \qquad (1)$$

where μ_O is the "outbred mean" and μ_I is the "inbred mean" (Wright 1951; Kempthorne 1957; Kelly and Williamson 2000). The statistics μ_O and μ_I are functions of genotypic effects and allele frequencies at QTL, and each will evolve under selection. The relative contribution of each component is determined by *f*, the average inbreeding coefficient within the population. The value of *f* can change both in the short term, due to the varying reproductive successive of individuals that are inbred to different levels, and also in the long term, with the evolution of traits that affect the selfing rate (Wright and

Cockerham 1985). As a consequence, $\Delta\bar{z}$ is a complex mixture of changes in different quantities unless $\mu_O = \mu_I$, i.e. the trait exhibits no directional dominance. Changes in μ_O and μ_I cannot be distinguished without controlled matings/self-fertilizations, generating collections of individuals with different but known values for *f*. We employ this approach here to monitor the evolution of both μ_O and μ_I under different mating systems.

The partitioning of the mean phenotype (eq 1) can also be used to predict response to multi-variate selection with inbreeding. Each trait is associated with a specific value for μ_O and μ_I and the population is described by the two vectors of means (Kelly and Williamson 2000). A distinct matrix of genetic quantities is used to predict changes in the values of μ_O and μ_I for each trait (Kelly 1999b). Like *G* of the random mating model, the elements of these matrices can be estimated from comparisons among relatives.

The diversity of potential evolutionary consequences associated with inbreeding is impressive. However, the empirical importance of these myriad factors has yet to be determined. To evaluate the most immediate effects of mating system, experimental populations of *Mimulus guttatus* were established from a common source and subjected to the same phenotypic selection regime. We imposed artificial selection on replicate populations of three different mating systems, fully outcrossing, mixed mating, and fully selfing (Figure 2-1). We documented evolution of six quantities within each experimental populations, μ_O and μ_I for each of three traits. These data address two basic questions: Does mating system affect the rate and pattern of quantitative evolution? Does mating system affect the rate that replicate populations diverge?

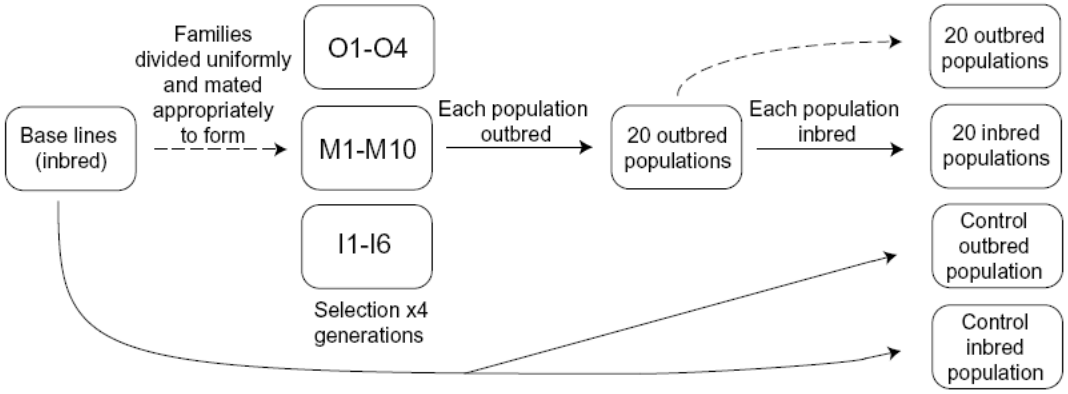


Figure 2-1. A schematic of the experimental design: evolutionary change was measured within four fully outcrossing populations (O1-O4), ten mixed-mating populations (M1-M10), and six fully selfing populations (I1-I6).

Materials and Methods

Study species and source population—Mimulus guttatus (2n = 28; Phrymaceae) is a self-compatible wildflower that occupies a variety of moist, open habitats throughout western North America. The species is mixed mating and the estimated selfing rate varies from 0 to 0.75 among populations and it may be annual, winter annual, or perennial (Ritland and Ganders 1987; Willis 1993c; Awadalla and Ritland 1997; Sweigart et al. 1999). Previous genetic studies have demonstrated substantial genetic variation in floral traits (Carr and Fenster 1994; Robertson et al. 1994) and inbreeding depression in fitness components (Dole and Ritland 1993; Willis 1993c; Latta and Ritland 1994; Carr and Dudash 1996; Dudash and Carr 1998; Willis 1999c, d). There have also been a number of studies

examining the genetic basis of differences in floral morphology and mating system between *M. guttatus* and closely related species (Vickery 1978; Fenster and Ritland 1994; Fishman et al. 2002b).

Experimental populations were initiated from the same source population, a large collection of randomly extracted, highly inbred lines. John H. Willis initiated approximately 1200 independent lines from 'Iron Mountain' in August of 1995. The Iron Mountain population is located in Oregon's Western Cascades and contains several hundred thousand individuals continuously distributed over an area of approximately 400 square meters (see Willis 1996, 1999a,b). Each line was founded from the seed set of a separate field-collected plant and subsequently maintained by single seed descent (self-fertilization) for 7-9 generations. The lines have an inbreeding coefficient of greater than 0.99 ($f \cong 1$), and as expected, are almost completely homozygous at highly polymorphic microsatellite loci with different lines fixed for different alleles (Willis 1999c; Holeski, unpublished results).

We established a total of 20 experimental populations, six within the fully selfing category (I1, I2, … I6), ten within the mixed-mating category (M1-M10), and four within the fully outcrossing category (O1-O4). The 20 populations were established by sampling progeny from a breeding design (based on the inbred lines) in a way that maximized the genetic variance within populations and minimized allele frequency differences among populations. One hundred and ninety four inbred lines, were randomly selected from our collection, self-fertilized, and then randomly paired and

crossed to produce F1 individuals. The F1 plants were then selfed to produce F2 families and backcrossed to each parental line (see Kelly 2005a for a detailed description of the breeding design). Selfed progeny of each line were sampled to initiate populations I1–I6 with each line contributing at most one plant to each population. The fully outcrossing populations (O1-O4) were founded by F1 plants with each line pair contributing at most two plants to each population. The mixed-mating populations were founded by F2 and backcross individuals (both of which have $f = 0.5$ relative to the ancestral population), again maximally dispersing lines across populations.

Populations were founded by different line-cross types (e.g. F1, F2, backcross…) to replicate the distribution of genetic variation generated by their respective mating systems. In a randomly mating population, we expect alleles to be distributed into genotypes according to Hardy-Weinberg proportions. These proportions obtain within the population of F1 families of the breeding design. With complete selfing, variation should be aggregated into fully homozygous inbred lines and our fully selfing populations were founded from such lines. In a mixed-mating species with a constant selfing rate S, the mean inbreeding coefficient (f) is S / (2 - S) (Hartl and Clark 1989 p. 262). In contrast to the other categories, plants within the mixed-mating populations will vary in the extent to which they are inbred. The *initial* mean inbreeding coefficient of plants initiating our mixed mating populations is slightly greater than the predicted equilibrium ($f = 0.5$ instead of 0.33), but variation in individual inbreeding coefficients is established after the first generation of selection and reproduction. While the differing initial f values of populations within different mating system types imply differences in

genotype frequencies, the expected allele frequencies do not differ among populations. Of course, there was stochastic variation due to sampling, but such differences would not be associated with mating system category. The implication is that the expected values for μ_O and μ_I (which are functions of allele frequencies and genotypic effects) of each trait are initially the same in each population.

Each population consisted of approximately 120 individuals prior to selection, all of which were measured for number of days to first flower (from day of seeding) and corolla width. These measurements were then used to calculate *I*, the trait index:

$$I = (\text{corolla width} / \sigma_{\text{corolla width}}) - (\text{number of days to first flower} / \sigma_{\text{day}}) \quad (2)$$

where the σ terms are the estimated trait standard deviations within the original base population: $\sigma_{\text{corolla width}} = 2.34$ mm and $\sigma_{\text{day}} = 3.05$ days. We imposed truncation selection on *I* values (the top 25% selected to reproduce) within each population subsequent to the completion of flowering. Several 'alternates', plants just below the cut-line, were also preserved from each population and included only if plants in the top 30 failed to produce seed.

Within the outcrossing and mixed-mating populations, selected plants were randomly paired and crossed via hand pollination. The cross was unidirectional with pollen transferred from the randomly assigned sire to dam. In the mixed-mating populations, each plant (both sires and dams) was also self-fertilized by hand, as were all selected plants in the fully selfing populations. In the mixed-mating populations, the progeny generation was founded by an equal number of outcrossed and selfed offspring thus

yielding a realized selfing rate of 0.5. Each generation, seed from the selected parents of the previous generation were seeded into 2-inch pots in the University of Kansas greenhouse (18 hours day lengths). 11 days after seeding, the appropriate number of individuals from each family were randomly selected from each 2-inch pot, transferred to 98-well trays, and placed in a growth room (18 day length). Seedling mortality was generally low.

Selection was imposed on four successive generations, indexed as generations 0, 1, 2, and 3. The selected plants of generation 3 were randomly paired (within all 20 populations) and crossed. The resulting progeny, 20 outbred populations, were grown to maturity without selection in generation 4. Within each population, plants were randomly paired and crossed, and each was also self-fertilized. This produced a collection of outbred and inbred progeny for each experimental population. The latter were all inbred to the same extent ($f = 0.5$ for selfed progeny of outbred plants) and are thus comparable across populations. Selfed and outbred progeny were grown to maturity and measured in generation 5 and these measurements were used to estimate μ_O and μ_I of each experimental population.

To produce sufficient sample sizes for accurate estimates, generation 5 consisted of two successive grow-ups from the same seed sets, each involving about 2150 individuals. The first grow-up (G-1) consisted of 617 individuals from the inbreeding populations, 1100 individuals from the mixed-mating populations, and 463 individuals from the outcrossing populations. The second grow-up (G-2) consisted of 628 individuals from

the inbreeding populations, 1043 and 446 individuals from the mixed-mating and outcrossing populations, respectively). A total of 941 plants from the base population, 436 F1 individuals and 505 F2 individuals, were grown simultaneously with plants from the experimental populations in G-1 and G-2 of generation 5. The mean of F1 plants estimates the original value for μ_O and the mean of F2 plants estimates μ_I.

Statistical analyses—We separately analyze data from outbred and inbred plants of each population because they estimate responses of distinct genetic quantities (μ_O and μ_I of eq 1). The General Linear Model (GLM) was fit to each response variable (Index, corolla width, and days to flower) using maximum likelihood. Let Y_{ijkm} denote the m'th measurement within population *k* of mating system *j* in grow-up *i*:

$$Y_{ijkl} = \mu + \delta_i + \alpha_j + P_{jk} + \varepsilon_{ijkm} \qquad (3)$$

where μ is the grand mean, δ_i is the (fixed) effect of grow-up (i = 1, 2), α_j is the (fixed) effect of mating system (j = S, M, O), P_{jk} is the (random) effect of population (nested within mating system), and ε_{ijkm} is a normally distributed error. This is a mixed model and the log-likelihood, l, is

$$l = C - \frac{1}{2}\ln|V| - \frac{1}{2}(y - X\eta)^T V^{-1}(y - X\eta) \qquad (4)$$

where V is the variance-covariance matrix of individual measurements, $|V|$ is the determinant of V, y is the vector of values for the response variable, X is an incidence matrix for fixed effects, η is the vector of fixed effects, and C is a constant determined by

the total sample size (Searle et al. 1992, pg 234). We ignore C in subsequent calculations given that it has no effect on the difference between log-likelihoods of comparable models.

Our primary aims are to determine if the average responses differ among mating systems (can we reject $\alpha_j = 0$ for all j?) and does the divergence among populations differ among mating systems (is the variance of P_{jk} heterogeneous across mating systems?). We address these hypotheses by comparing the 'full model' to a set of restricted models. The full model has eight estimated parameters, four fixed effects (the means associated with each mating system plus the effect of grow-up) and four variance parameters for random effects (the error variance, σ^2, and inter-population variances $\sigma^2_{P,S}$, $\sigma^2_{P,M}$, and $\sigma^2_{P,O}$). Reduced Model 1 stipulates that mating system has no effect on the mean phenotypes of populations ($\alpha_j = 0$ for all j) and thus has six parameters: 2 fixed effects (the grand mean and the effect of grow-up) and 4 variance components. Reduced Model 2 stipulates that mating system has no effect on the stochastic divergence of populations. It also has six parameters: the 4 fixed effects and 2 variance components (σ^2 and σ^2_P). Reduced Model 3 stipulates that mating system has no effect on either means or inter-population variances and thus has only 4 parameters (2 fixed effects and 2 variance components).

We compare models using two standard approaches, the Akaike Information Criterion (AIC) and likelihood ratio tests. The AIC value for a model is

$$AIC = -2l + 2K \qquad (5)$$

where K is the number of parameters (Burnham and Anderson 2002, pg 61). The 'selected model' has the lowest AIC. The likelihood ratio statistic is equal to twice the difference between the log-likelihood of the 'alternative model' and the log-likelihood of the 'null model'. Here the null model is derived from the alternative model by eliminating a particular effect. The effect of mating system on the means of populations can be evaluated with two different model comparisons: the Full Model vs. Reduced Model 1 or Reduced Model 2 vs. Reduced Model 3. Likewise, the other null hypothesis, that mating system has no effect on the inter-population variance, can be evaluated with either of two different contrasts: the Full Model vs. Reduced Model 2 or Reduced Model 1 vs. Reduced Model 3. For each trait and type of plant (outbred and inbred), we calculate both likelihood ratio test statistics for each null hypothesis. We reject the null if the statistic is greater than 5.99 (the critical value from the chi-square distribution with two degrees of freedom) because the alternative and null models differ by two parameters in each case.

We analyzed the measurements from generation 5 using maximum likelihood instead of Analysis of Variance (ANOVA) for two reasons. First, the standard nested ANOVA assumes that the variance among sub-groups (populations) within groups (mating systems) is homogeneous. It thus does not allow us to address one of our primary questions. The ANOVA test for the effect of group (mating system) is essentially a contrast between Reduced Models 2 and 3 described above. Second, even with homogeneous inter-population variance, maximum likelihood is a preferable approach for the analysis of mixed models when the design is unbalanced (Searle et al. 1992, pg 254).

The present design is unbalanced at both levels: the number of populations varies among mating system categories, as does the number of measurements per population.

We used ANOVA to test for differences in selection parameters and in the realized heritability of the Index among mating systems. For each of the 20 experimental populations in each of the four generations of selection, we calculated the selection differential from the difference in mean phenotype between selected individuals and the entire population. Using estimates for the phenotypic variances and covariances within each population, we subsequently calculated the selection gradients on corolla width and days to flower according to the formula $\beta = P^{-1}S$. [The selection gradient for I is S divided by the phenotypic variance in I.] Cumulative selection differentials and gradients for each trait were then obtained by summing across generations within each population. Two realized heritability estimates were obtained from each population by dividing the cumulative responses, $\Delta\mu_O$ and $\Delta\mu_I$, for the trait index by the cumulative selection differential. In each of these analyses, all measurements from a population distill into a single value per analysis, e.g. the cumulative selection gradient on corolla width.

Results

Figure 2-2 illustrates the change in the mean index value of populations in each mating system category over the first three generations of selection. The differences in generation 0 (prior to any selection) reflect the direct effect of inbreeding on trait mean values. While there is clearly a response to selection, this response involves a mixture of changes in μ_O, μ_I, and f (although f is only changing in the mixed-mating populations).

The controlled crosses and self-fertilizations of adult plants in generations 3 and 4 serve to disentangle these changes. The direct evaluation of mating system effects on the evolution of μ_O and μ_I is given in Table 2-1, the parameter estimates from the full model in Table 2-2.

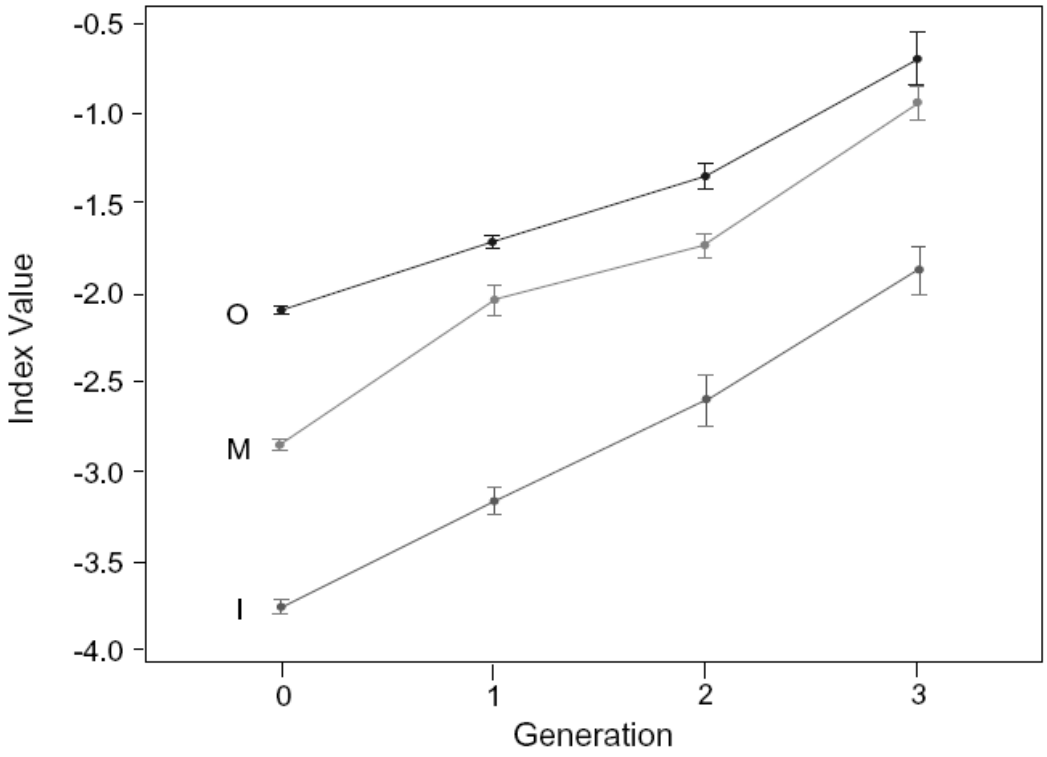


Figure 2-2. The mean index values (*I*) of populations within each mating system category are given for Generations 0, 1, 2, and 3 (error bars denote +/- one SE).

	Corolla Width		Days to Flower		Index	
Outbred plants: $\Delta\mu_O$	Log-likelihood	AIC	Log-likelihood	AIC	Log-likelihood	AIC
Full Model	-1003.56	**2015.12**	-1004.79	**2017.58**	-1038.00	2084.00
Reduced 1: MS has no effect on mean divergence	-1006.14	2018.28	-1011.61	2029.22	-1038.88	**2083.76**
Reduced 2: MS has no effect on variance in divergence	-1007.60	2021.20	-1010.78	2027.56	-1042.51	2091.02
Reduced 3: MS has no effect on mean or variance in divergence	-1010.68	2025.36	-1016.12	2036.24	-1042.79	2089.58
Inbred plants: $\Delta\mu_I$						
Full Model	-951.93	**1911.86**	-969.43	**1946.86**	-1002.20	**2012.40**
Reduced 1: MS has no effect on mean divergence	-954.17	1914.34	-975.96	1957.92	-1004.14	2014.28
Reduced 2: MS has no effect on variance in divergence	-954.15	1914.30	-970.60	1947.20	-1005.31	2016.62
Reduced 3: MS has no effect on mean or variance in divergence	-956.51	1917.02	-983.36	1970.72	-1008.46	2020.92

Table 2-1: The log-likelihood and AIC values are given for each model predicting variation in corolla width, days to flower and the trait index. The upper panel gives model fits for outbred plants grown in generation 5 ($\Delta\mu_O$), the lower panel for inbred plants ($\Delta\mu_I$). Bold type indicates the lowest AIC value, i.e. the selected model.

Outbred plants

	Corolla width (mm)		Days to flower		Index	
Fixed Effects	Estimate	SE	Estimate	SE	Estimate	SE
Grow-up	0.70	0.11	0.53	0.12	0.12	0.06
Selfing mean	18.64	0.41	25.43	0.48	-0.36	0.21
Mixed mating mean	17.82	0.20	24.49	0.17	-0.40	0.11
Outcrossing mean	17.59	0.13	23.68	0.15	-0.24	0.07
Var. components						
σ^2	6.64	0.14	8.18	0.18	1.74	0.04
$\sigma^2_{P,S}$	0.91	0.40	1.30	0.56	0.24	0.10
$\sigma^2_{P,M}$	0.32	0.12	0.17	0.08	0.10	0.04
$\sigma^2_{P,O}$	0.00	0.03	0.00	0.04	0.00	0.01

Inbred plants

	Corolla width (mm)		Days to flower		Index	
Fixed Effects	Estimate	SE	Estimate	SE	Estimate	SE
Grow-up	1.29	0.13	0.31	0.16	0.46	0.07
Selfing mean	17.26	0.40	27.47	0.33	-1.63	0.16
Mixed mating mean	16.62	0.20	25.44	0.19	-1.24	0.09
Outcrossing mean	16.29	0.19	25.24	0.20	-1.31	0.08
Var. components						
σ^2	8.52	0.19	12.54	0.28	2.35	0.05
$\sigma^2_{P,S}$	0.82	0.37	0.49	0.25	0.12	0.06
$\sigma^2_{P,M}$	0.28	0.12	0.18	0.09	0.05	0.02
$\sigma^2_{P,O}$	0.05	0.06	0.03	0.07	0.00	0.01

Table 2-2. Parameter estimates from the Full Model with standard errors extracted from the asymptotic dispersion matrix (Searle et al. 1992). Estimates for fixed effects are given as the following functions of the terms in equation 3: Effect of grow-up = $\delta_2 - \delta_1$, mean of selfing populations = $\mu + \alpha_S$, mean of mixed mating populations = $\mu + \alpha_M$, mean of outcrossing populations = $\mu + \alpha_O$.

The Full Model produces the lowest AIC value in five of six cases, the exception being μ_O for the trait index where Reduced Model 1 is selected. In all cases, the selected model allows the variance among replicate populations to differ between mating system categories. Inbreeding increases variability, $\sigma^2_{P,S} > \sigma^2_{P,M} >$ and $\sigma^2_{P,O}$ (Table 2). Mating system affected the mean values of μ_O for corolla width and days to flower, but not for the trait index. Mating system means differed for all three response variables of μ_I (Table 2-1, lower panel). Likelihood ratio tests are consistent with AIC selections. Focusing first on the effect of mating system effects on inter-population variation in μ_O, the likelihood ratio statistic for all six of the possible comparisons is significant (Full Model vs. Reduced Model 2 and Reduced Model 1 vs. Reduced Model 3 for each trait). The corresponding tests for inbred plants are significant for 'days' and the index, but not for corolla width. Likelihood ratio tests of mating system effects on the means are more variable in outcome. For outbred plants, three of six comparisons are significant: Reduced Model 2 vs. Reduced Model 3 for both corolla with and 'days' and Full Model vs. Reduced Model 1 for 'days'. For inbred plants, three of six are also significant: Reduced Model 2 vs. Reduced Model 3 for both the trait index and 'days' and Full Model vs. Reduced Model 1 for 'days'.

Variation among populations in $\Delta\mu_O$ (evolution of the outbred mean) for the trait index is illustrated in Figure 2-3. The estimated $\Delta\mu_O$ within each experimental population was obtained by subtracting the mean of F1 (control) plants from the mean of outbred plants (for each response variable). Estimates for $\Delta\mu_I$ (evolution of the inbred mean) were

obtained by calculating the mean phenotype of the inbred progeny within each experimental population and then subtracting the mean of F2 control plants. The F2 plants of the base population, with $f = 0.5$, are inbred to the same extent as the selfed progeny of each experimental population. One technical note: the μ_I estimates from generation 5 of our experiment are not equivalent to the variable introduced in eq (1), because $f = 0.5$ for generation 5 plants. However, this is sufficient to predict the mean of fully inbred plants ($f = 1$) when the relationship between trait means and f is linear (which is assumed in the derivation of equation 1). Given linearity, one can predict the mean of fully inbred plants (the μ_I of eq. 1) simply by doubling the difference between our reported estimates of μ_I and μ_O. A previous experiment demonstrated that both flower size and days to flower change in an approximately linear way with f (Kelly 2005a).

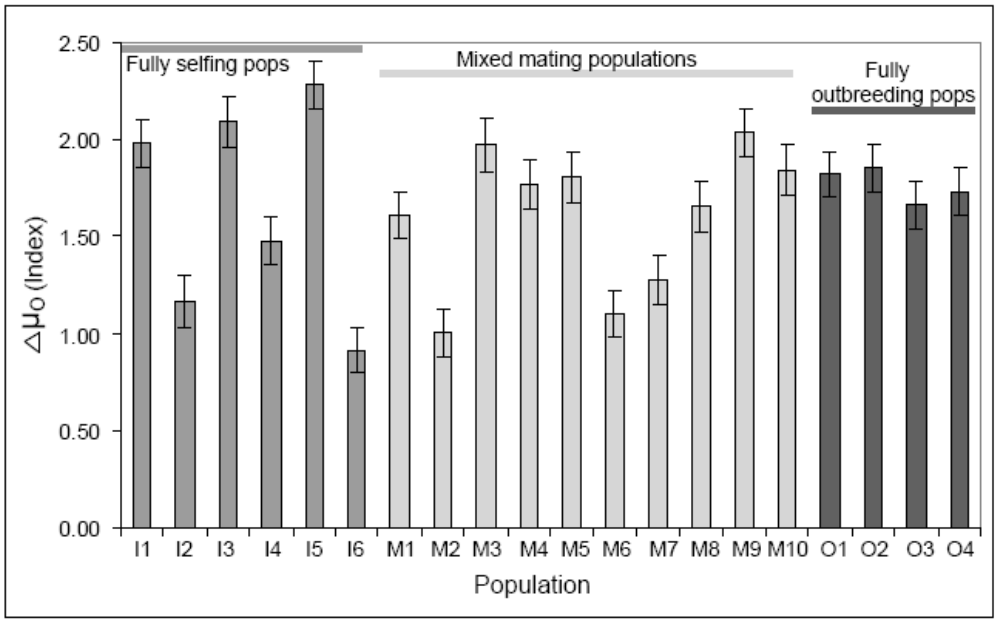


Figure 2-3. The estimated $\Delta\mu_O$ for the index value (I) of each population (error bars denote +/- one SE using the pooled within population variance).

The nature of mating system effects on rates of evolution, $\Delta\mu_O$ and $\Delta\mu_I$ (averaging across populations within each mating system category), is demonstrated in Figure 2-4. For the trait index, $\Delta\mu_O$ did not differ significantly among mating systems, but inbreeding and outcrossing populations achieved high index values in different ways. The fully inbreeding populations responded mainly by evolving larger flowers while outcrossing populations evolved both traits. Development time was substantially reduced in outcrossing populations. Mixed mating populations were intermediate. The response of the inbred mean, $\Delta\mu_I$, for the trait index was greatest for mixed mating populations and least for fully selfing populations (Figure 2-4, Part B). As with the outbred means, the fully selfing populations yielded the greatest response in $\Delta\mu_I$ for corolla width. Fully outcrossing populations evolved the least for corolla width and mixed mating populations were intermediate. The opposite trend obtained for $\Delta\mu_I$ of days to flower. In fact, the inbreeding populations evolved opposite the direction of selection for this character, i.e. populations I1-I6 exhibit significantly delayed flowering (on average) relative to the controls.

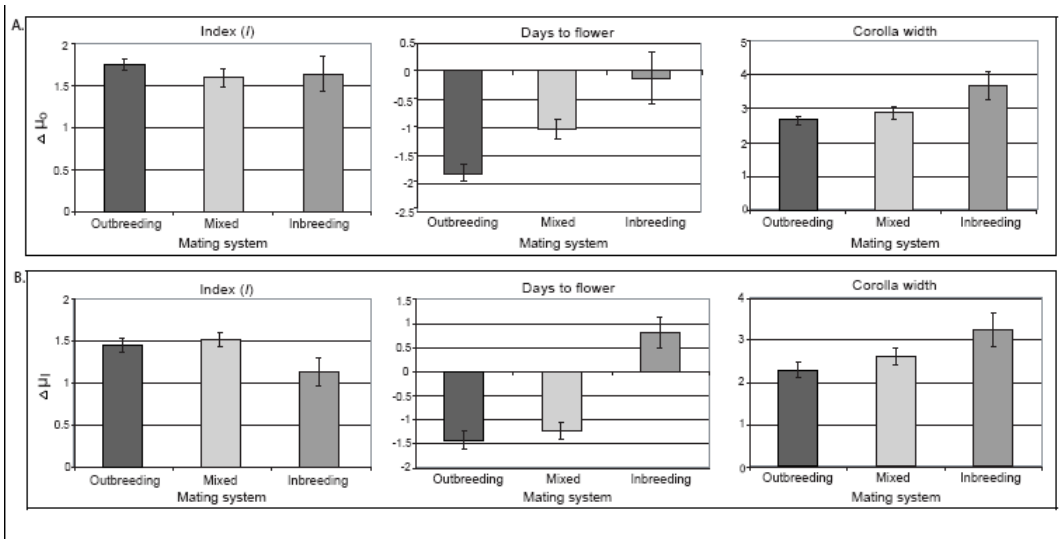


Figure 2-4. Mean responses to selection within each mating system category (error bars denote +/- one SE) for the trait index, days to first flower, and corolla width (in mm). Part A demonstrates the changes in outbred mean values ($\Delta\mu_o$) while Part B demonstrates the inbred mean values ($\Delta\mu_I$).

The pattern of selection, as measured by cumulative selection gradients and differentials, differed significantly among mating system categories (Table 2-3, Figure 2-5). The cumulative selection differential on *I* was significantly lower in the fully outcrossing populations than in the mixed-mating or fully selfing categories. The opposite trend was obtained for the cumulative selection gradient. This reversal reflects the fact that the variance in *I* within populations was substantially elevated by inbreeding. For days to flower, cumulative differentials did not differ among categories, but the cumulative gradients for fully outcrossing populations were significantly greater than for mixed-mating or fully selfing populations. For corolla width, the magnitude of differentials increases with inbreeding while gradients decline. The realized heritability of the trait index differed significantly among mating systems when evaluated using inbred plants but not outbred plants (Table 2-3). The estimated mean heritabilities derived from $\Delta\mu_O$

are 0.27, 0.26, and 0.33 for fully selfing, mixed mating, and outcrossing populations, respectively. The comparable values from $\Delta\mu_I$ are 0.18, 0.25, and 0.27.

	Source	DF	SS	MS	F	p
Section parameters:						
	Corolla					
β	**width**	Mating system	2	2.180	1.090	30.53	0.000
		Error	17	0.607	0.036		
	day	Mating system	2	3.695	1.847	13.37	0.000
		Error	17	2.349	0.138		
	Index	Mating system	2	6.694	3.347	15.12	0.000
		Error	17	3.763	0.221		
	Corolla					
S	**width**	Mating system	2	0.698	0.349	2.14	0.15
		Error	17	2.777	0.163		
	day	Mating system	2	0.210	0.105	3.57	0.05
		Error	17	0.501	0.029		
	Index	Mating system	2	0.616	0.308	10.89	0.001
		Error	17	0.481	0.028		
Realized heritability for Index:						
		Mating system	2	0.01412	0.00709	1.98	0.17
$\Delta\mu_O$		Error	17	0.06090	0.00358		
		Mating system	2	0.02385	0.01193	5.73	0.01
$\Delta\mu_I$		Error	17	0.03539	0.00208		

Table 2-3. Analysis of variance results with selection parameters (cumulative gradients, β, or differentials, S) or realized heritabilities as the response variables and mating system as the factor.

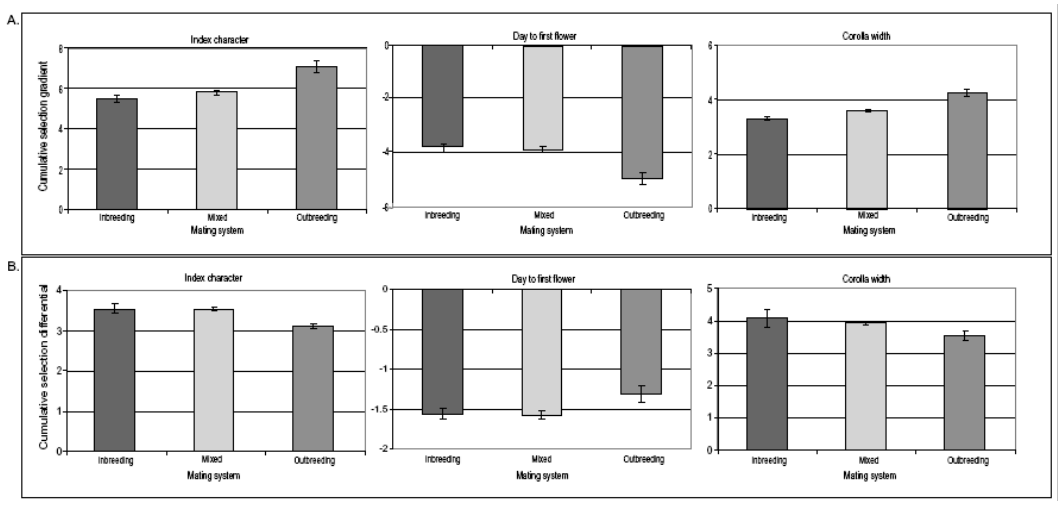


Figure 2-5. The average cumulative selection gradients (Part A) and differentials (Part B) are given for each trait for each mating system category (error bars denote +/- one SE).

Discussion

This experiment demonstrates that populations experiencing the same selection regime may diverge in their responses due only to differences in mating system. Phenotypic divergence is notable given that our experimental design excluded many of the hypothesized influences of mating system. Our populations were founded from the same source and each had the same initial allele frequencies (approximately). The important effect of mating system on the amount of genetic variation was thus excluded (Stebbins 1957; Lande and Schemske 1985; Charlesworth 2003). The experiment was short in duration, and as a consequence, standing variation determined response. Mating system would likely impact the dynamics of new mutations contributing to long term response (Caballero and Hill 1992b; Charlesworth 1992; Caballero and Santiago 1995). Finally,

we experimentally controlled the fitness of both inbred and outbred plants based on their phenotypic values. In nature however, inbreeding often directly affects fitness via inbreeding depression, complicating the relationships between genotype, phenotype, and fitness (Willis 1996a).

The experiment identified mating system effects on both the mean and variance of evolutionary responses to selection. Average responses in both outbred and inbred means, i.e. the means for $\Delta\mu_O$ and $\Delta\mu_I$ across replicate populations, differed in both magnitude and nature among mating system categories (Figure 2-4). In interpreting these differences, it is useful to distinguish the effects of inbreeding on the initial presentation of genetic variation (evident in generation 0) and the subsequent interaction of selection and inbreeding (accruing over generations 0-4). As described below, two sets of genetic estimates suggest that the immediate effect of inbreeding is to inflate genetic variances. The initial V_G for *I* and its component traits was thus greater in the fully selfing populations (I1-I6) than in the outcrossing populations (O1-O4). It is therefore noteworthy that inbreeding did not *generally* accelerate response to selection. For the trait index (*I*), there was no difference among mating systems in mean $\Delta\mu_O$, while $\Delta\mu_I$ was substantially lower in I1-I6 than in O1-O4 (Figure 2-3).

The effect of inbreeding on the genetic variance of a quantitative character can be predicted using genetic variance components (Weir and Cockerham 1977; Cockerham 1983; Cockerham and Weir 1984; Shaw et al. 1998). Previously, Kelly and Arathi (2002b) used a breeding design with both inbred and outbred plants from the Iron

Mountain population to estimate the relevant components for flower size and the rate of development. These estimates predict that the genetic variance of corolla width should double as *f* increases from 0 to 1 while the variance in days to flower should change little (see Table 2 of Kelly and Arathi 2003). Importantly, values for both traits were transformed in that study (using square-root for corolla width and the logarithm for days) while selection was imposed on an index of untransformed values in the present experiment. In addition, the plants measured by Kelly and Arathi (2003) were grown in a greenhouse (with a mixture of natural and artificial light) while those of this study matured in a growth chamber. The relevant characters, particularly development rate, are sensitive to growth conditions.

For the growth conditions of this study, we can estimate V_G for both outbred and fully inbred genotypes from measurements of plants in generation 0. This generation consisted of family groups. Each inbred line, as well as the F1 families derived from crosses between lines, represents a set of genetically identical individuals (or at least nearly identical, see (Kelly 2005a)). We can thus partition variation within and among families to estimate genetic and environmental variances (procedure described in Appendix A). For corolla width, the estimated outbred V_G is 2.54. The variance among fully inbred lines is 3.11, a 22% increase. For days to flower, the estimated V_G is 1.83 among outbred genotypes and 4.44 among fully inbred lines (a 143% increase). For the trait index, the estimated V_G among inbred genotypes is slightly less than twice the outbred V_G (0.95 vs. 0.53). These estimates are associated with sizable standard errors, and unlike those of (Kelly and Arathi 2003), they are potentially contaminated by maternal effects.

However, taken in aggregate, these studies suggest that the immediate effect of inbreeding is to inflate the genetic variance in both *I* and its component traits.

Given the initial effect of inbreeding, the observed $\Delta\mu_O$ and $\Delta\mu_I$ (Figure 2-3) suggests that the interaction between selection and inbreeding over the course of the experiment (generations 0-4) must have substantially retarded responses. The realized heritability of *I* did not differ among mating systems (for $\Delta\mu_O$) or was significantly lower in the fully selfing populations (for $\Delta\mu_I$, Table 2-3). This result is fully consistent with theoretical predictions (see Introduction of this chapter). Linkage disequilibrium among QTL is one manifestation of the interaction between inbreeding and selection. Truncation selection reduces the phenotypic variance and generates negative associations among alleles (Bulmer 1980). This reduces V_G, an effect that is greatly amplified with inbreeding due to the reduced efficacy of recombination. Reduced V_G limits response to selection.

The most surprising result concerns $\Delta\mu_I$: the inbred mean for *I* evolved least in the fully selfing populations. 'Days to flower' actually evolved in the opposite direction of selection (Figure 2-4, Part B), i.e. the selfed progeny from experimental populations I1-I6 developed more slowly (on average) than selfed progeny from the original control population. This result is counter-intuitive because μ_I is determined by the effects of alleles when in homozygous form (Wright 1951). Selection acted most directly on the homozygous effects of alleles in the fully selfing populations and least directly on these effects in the fully outcrossing populations.

This paradoxical result may represent a case of "genetic slippage", wherein selection gains are reduced ($\Delta\mu_I$ is lowered in this case) because recombination eliminates favorable gene combinations produced by selection (Lynch and Deng 1994; Pfrender and Lynch 2000). Selected adults within populations I1-I6 were randomly paired and mated at the end of generation 3. These outbred progeny (generation 4) were then both outcrossed and selfed to produce the plants used to estimate $\Delta\mu_O$ and $\Delta\mu_I$ (Figure 2-1). Because there was no selection, these two meiotic episodes should not have altered allele frequencies in a deterministic way within populations I1-I6. Thus, the same single locus genotypes present at the end of generation 3 are reproduced in the inbred plants of generation 5. However, the multi-locus genotypic combinations are not. Recombination in these two generations might have eliminated, or at least reduced the frequency of, favorable gene combinations established by selection within populations I1-I6. Inbreeding reduces the effect of recombination and facilitates selection on gene combinations if there is epistasis (Allard 1975). Despite the fact that corolla width decreases linearly with *f* while days to flower increases linearly with *f* (consistent with Equation 1), both traits exhibit considerable epistasis (Kelly 2005a). Experiments are currently underway to determine if this epistasis is of the proper nature to yield genetic slippage.

The second prominent effect of mating system was how selection affected the evolution of the component characters, flower size and rate of development. The regime favored large-flowered plants that develop rapidly. Fully selfing populations responded almost entirely by evolving larger flowers, whereas fully outcrossing populations also evolved

more rapid development. Plants from the mixed mating populations evolved flowers with intermediate values for both flower size and development rate. We would like to interpret this differential response in terms of mating system effects on genetic parameters, i.e. the elements of *G* or its generalization (Kelly 1999b), and/or on selection parameters, e.g. β. Mating system did alter selection differentials and gradients (Figure 2-5), but these effects do not explain the differential responses of μ_O and μ_I. The cumulative selection differentials for corolla width, days to flower, and the character index were smaller in magnitude for the outcrossing populations than in either mixed or fully selfing populations. In contrast, the cumulative selection gradients of outcrossing populations were consistently the largest in magnitude. These differences are due mainly to the inflation of the phenotypic variance (in the index and each component character) caused by inbreeding. Both the genetic and environmental variances of quantitative traits can change with inbreeding (Lerner 1954; Wright 1977; Whitlock and Fowler 1999; Kelly and Arathi 2003).

Can inbreeding-induced changes in the genetic variances of, and covariance between, corolla width and development rate explain their differential responses to selection? As described above, the *immediate* effects of inbreeding on genetic variances do not obviously explain the result. Inbreeding does not simultaneously inflate the variance in corolla width (accelerating response) and reduce the variance in days to flower (retarding response). Inbreeding could have altered genetic correlations among traits (e.g. Phillips et al. 2001) and the extent to which each component trait determines the index. The immediate effect of inbreeding is to increase the magnitudes of genetic covariances

between both component traits and the index (see Appendix A). However, just as with variances, the interaction between selection and inbreeding can dramatically alter genetic covariances (Kelly 1999b). This interaction is likely responsible for the differential response.

The third major mating system effect was that inbreeding consistently increased the variance among replicate populations (Figure 2-3; Table 2-2). This observation is also consistent with theoretical predictions (see Introduction). Drift is an important factor in most selection experiments (Robertson 1961) and its effect is magnified by inbreeding of selected adults (Caballero and Santiago 1995). We expect drift to limit long term response, but it is unlikely that the inbreeding effect on drift can explain the differences in mean responses of mating system categories (Figure 2-4). First, the experiment was short in duration (4 generations) with response based on standing variation. As a consequence, the pronounced effect of drift on new mutations is not relevant (see Caballero and Hill 1992b; Merchante et al. 1995). Second, the responses of inbreeding populations were not uniformly lower than those of fully outcrossing populations. Fully selfing populations produced the most evolutionary change in corolla width (both $\Delta\mu_O$ and $\Delta\mu_I$), while mixed mating populations exhibited the highest average $\Delta\mu_I$ for the trait index (Figure 2-4).

Implications—The experiment demonstrates short term changes in trait means, variances, and covariances that result from the interaction of selection, genetic drift, and mating system. These results bear, at least indirectly, on broader examinations of evolutionary

pattern. Genetic variances and covariances provide a critical linkage between micro-evolutionary processes and macro-evolutionary patterns (Arnold 1992). Lande (1979) developed an explicit quantitative method for relating genetic variances and covariances within species to differences in mean trait values among species. This approach assumes constancy of the relevant genetic parameters, an assumption that has received substantial empirical scrutiny (Steppan et al. 2002; Phelan et al. 2003; Begin and Roff 2004; Manuel Cano et al. 2004). Our results demonstrate that genetic variances and covariances are likely to change rather dramatically, over short time spans, if the mating system of a population changes simultaneously with selection.

Consider the evolutionary transition from outcrossing to self-fertilization, one of most common in the history of Angiosperms (Stebbins 1950; Stebbins 1974; Barrett 2002). Selfing species are typified by numerous physiological and morphological characteristics including accelerated floral development, smaller corollas, decreased stigma and anther separation, and changes in timing of anther dehiscence and stigma receptivity (Wyatt 1983; Karron et al. 1997; van Kleunen and Ritland 2004). These characteristics may evolve prior to, simultaneous with, or following the transition to a self-fertilizing mating system. Given that many of these characters will exhibit genetic correlations, determining the actual targets of selection at different stages in the progression becomes difficult. Disentangling cause and consequence requires a detailed consideration of the interaction between mating system and natural selection in determining phenotypic evolution.

References

Allard, R. W. 1975. The mating system and microevolution. Genetics 79:S115-S126.

Arnold, S. 1992. Constraints on phenotypic evolution. American Naturalist 140:S85-S107.

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.

Barrett, S. C. H. 2002. The evolution of plant sexual diversity. Nature Reviews Genetics 3:274-284.

Barrett, S. C. H. 2003. Mating strategies in flowering plants: The outcrossing-selfing paradigm and beyond. Phil. Trans. R. Soc. Lond. B 258:991-1004.

Begin, M., and D. A. Roff. 2004. From micro- to macroevolution through quantitative genetic variation: Positive evidence from field crickets. Evolution 58:2287-2304.

Bennett, J. H., and F. E. Binet. 1956. Associations between mendelian factors with mixed selfing and random mating. Heredity 10:51-55.

Bulmer, M. G. 1980. The mathematical theory of quantitative genetics. Clarendon Press, Oxford.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: A practical information-theoretic approach. Springer-Verlag, New York.

Byers, D. L., and D. M. Waller. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. Annual Review of Ecology and Systematics 30:479-513.

Caballero, A., and W. G. Hill. 1992a. Effective size of nonrandom mating populations. Genetics 130:909-916.

Caballero, A., and W. G. Hill. 1992b. Effects of partial inbreeding on fixation rates and variation of mutant-genes. Genetics 131:493-507.

Caballero, A., P. D. Keightley, and W. G. Hill. 1991. Strategies for increasing fixation probabilities of recessive mutations. Genetical Research 58:129-138.

Caballero, A., and E. Santiago. 1995. Response to selection from new mutations and effective size of partially inbred populations. I. Theoretical results. Genetical Research 66:213-225.

Carr, D. E., and M. R. Dudash. 1996. Inbreeding depression in two species of *Mimulus* (Scrophulariaceae) with contrasting mating systems. Am. J. Bot. 83:586-593.

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

Charlesworth, B. 1992. Evolutionary rates in partially self-fertilizing species. American Naturalist 140:126-148.

Charlesworth, D. 2003. Effect of inbreeding on genetic diversity of populations. Phil. Trans. R. Soc. Lond. B 358:1051-1070.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annual Review Of Ecology and Systematics 18:237-268.

Charlesworth, D., and B. Charlesworth. 1995. Quantitative genetics in plants - the effect of the breeding system on genetic variability. Evolution 49:911-920.

Charlesworth, D., and S. I. Wright. 2001. Breeding systems and genome evolution. Current Opinion in Genetics and Development. 11:685-690.

Clegg, M. T., R. W. Allard, and A. L. Kahler. 1972. Is the gene the unit of selection? Evidence from two experimental plant populations. Proc. Natl. Acad. Sci. USA 69:2474-2478.
Cockerham, C. C. 1983. Covariances of relatives from self-fertilization. Crop science 23:1177-1180.
Cockerham, C. C., and D. F. Matzinger. 1985. Selection response based on selfed progenies. Crop science 25:483-488.
Cockerham, C. C., and B. S. Weir. 1984. Covariances of relatives stemming from a population undergoing mixed self and random mating. Biometrics 40:157-164.
Darwin, C. R. 1876. The effects of cross- and self-fertilisation in the vegetable kingdom. John Murray, London.
Dole, J. A., and K. Ritland. 1993. Inbreeding depression in 2 *Mimulus* taxa measured by multigenerational changes in the inbreeding coefficient. Evolution 47:361-373.
Dudash, M. R., and D. E. Carr. 1998. Genetics underlying inbreeding depression in Mimulus with contrasting mating systems. Nature 393:682-684.
Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. Prentice Hall, London.
Fenster, C. B., P. K. Diggle, S. C. H. Barrett, and K. Ritland. 1995. The genetics of floral development differentiating 2 species of *Mimulus* (Scrophulariaceae). Heredity 74:258-266.
Fenster, C. B., and K. Ritland. 1994. Quantitative genetics of mating system divergence in the yellow monkeyflower species complex. Heredity 73:422-435.
Fishman, L., A. J. Kelly, and J. H. Willis. 2002. Minor quantitative trait loci underlie floral traits associated with mating system divergence in *Mimulus*. Evolution 56:2138-2155.
Haldane, J. B. S. 1949. The association of characters as a result of inbreeding and linkage. Annual of Eugenics 15:15-23.
Hamrick, J. L., and M. J. W. Godt. 1996. Effects of life history traits on genetic diversity in plant species. Phil. Trans. Roy. Soc. London Biol. Sci. 351:1291-1298.
Harris, D. L. 1964. Genotypic covariances between inbred relatives. Genetics 50:1319-1348.
Hartl, D. L., and A. G. Clark. 1989. Principles of population genetics. Sinauer Associates, Sunderland, Massachusetts.
Hayashi, T., and Y. Ukai. 1994. Change in genetic variance under selection in a self-fertilizing population. Genetics 136:693-704.
Jacquard, A. 1974. The genetic structure of populations. Springer-Verlag, New York.
Karron, J. D., R. T. Jackson, N. N. Thumser, and S. T. Schlicht. 1997. Outcrossing rates of individual *Mimulus* ringens genets are correlated with anther-stigma separation. Heredity 79:365-370.
Kelly, J. K. 1999a. Response to selection in partially self fertilizing populations. I. Selection on a single trait. Evolution 53:336-349.
Kelly, J. K. 1999b. Response to selection in partially self fertilizing populations. II. Selection on multiple traits. Evolution 53:350-357.
Kelly, J. K. 2005. Epistasis in monkeyflowers. Genetics in press
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 S. Williamson. 2000. Predicting response to selection on a quantitative trait: A comparison between models for mixed-mating populations. Journal of Theoretical Biology 207:37-56.
Kempthorne, O. 1957. An introduction to genetic statistics. Wiley, New York.
Lande, R. 1979. Quantitative genetic analysis of multivariate evolution applied to brain: Body allometry. Evolution 33:402-416.
Lande, R., and S. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210-1226.
Lande, R., and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I.Genetic models. Evolution 39:24-40.
Latta, R., and K. Ritland. 1994. The relationship between inbreeding depression and prior inbreeding among populations of 4 *Mimulus* taxa. Evolution 48:806-817.
Lerner, I. M. 1954. Genetic homeostasis. John Wiley and Sons, New York.
Lynch, M., and H.-W. Deng. 1994. Genetic slippage in response to sex. American Naturalist 144:242-261.
Manuel Cano, J., A. Laurila, J. Palo, and J. Merila. 2004. Population differentiation in g matrix structure due to natural selection in *Rana temporaria*. Evolution 58:2013-2020.
Merchante, M., A. Caballero, and C. Lopez-Fanjul. 1995. Response to selection from new mutation and effective size of partially inbred populations. II. Experiments with *Drosophila melanogaster*. Genetical Research 66:227-240.
Mitchell-Olds, T., and J. J. Rutledge. 1986. Quantitative genetics in natural plant populations: A review of the theory. American Naturalist 127:379-402.
Nomura, T. 2005. Joint effect of selection, linkage, and partial inbreeding on additive genetic variance in an infinite population. Biometrical Journal 47:527-547.
Pederson, D. G. 1969. The prediction of selection response in a self-fertilizing species i. Individual selection. Australian Journal of biological science 22:117-129.
Pfrender, M. E., and M. Lynch. 2000. Quantitative genetic variation in *Daphnia*: Temporal changes in genetic architecture. Evolution 54:1502-1509.
Phelan, J. P., M. A. Archer, K. A. Beckman, A. K. Chippindale, T. J. Nusbaum, and M. R. Rose. 2003. Breakdown in correlations during laboratory evolution. I. Comparative analyses of *Drosophila* populations. Evolution 57:527-535.
Phillips, P. C., and S. J. Arnold. 1989. Visualizing multivariate selection. Evolution 43:1209-1222.
Phillips, P. C., M. C. Whitlock, and K. Fowler. 2001. Inbreeding changes the shape of the genetic covariance matrix in *Drosophila melanogaster*. Genetics 158:1137-1145.
Pollak, E., and M. Sabran. 1992. On the theory of partially inbreeding finite populations. III. Fixation probabilities under partial selfing when heterozygotes are intermediate in viability. Genetics 131:979-985.
Ritland, K., and F. R. Ganders. 1987. Covariation of selfing rates with parental gene fixation indexes within populations of *Mimulus guttatus*. Evolution 41:760-771.
Robertson, A. 1952. The effect of inbreeding on the variation due to recessive genes. Genetics 37:189-207.
Robertson, A. 1961. Inbreeding in artificial selection programmes. Genetical Research 2:189-194.

Robertson, A. W., A. Diaz, and M. R. MacNair. 1994. The quantitative genetics of floral characters in *Mimulus guttatus*. Heredity 72:300-311.
Searle, S. R., G. Casella, and C. E. McCulloch. 1992. Variance components. John Wiley and sons Inc., New York.
Shaw, R. G., D. L. Byers, and F. H. Shaw. 1998. Genetic components of variation in *Nemophila menziesii* undergoing inbreeding: Morphology and flowering time. Genetics 150:1649-1661.
Sprengel, C. K. 1793. Das endeckte geheimniss des natur in bau und in der befurchtung der blumen. F. Vieweg aelt., Berlin.
Stebbins, G. L. 1950. Variation and evolution in plants. Columbia University Press, New York.
Stebbins, G. L. 1957. Self-fertilization and population variability in higher plants. Am. Nat. 91:337-354.
Stebbins, G. L. 1974. Flowering plants: Evolution above the species level. Harvard University Press, MA.
Steppan, S. J., P. C. Phillips, and D. Houle. 2002. Comparative quantitative genetics: Evolution of the g matrix. Trends in Ecology & Evolution 17:320-327.
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 83:625-632.
Turelli, M., and N. H. Barton. 1994. Genetic and statistical-analyses of strong selection on polygenic traits - what, me normal. Genetics 138:913-941.
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 Kleunen, M., and K. Ritland. 2004. Predicting the evolution of floral traits associated with mating system in a natural plant population. J. Evolutionary Biology 17:1389-1399.
Vickery, R. K. 1978. Case studies in the evolution of species complexes in *Mimulus*. Evol. Biol. 11:405-507.
Weir, B. S., and C. C. Cockerham. 1977. Two-locus theory in quantitative genetics. Pp. 247-262 *in* E. Pollack, O. Kempthorne and T. B. Bailey, eds. Proceedings of the International Conference on Quantitative Genetics. Iowa State University Press, Ames, Iowa.
Whitlock, M. C., and K. Fowler. 1999. The changes in the genetic and environmental variance with inbreeding in *Drosophila melanogaster*. Genetics 152:345-353.
Willis, J. H. 1993. Partial self fertilization and inbreeding depression in two populations of *Mimulus guttatus*. Heredity 71:145-154.
Willis, J. H. 1996. Measures of phenotypic selection are biased by partial inbreeding. Evolution 50:1501-1511.
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.
Wright, A. J., and C. C. Cockerham. 1985. Selection with partial selfing I. Mass selection. Genetics 109:585-597.

Wright, S. 1951. The genetical structure of populations. Annals of eugenics 15:323-354.
Wright, S. 1977. Evolution and the genetics of populations. Vol. 3. Experimental results and evolutionary deductions. University of Chicago Press, Chicago.
Wyatt, R. 1983. Pollinator-plant interactions and the evolution of breeding systems. Pp. 51-95 *in* L. A. Real, ed. Pollination biology. Academic press, Orlando, FL.

Chapter 3. Genetic and environmental variation in trichome density of *M. guttatus*

Abstract

Mimulus guttatus (yellow monkeyflower) frequently produce glandular trichomes, a trait that may resist herbivory. Constitutive production of trichomes is variable both within and among populations of *M. guttatus* and there is genetic variation for it both within and among geographically distinct natural populations. Damage on early leaves can induce increased trichome production on later leaves, a plastic response that is likely adaptive. In addition, it is shown that this induction can be maternally transmitted by a yet undescribed epigenetic mechanism. There is genetic variation among plants in the capacity for both *within* and *between* plant generation induction. Despite the clear evolutionary importance of variation in constitutive and induced herbivory-resistance traits, few other studies have noted genetic variation in both within a plant species.

Introduction

Part 1: *Genetic variation in trichome density within and among geographically distinct natural populations.*

Most plant species are consumed by insects and this interaction has been extensively studied (Berenbaum et al. 1986; Rausher and Simms 1989; Simms and Rausher 1989; Simms and Fritz 1990; Marquis 1991, 1992; Nunez-Farfan and Dirzo 1994; Hare and Elle 2002; Fornoni et al. 2003a). Geographical variation in plant-insect herbivore interactions is a widespread phenomenon, as distributions of particular insect and plant populations may overlap in only portions of their ranges (Thompson 1994). Differing severities and types of herbivory are likely to impose differing selection regimes among populations of a species for resistance and tolerance traits. The evolution of geographical

trait variation is a precursor to speciation and a critical link between micro- and macro-evolutionary processes. It is thus surprising that there is relatively little data on the nature and extent of genetic differentiation among geographical populations of natural plant species in their responses to herbivory (Mopper et al. 1992; Valverde et al. 2001; Fornoni et al. 2003b; Valverde et al. 2003; Fornoni et al. 2004).

In response to natural selection imposed by herbivores, plants have evolved a wide range of defensive traits. 'Resistance characters' are mechanical or chemical features that prevent herbivory or limit its extent. These characters can be further subdivided depending on whether they affect insect preference (antixenosis) or performance (antibiosis). Defensive traits can be further classified as constitutive or induced. Constitutive resistance is always present, while induced resistance is triggered by herbivory (Morris et al. 2006). An alternative response to herbivory is through the evolution of 'tolerance'. Tolerance characters reduce the effect of herbivory on fitness through mechanisms such as increased photosynthetic rates and/ or compensatory regrowth (Fineblum and Rausher 1995; Strauss and Agrawal 1999; Weinig et al. 2003; Leimu and Koricheva 2006).

Plant trichomes are hairlike structures that extend from the plant epidermis and occur in a variety of types including glandular or non-glandular, hooked or straight. Trichomes may serve a variety of defensive and physiological functions. Leaf trichomes have been shown to reduce insect herbivory in a number of plant species (Levin 1973; Marquis 1992; van Dam and Hare 1998; Romeis et al. 1999; Valverde et al. 2001). They interfere

with insect movement and feeding, and often secrete glandular fluids that may trap, poison, or repel herbivores (Levin 1973; Elle and Hare 2000). For plants in xeric habitats, trichomes reflect light and can reduce transpiration (Ehleringer 1984; Larcher 2001). Geographic and genetic variation in trichome density and type has been noted within a number of other plant species (Grant 1950; Levin 1973; van Dam et al. 1999; Valverde et al. 2001).

Trichome density is highly variable in *Mimulus guttatus* (yellow monkeyflower), a wildflower common throughout western North America. Trichomes in *M. guttatus* are straight and occur mostly in the glandular form, often secreting a sticky and potentially noxious fluid. Trichome density is environmentally sensitive in *M. guttatus*, and the first part of this study demonstrates that substantial genetic variation exists in this trait, both within and among populations. This is accomplished using a common garden experiment involving six natural populations.

Environmental variation in trichome density in M. guttatus

Phenotypic plasticity is environmentally-dependant phenotypic variation in the growth or development of an organism (Bradshaw 1965; West-Eberhard 1989; DeWitt and Scheiner 2004). Plasticity is well-documented in plants and is commonly hypothesized to be an adaptive response of these sessile organisms to environmental heterogeneity (Bradshaw 1965; Dudley 2004). Plasticity is adaptive only if a particular change in growth/development improves fitness. To determine whether or to what extent phenotypic plasticity affects the evolutionary trajectory of a population or species, it is

essential to know not only whether it is adaptive, but whether there is variation for plasticity and what proportion of this variation is heritable (Schlichting 2004).

Physiological benefits (i.e. to reduce light radiation or transpiration rates) are unlikely to explain the high trichome densities of plants in a number of the populations, as they are located in the fog belt of coastal Northern California and should not suffer extreme drought or intense sunlight. These high trichome density populations are perennial, and experience heavy herbivory by a number of insect herbivores. This contrasts with annual, alpine *M. guttatus* populations where plants only live for 6-10 weeks. Frequently, plants in these populations have few or no trichomes and experience little insect herbivory in their short lifetimes. One might thus predict presence of inter-population genetic variation for trichome density or other putative resistance traits between the alpine annual and coastal perennial *M. guttatus* populations.

Several studies have shown that leaf damage can induce increased trichome production. Both vertebrate herbivore damage and mechanical damage induced higher trichome densities in stinging nettle (*Urtica dioica*) (Pullin and Gilbert 1989). They also found induction to depend on soil nutrient availability. Damage by cabbage butterfly larvae (*Pieris rapae*) on the fifth leaf of black mustard plants (*Brassica nigra*) induced higher trichome densities on the seventh leaf of these plants (Traw and Dawson 2002). Adult leaf beetle grazing (*Phratora vulgatissima*) on willow (*Salix cinerea*) induced significantly greater trichome densities on later leaves, which reduced herbivory by the next generation of beetle larvae of the same species later in the season (Dalin and

Bjorkman 2003). Mechanical defoliation of a tropical shrub (*Cnidoscolus aconitifolius*) induced higher trichome density in leaves that developed between 30 days and 3 months after the defoliation (Abdala-Roberts and Parra-Tabla 2005). Despite the apparent frequency of the response across plant species, no studies previous to this have documented genetic variation in within-generation trichome induction.

When there is consistency of environmental conditions across generations, it may be advantageous to transmit a trait-induction signal to progeny. Transgenerational induction of a trait differs from typical maternal effects in both mechanism and effect. Maternal effects often reflect differences in the quality of the maternal environment, resulting in differential partitioning of resources to the offspring. This, in turn, affects the fitness of these offspring (Lynch and Walsh 1998). Transgenerational plasticity, in contrast, is epigenetically based; the expression of genes influencing trait development in the offspring is maternally activated. Transgenerational induction of a plant trait has been demonstrated only once before, by Agrawal (1999, 2001) in wild radish (*Raphanus raphanistrum*). Transgenerational induction is only to be adaptive if environmental cues received by the parent are predictive of the fitness environment that will be experienced by their progeny.

Here, I describe experiments that investigate phenotype plasticity in trichome density in *Mimulus guttatus* (yellow monkeyflower). With these experiments, I address the following questions: 1) Is trichome production in *M. guttatus* phenotypically plastic; i.e. can foliar damage on early leaves induce increased trichome densities on later leaves? 2)

Is there transgenerational plasticity, i.e can induction be transmitted maternally? 3) Is there genetic variation for either within-generation or transgenerational trichome induction in *M. guttatus*? 4) Is constitutive production negatively related to the capacity for induction?

Materials and Methods

Yellow monkeyflower (*Mimulus guttatus*; Phrymaceae, (Beardsley and Olmstead 2002)) ranges from Mexico to Alaska in western North America and typically inhabits wet areas such as stream banks. It is a self-compatible, hermaphroditic plant that reproduces by a mixture of outcrossing and self-fertilization. Local populations differ extensively in morphology, life history, and selfing rate (Fenster and Ritland 1994; Kelly and Arathi 2003; Hall et al. 2006; Hall and Willis 2006).

Part 1:

The experiment described here uses plant populations established in the greenhouse from seed collected from seven natural populations (Table 3-1, Figure 3-1). This list includes both annual and perennial populations that occupy diverse habitats over a range of altitudes (from 4 to 1372 meters above sea level).

Population	Location	Latitude and Longitude	Elevation (meters above sea level)	Population type (annual or perennial)
A	HW 4, E of Copperopolis, CA	N 37°57.36 W120°41.024	268	Annual
B	HWY 120, E of Don Pedro Reservoir	N 37°52.452 W120°30.496	310	Annual
C	HW 4, W of Copperopolis, CA	N 38°02.786 W120°38.800	490	Annual
D	Muir Beach, CA	N 37°51.662, W122°34.525	3.5	Perennial
E	Point Reyes National Seashore, CA	N 38°02.925, W122°52.185	35	Perennial
F	Oregon Dunes National Recreation Area, OR	N 43°57.594, W124°07.847	8	Perennial
G	Metolius River headwaters, OR	N 44°26.438, W121°38.225	922	Perennial
H	Cougar Reservoir, OR	N 44°07.250, W122°14.280	519	Annual
I	Dexter Reservoir, OR	N 43°55.032, W 122°45.168	301	Annual
IM	Iron Mountain, OR	N 44°24, W122°09	1372	Annual
J	Campbell Lake Road, WA	N 48°26.832, W122°37.937	33	Annual
K	Pass Lake, WA	N 48°25.022, W122°38.243	38	Annual

Table 3-1. Longitude, latitude, population type, and elevation for each population.

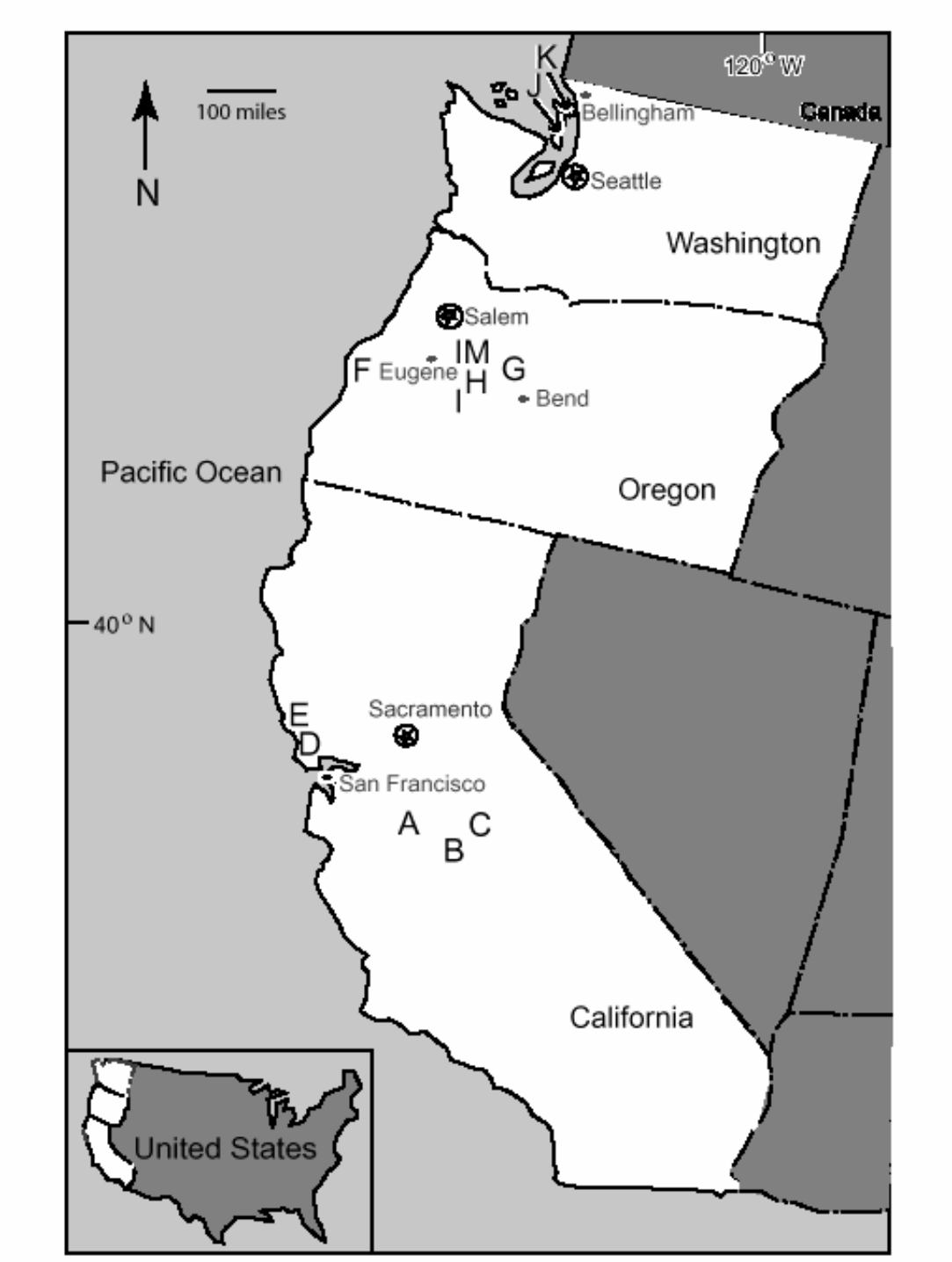


Figure 3-1. Map of the locations of 12 natural *M. guttatus* populations from which seed was collected, June-July 2003.

Field-collected seed from 20-30 plants from the MB, PR, CR, and DR populations was germinated in the greenhouse at the University of Kansas under standard greenhouse conditions (see Arathi and Kelly 2004). Within each population, plants were randomly paired and cross-fertilized to produce outbred families. Plants from the OD and MR populations had not yet set seed at the time of collection, so live plants from each of these populations were shipped back to the University of Kansas greenhouse. There they were outcrossed within each population to form outbred families. Seed from the IM population used in Experiment 2 was not collected by the author (See Methods, Experiment 2 for details) and was omitted from Experiment 1.

Two to three outbred families from each population were grown from seed in the greenhouse. Trichome counts were completed on a defined measuring area of 1 cm^2 on the basal central part of the adaxial side of a leaf from the second leaf pair, immediately after the leaves in the third leaf pair were fully expanded. Trichome density was right-skewed with a large number of zero values and was transformed as log (trichome count +1) prior to analysis of results. Analysis of trichome density was performed as nested ANOVA with full-sib families (a random factor) nested within population, which was treated as a fixed factor because populations were selected at least partially because they differed in trichome density.

Part 2:

The experiments described here used offspring of plants collected from two natural populations, Iron Mountain (IM) and Point Reyes National Seashore (PR). IM is an

annual population in the Cascade Mountains of central Oregon. Plants have a short life span (1-2 months from germination to death) and typically experience little insect herbivory. PR is a perennial, low elevation population in the fog belt of coastal northern California. These plants experience heavy herbivory. The IM plants were from a highly inbred line (IM 767) extracted from the natural population (see Willis 1999 for details). The PR plants were full siblings from a single outbred family.

I developed recombinant inbred lines (RILs) from a cross between an IM 767 plant (father) and a PR plant (mother). A single F1 individual from this cross was self-fertilized to generate 1000 F2 individuals, each of which founded a distinct recombinant lineage. These lines were propagated through single-seed descent for 4 subsequent generations. Due to line loss, approximately 450 RILs remained in the F6 generation. Experiment 1 was conducted (in part) over the course of RIL development while Experiment 2 used only the F6 generation plants and the parentals.

Experiment 1: Constitutive trichome production and trichome induction.

I used 3 categories of plants for this experiment: plants from the two parental populations (PR and IM) and RILs in the F3, F4, and F6 generations. F3 and F4 RILs used had almost complete overlap, while F3/F4 and F6 RILs used had only partial overlap. Replicates from each of these plant categories experienced some or all of the following treatments: (1) constitutive 2^{nd} leaf trichome count, (2) constitutive 5^{th} leaf trichome count, (3) induced 5^{th} leaf trichome count.

All trichome counts were performed on one leaf from the $2^{nd}/5^{th}$ leaf pair on a defined measuring area of 1 cm^2 on the basal central part of the adaxial side of the leaf, immediately after the leaves in the $3^{rd}/6^{th}$ leaf pair were fully expanded. For the induced 5^{th} leaf trichome counts, I simulated insect-chewing damage (punched two holes of approximately 6 mm in diameter) on both leaves of the 2^{nd} leaf pair as soon as the 3^{rd} leaf pair was unfurled. Fifth leaf trichome counts were performed on these plants following the same method as the constitutive 5^{th} leaf counts. Because simulated damage and all trichome counts were done on a specific leaf when the leaf pair immediately above it was unfurled, both damage and response were measured at the same particular development stage across plants (Orians 2005; Morris et al. 2006). Fifth leaf trichome counts were used to assess induction because a preliminary experiment showed that induction was more pronounced in 5^{th} leaves than in later (7^{th}) leaves (unpub data).

This experiment involved 25 PR full-siblings, 10 IM 767 plants, 3 replicates from each of 99 F3 RILs, 3 replicates from each of 99 F4 RILs, and 10 replicates from each of 82 F6 RILs. The same RILs were used in the F3 and F4, and there was partial overlap of lines between the F3/F4 set and the F6 RILs used. I measured these plants as follows: Constitutive 2^{nd} leaf trichome density was measured on 1 replicate from each of the 99 F3 RILs and 1 replicate from each of the F4 RILs. Constitutive 5^{th} leaf trichome density was measured on 11 PR plants, 5 IM plants, 1 replicate from each of the 99 F3 RILs, 1 replicate from each of the 99 F4 RILs, and 5 replicates from each of the 82 F6 RILs. Induced 5^{th} leaf trichome density was measured on 14 PR plants, 5 IM plants, 1 replicate

from each of the 99 F3 RILs, 1 replicate from each of the F4 RILs, and 5 replicates from each of the 82 F6 RILs.

The remaining RILs from the F6 generation were measured for 2nd leaf constitutive and 5th leaf induced trichome density only. 8 replicates were grown for each RIL, in 5 planting rounds in the greenhouse (all replicates from a particular RIL were grown in the same round) with 2nd leaf counts on 4 replicates and damaged 2nd leaves with induced 5th leaf counts on the other 4 replicates. In all cases, plants were grown in 2 inch pots. Plants were randomized within and among categories and were rotated daily on the greenhouse bench to minimize environmental effects.

Experiment 2: Transgenerational trichome induction.

I germinated two replicates from each of 90 F6 RILs, 12 IM 767 plants, and 12 PR full-siblings in 2-inch pots in the greenhouse. One replicate from each RIL, 6 of the IM 767 plants, and 6 of the PR plants were in the "No damage" category. For these, I grew, self-pollinated, and collected seed from each plant. One replicate from each RIL, 6 IM 767 plants, and 6 PR plants were in the "Damaged" category in which I damaged leaves using the simulated insect-chewing method described in Experiment 1. I began the damage of each plant on the leaves from the 2nd leaf pair as soon as the 3rd leaf pair was unfurled and continued to damage the consecutive leaf pairs in the same manner during the self-pollination and seed maturation of these plants. I randomized the position of all plants at the beginning of the experiment on the greenhouse bench and rotated them daily. In the following generation (F7), I grew 4 offspring from each maternal plant (damaged and

undamaged parents of each genotypic class) to maturity and counted trichomes on 5th leaf pair. Preliminary results with a smaller subset of RIL indicated that maternal damage has no clear impact on the ability of damaged progeny to induce higher trichome densities when they (the progeny) receive 2nd leaf simulated leaf damage, so this was omitted from the larger experiment (L. Holeski, unpublished results).

Analysis:

Trichome counts exhibited a right-skew with a large number of zero values. For analysis, I transformed the counts as log (trichome count +1). All calculations were performed using Minitab 14.0 (SAS).

Experiment 1

Parental populations

The significance testing for the direct effect of treatment on the parental population was accomplished via one-way ANOVA. The IM plants had values of zero for transformed trichome density in all of the control and treatment plants, so an ANOVA was done only for the PR plants. The interaction effect of population x treatment was tested in a General Linear Model ANOVA (population and treatment as fixed factors).

RILs

A mean value was calculated for constitutive 2nd, constitutive 5th, and post-damage 5th leaf trichome counts for each RIL. Induced 5th leaf counts were calculated for each RIL as the difference between the means of 5th leaf counts between damaged and undamaged

plants. Significant testing was done via a General Linear Model ANOVA with generation and treatment as fixed factors, RIL as a random factor, and 5th leaf trichome density as the response variable. The direct effect of treatment was used as a test for trichome induction; the interaction effect of RIL x treatment tests for genetic variation in induction.

To evaluate the ontological relationship between 2nd leaf constitutive trichome density and the extent of 5th leaf trichome induction, I regressed the induced 5th leaf trichome counts onto the 2nd leaf counts for all of the RILs. I also coded 2nd leaf trichome counts and induced 5th leaf trichome counts into a discrete form to evaluate the relationship between presence or absence of 2nd leaf trichomes and presence or absence of 5th leaf trichome induction through a Chi-square table analysis. Only F3 and F4 RIL data was used for the regression and table analysis, because 2nd leaf trichome counts were not available for the F6 plants used for the 5th leaf constitutive/damaged counts.

Experiment 2

For Experiment 2, I used a General Linear Model ANOVA (maternal treatment, fixed factor; RIL random factor; 5th leaf trichome density response variable) to determine whether offspring of damaged maternal plants had higher constitutive 5th leaf trichome densities than do offspring of undamaged maternal plants. As in Experiment 1, the direct effect of treatment was tests for (maternal) induction, while the treatment x RIL interaction tests for genetic variation in induction.

Results

Part 1:

The untransformed mean trichome density (counts per $cm^2 \pm 1$ standard deviation) for each population were: MB (641 ± 472), OD (8.77 ± 15.3), MR (43.5 ± 56.8), CR (39.5 ± 112), DR (149 ± 268), PR (1817 ± 631). Following transformation, second leaf trichome counts were significantly different among populations, as well as among families nested within populations (Table 3-2). This indicates genetic variation among populations and among individuals within populations. The variance among families (within populations) estimates the covariance of full siblings. Provisionally neglecting genetic complexities such as dominance and epistasis and maternal effects, this covariance is equal to one half the additive genetic variance, Va, of log-trichome number within populations (Lynch and Walsh 1998, Ch. 7). Estimates from Table 3-2 suggest Va = 0.408 and the corresponding narrow sense heritability, h^2, is equal to 0.57.

Source	DF	Adj SS	Adj MS	F	P
Population	5	132.366	26.473	12.73	**<0.001**
Family(Population)	10	21.288	2.129	4.15	**<0.001**
Error	113	57.973	0.513		
Total	128				

Table 3-2. Nested ANOVA for trichome counts. Significant p-values are in bold type.

Part 2:

Experiment 1:

Simulated insect damage on the second leaf pair of plants significantly induced higher 5th leaf trichome densities in the PR plants (p-value = 0.001) and RILs (Table 3-3), but not in the IM plants (Figure 3-2). There was a significant population by treatment interaction and a non-significant RIL by treatment interaction (Table 3-4).

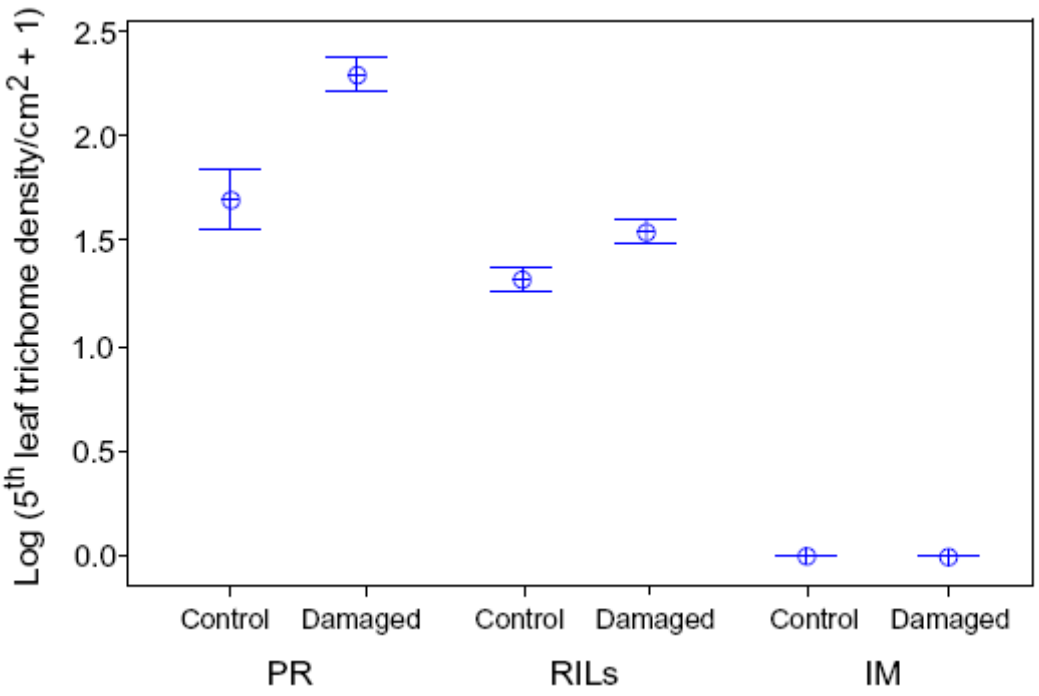


Figure 3-2. Mean 5th leaf trichome count per cm^2 for parental (PR and IM) and RIL plants in Experiment 1 that did ("Damaged") or did not ("Control") receive 2nd leaf damage. In this and subsequent figures, error bars represent 1 standard error (SE).

Source	DF	MS	F	P
Population (PR or IM)	1	28.315	257.83	**< 0.001**
2nd leaf damage?	1	0.621	5.65	**0.024**
Population x 2nd leaf damage?	1	0.621	5.65	**0.024**
Error	31	0.110		
Total	34			

Source	DF	MS	F	P
Generation	2	1.423	2.42	0.091
RIL	87	3.724	6.29	**< 0.001**
2nd leaf damage?	1	6.335	10.73	**0.001**
RIL x 2nd leaf damage?	87	0.592	1.01	0.470
Error	247	0.587		
Total	424			

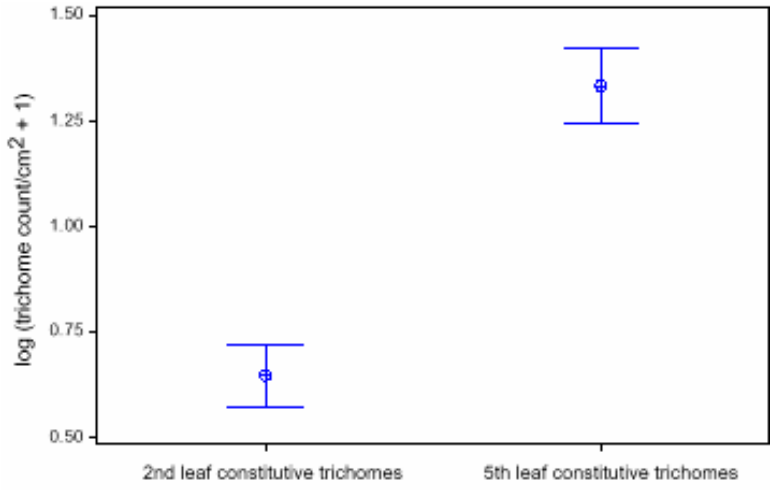
Table 3-3. ANOVA table of factors affecting 5th leaf trichome density in the parental populations (PR and IM) and RILs from Part 2, Experiment 1. Causal variables include the direct effects of RIL generation, direct effects of population or RIL, direct effects of treatment (presence or absence of 2nd leaf damage), and a population or RIL x treatment interaction. Significant p-values are in bold type.

Source	DF	MS	F	P
Maternal treatment	1	1.052	2.79	0.096
RIL	79	4.310	11.42	**< 0.001**
Maternal treatment x RIL	79	0.926	2.45	**< 0.001**
Error	488	0.377		
Total	647			

Table 3-4. ANOVA table of factors affecting 5th leaf trichome density in the offspring of damaged or undamaged maternal plants in Experiment 2. Causal variables include the direct effects of maternal treatment (damage or no damage), direct effects RIL, and a RIL x maternal treatment interaction. Significant p-values are in bold type.

The mean constitutive 2nd leaf trichome density is significantly lower than the mean constitutive 5th leaf trichome density in RILs ($F_{1, 313} = 35.43$, p-value < 0.001; Figure 3-4A). Constitutive 2nd leaf and constitutive 5th leaf trichome densities are non-significantly positively related (p-value = 0.443; $r^2 = 0.4\%$). There is also no significant relationship between constitutive trichome density (2nd leaf) and induced 5th leaf trichome density when data is treated as continuous (p-value = 0.401; $r^2 = 0.6\%$; Figure 3-4B) or as discrete ($X^2_1 = 1.001$, p-value = 0.317).

A.



B.

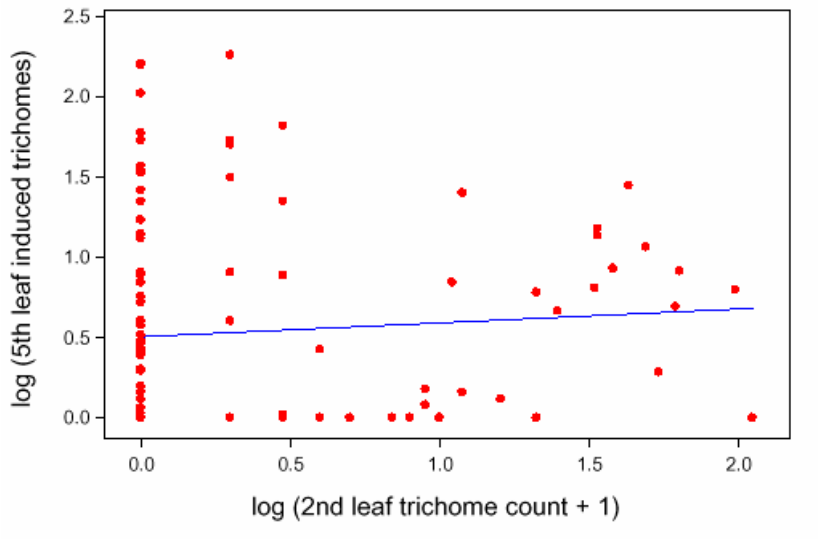


Figure 3-4: **A.** Mean 2nd leaf and 5th leaf constitutive trichome densities for F3 and F4 RILs. **B.** 5th leaf induced trichomes regressed on 2nd leaf constitutive trichomes for RILs in the F3 and F4 generations; $r^2 = 0.6\%$. Induced 5th leaf trichomes are calculated as: log (5th leaf trichome count (treatment) +1) – log (5th leaf trichome count (control) + 1). All trichome counts are per cm^2.

Experiment 2:

Within the RILs and within the Point Reyes population, offspring of damaged maternal plants had significantly higher constitutive 5th leaf trichome densities than did offspring of undamaged maternal plants (Figure 3-5; RILs: $F_{1, 647} = 4.13$, p-value = 0.043; PR: $F_{1, 35} = 4.48$, p-value = 0.042). There was a significant RIL by treatment interaction (Table 3-3). There was no significant difference in constitutive 5th leaf trichome production between offspring of damaged and undamaged IM 767 maternal plants (Figure 3-5; $F_{1, 38} = 0.31$, p-value = 0.58).

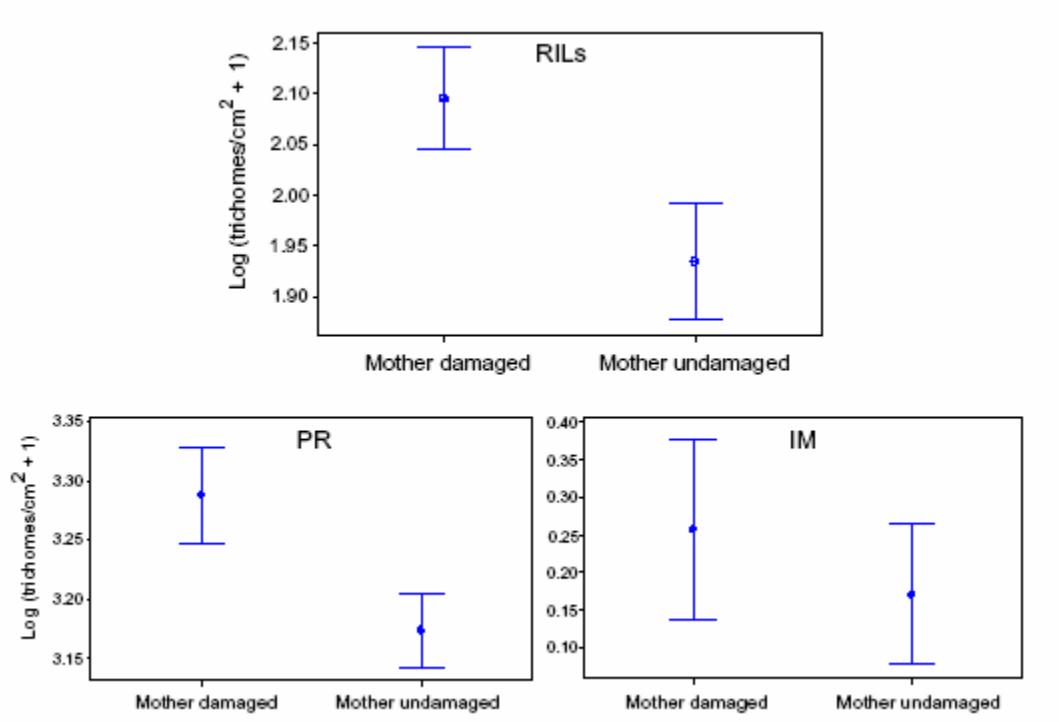


Figure 3-5. 5th leaf trichome density for parental population (PR and IM) and RIL progeny with damaged versus undamaged mothers. Differences between progeny types are significant within both the PR and RIL population. It is non-significant within the IM population. Note differences in y-axis scale between population plots.

Only 8 RILs were used in both experiments 1 and 2, so I cannot test for a relationship between induction within and across generations for a particular genotype. For these 8 RILs, 2 had both within and across generation induction, 2 had induction neither within nor across generations, 2 had within generation induction but not across, and 2 had no within generation induction but did have induction across generations.

Discussion

Part 1 demonstrates that differences in trichome density among populations of *Mimulus guttatus* have a genetic basis. This differentiation may reflect a history of differing selection pressures from herbivores. The common garden in Part 1 also demonstrated genetic variation in trichome production within populations. The estimated intra-population heritability of log-transformed trichome density is 0.57, although this is almost certainly an average of differing population specific values. An important feature of the common garden design was the intermediate greenhouse generation between field collection and growth of progeny. This generation served two purposes. First, growing the parents under common conditions reduces maternal effects (Lynch and Walsh 1998). Second, generating the progeny from controlled crosses within the greenhouse insured that the measured plants were all outbred. Because *Mimulus guttatus* is mixed-mating and the selfing rate varies among populations (Sweigart 1998; Sweigart et al. 1999), this would not likely be true if progeny were grown from field collected fruits.

In Part 2, Experiment 1 demonstrates that trichome density is inducible in *Mimulus guttatus* (within generation plasticity) and that there is genetic variation for the extent of induction (Figure 3-2, Table 3-2). Experiment 2 shows that trichome induction can be maternally transmitted, and that there is genetic variation in the capacity for maternal transmission. While genetic variation for induced chemical defenses has been demonstrated (Agrawal et al.1999a), previous studies of trichome induction have usually failed to show genetic variation. However, this may be due to the fact that most of these experiments were not designed to detect it. Genetic variation is a prerequisite for evolution of a trait, and therefore should be focus of studies assessing constitutive and induced defense.

The genetic variance demonstrated in Part 2 is inter-populational. The RILs are derived from a cross between two divergent populations, the trichome dense PR and trichome depauperate IM. Not only does the IM genotype produce no trichomes constitutively, it does not respond to damage either within or between generations (Figures 3-2 and 3-4). Herbivory levels are low in the natural IM population, and it is hypothesized that they escape herbivory through rapid development. Rapid development has likely evolved as a method of drought escape in the IM population (Franks et al. 2007). The absence of trichomes in the IM population may be due to the fact that they are costly to produce or simply because selective pressures have not necessitated them.

In contrast, the PR population plants constitutively produce trichomes and greatly increase production in response to damage (Figures 3-2 and 3-4). Induction of plant

defenses (chemical or physical) has been shown to reduce subsequent herbivory in a number of field studies (Denno et al. 1995 and references therein). In the present study, plants responded to leaf punctures that mimic the effect of chewing insects. Herbivores can emit chemicals that prevent or delay signaling pathways in the plant responsible for induction response (Agrawal 1998, 1999, 2000; Schultz and Appel 2004). However, other studies have found induction in defense traits with insect damage but no induction with simulated damage (Agrawal 1998). Thus, the induction response to particular forms of simulated or insect damage seems to often be species/ herbivore specific (Agrawal 1998, 1999, 2000).

Plasticity and defense against herbivory

I found no relationship between the level of constitutive trichome production and the capacity for induction across Recombinant Inbred Lines (Figure 3-3b). This result is inconsistent with the prediction of optimal defense theory, but is not incompatible with models of generalist/specialist herbivory trade-off. Optimal defense theory (Rhoades 1979; Zangerl and Bazzaz 1992; Zangerl and Rutledge 1996) predicts patterns of defense based on the costs and benefits of defense, as well as the probability of attack. Assuming that defense carries a cost (due to the allocation of resources to the development of the trait that would otherwise be used for growth or reproduction), a negative correlation is expected between plant constitutive and induced levels of defense. To maximize resource use, plants that frequently and predictably experience herbivory are predicted to have high constitutive levels of defense and low levels of induction. Populations subject to infrequent herbivory are predicted to have low constitutive levels of defense and

higher levels of induction. While data from other studies support these predictions (Zangerl and Berenbaum 1990; Lewinsohn et al. 1991; Traw 2002), my results do not (Figures 1 and 3; see also (Brody and Karban 1992; Havill and Raffa 1999; Alpert and Simms 2002; Agrawal 2005).

The generalist/specialist trade-off model suggests that variation in levels of constitutive and induced resistance in a particular trait is maintained because of differential effects of the trait on different herbivores. For example, some specialist herbivores feed with increased frequency on plants with high levels of induction (Chambliss and Jones 1966; Da Costa and Jones 1971; van Dam and Hare 1998; Agrawal 1999b). My results indicate such variation in constitutive and induced trichomes: trichome induction cannot be predicted by levels of constitutive trichomes (Figure 3-3B). While this is consistent with the generalist/specialist trade-off model, generalist/specialist herbivores could not be attributed as a causal factor of the observed pattern without more information. Variation in levels of constitutive and induced resistance may also be explained in part by complex interactions between the resistance trait and insect pollinators or other mutualists (Agrawal and Karban 1999).

In several plant species, resistance to herbivores changes as plants pass develop, although the direction of this change is variable (Price et al. 1987; Kearsley and Whitham 1989; Karban and Thaler 1999). Several species have increased chemical or physical resistance in the juvenile stage relative to the adult (Price et al. 1987; Kearsley and Whitham 1989), while others have increased adult resistance relative to their juvenile condition (Karban

and Thaler 1999). Juvenile true leaves and adult true leaves usually have different patterns of cellular differentiation and are anatomically and biochemically different (Poethig 1997; Mauricio 2005; Donaldson et al. 2006; Rehill et al. 2006). Here, I show that constitutive 5th leaf (adult stage) trichome density is significantly higher than 2nd leaf (juvenile stage) constitutive trichome density, indicating that at least insofar as trichomes affect herbivory, resistance is higher in adult *M. guttatus* plants relative to juvenile (Figure 3-3A).

Experiment 2 demonstrates transgenerational induction of trichomes (Table 3-3). There is also genetic variation in the capacity for this response. A well-documented example of this general phenomenon is passive acquired immunity in human infants. During pregnancy, the mother passes antibodies through the placenta to the infant, so that the infant has high levels of antibodies at birth (Saji et al. 1999). Although the specific mechanism differs between the transgenerational induction in plants seen here and human acquired immunity, the general effect is similar: progeny have increased resistance against common enemies in a particular environment before they have actually encountered them.

Transgenerational induction has been shown in only one other plant species (Agrawal 1999a, 2001; Agrawal 2002). Agrawal performed a series of experiments with wild radish (*Raphanus raphanistrum*), and demonstrated that both caterpillar (*Pieris rapae*) damage, and jasmonic acid treatment to maternal plants increased progeny resistance relative to control plants. Hydroxylated glucosinulate concentration increased in the

progeny of damaged plants, while other classes of glucosinulates declined in concentation in these progeny. In another experiment with the same plant and herbivore species, indole glucosinulates were induced significantly by maternal damage (Agrawal 2002). In these experiments, Agrawal found no genetic variation for transgenerational glucosinulate induction, but did find genetic variation for transgenerational trichome induction (Agrawal 2001, 2002).

The mechanism for transgenerational induction is not known for either *Raphanus raphanistrum* or *Mimulus guttatus*. Epigenetic inheritance allows organisms to respond to a particular environment through changes in gene expression (Jaenisch 2003; Rakyan 2003). Possible mechanisms include post-translational modification of DNA or proteins through processes such as demethylation and the effects of such processes could persist across more than one progeny generation (Jaenisch and Bird 2003; Rakyan and Whitlaw 2003). The genomic sequence of *M. guttatus*, in combination with extensive collection of candidate genes for trichome development in *Arabidopsis* and *Antirrhinum*, may allow mechanistic studies of transgenerational plasticity in this system.

Ecological implications

In a review of almost 200 phytophagous insect species pair-wise interactions, Denno et al. (1995) found that host plants mediated competitive interactions more frequently than did natural enemies, physical factors, or interspecific competition. Which herbivores are affected by a particular induction response is determined by the lag time between plant damage and defense induction, the length of time that the defense is expressed in its

induced/heightened form, and by the life history of the herbivore(s). This combination of factors creates potentially complex influences of induction on arthropod community structure (Moran and Whitham 1990; Dalin and Bjorkman 2003; Van Zandt and Agrawal 2004 a, b; Agrawal 2005; McGuire and Johnson 2006).

Transgenerational induction of defense introduces a new consideration to studies of plant defense and host-mediated competitive interactions. If densities of a particular herbivore species are consistent across seasons and maternal plants experience heavy damage, inducing higher constitutive defenses provides offspring with a fitness advantage. If herbivore communities vary across seasons, transgenerational induction of a defense trait could provide plant-mediated indirect competition, albeit with a longer time lag than within-generation defense trait induction. Transgenerational induction could thus alter herbivore community dynamics and species interactions on a plant genotype *across* seasons, in a manner similar to within generation induction.

References:

Abdala-Roberts, L., and V. Parra-Tabla. 2005. Artificial defoliation induces trichome production in the tropical shrub *Cnidoscolus aconitifolius* (Euphorbiaceae). Biotropica 37:251-257.

Agrawal, A. A. 1998. Induced responses to herbivory and increased plant performance. Science 279:1201-1202.

Agrawal, A. A. 1999. Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. Ecology 80:1713-1723.

Agrawal, A. A. 2000. Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. Oikos 89:493-500.

Agrawal, A. A. 2001. Transgenerational consequences of plant responses to herbivory: An adaptive maternal effect? American Naturalist 157:555-569.

Agrawal, A. A. 2002. Herbivory and maternal effects: mechanisms and consequences of transgenerational induced plant resistance. Ecology 83:3408-3415.

Agrawal, A. A. 2005. Future directions in the study of induced plant responses to herbivory. Entomologia Experimentalis et Applicata 115:97-105.

Agrawal, A. A., and R. Karban. 1999. Why induced defenses may be favored over constitutive strategies in plants. Pp. 45-61 *in* R. T. a. C. D. Harvell, ed. Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton.

Agrawal, A. A., C. Laforsch, and R. Tollrian. 1999a. Transgenerational induction of defenses in plants and animals. Nature 401:60-63.

Agrawal, A. A., P.M. Gorski, and D.W. Tallamy. 1999b. Polymorphism in plant defense against herbivory: constitutive and induced resistance in *Cucumis sativus.* Journal of Chemical Ecology 25:2285-2304.

Agrawal, A. A., S.Y. Strauss, and M.J. Stout. 1999c. Costs of induced responses and tolerance to herbivory in male and female components of wild radish. Evolution 53:1093-1104.

Alpert, P., and E. L. Simms. 2002. The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? Evolutionary

Arathi, H.S. and J. K. Kelly. 2004. Corolla morphology facilitates both autogamy and bumblebee pollination in *Mimulus guttatus*. International Journal of Plant Sciences 165:1039-1045.

Beardsley, P. and R.G. Olmstead. 2002. Redefining Phyrmaceae: the placement of *Mimulus*, tribe Mimulae, and Phyrma. American Journal of Botany 89:1093-1102.

Berenbaum, M. R., A. R. Zangerl, and J. K. Nitao. 1986. Constraints on chemical coevolution: wild parsnips and the parsnip webworm. Evolution 40:1215-1228.

Bradshaw, A. D. 1965. Evolutionary significance of phenotype plasticity in plants. Adv. Genet. 13:115-155.

Brody, A. K., and R. Karban. 1992. Lack of a tradeoff between constitutive and induced defenses among varieties of cotton. Oikos 65:301-306.

Chambliss, O. L., and C.M. Jones. 1966. Cucurbitacins: Specific insect attractants in Cucurbitaceae. Science 153:1392-1393.

Da Costa, C. P., and C.M. Jones. 1971. Cucumber beetle resistance and mite susceptibility controlled by the bitter gene in *Cucumis sativas* L. Science 172:1145-1146.

Dalin, P., and C. Bjorkman. 2003. Adult beetle grazing induces willow trichome defence against subsequent larval feeding. Oecologia 134:112-118.

Denno, R.F., M.S. McClure, and J.R. Ott. 1995. Interspecific interactions in phytophageous insects- Competition reexamined and resurrected. Annual Review of Entomology 40:297-331.

DeWitt, T. J. and S. M. Scheiner. 2004. Phenotypic variation from single genotypes. Pp. 1-9 *in* S.M. Scheiner and T. J. DeWitt, eds. Phenotype plasticity: Functional and conceptional approaches. Oxford University Press, Oxford.

Donaldson, J. R., Stevens, M.T., Barnhill, H.R., Lindroth, R.L. 2006. Age-related shifts in leaf chemistry of clonal aspen. Journal of Chemical Ecology 32:1415-1429.

Dudley, S. A. 2004. The functional ecology of phenotype plasticity in plants. Pp. 151-172 *in* S. M. Scheiner and T.J. DeWitt, eds. Phenotype plasticity: Functional and conceptual approaches. Oxford University Press, Oxford.

Ehleringer, J. 1984. Ecology and ecophysiology of leaf pubescence in North American desert plants. *in* E. Rodregues, P. L. Healey and I. Mehta, eds. Biology and Chemistry of Plant Trichomes. Plenum Press, New York.

Elle, E., and J. D. Hare. 2000. No benefit of glandular trichome production in natural populations of *Datura wrightii*? Oecologia 123:57-63.

Fenster, C.B. and K. Ritland. 1994. Quantitative genetics of mating system divergence in the yellow monkeyflower species complex. Heredity 73:422-435.

Fineblum, W. L., and M. D. Rausher. 1995. Tradeoff between resistance and tolerance to herbivore damage in a morning glory. Nature 377:517-520.

Fornoni, J. J., J. Nunez-Farfan, and P. L. Valverde. 2003a. Evolutionary aspects of tolerance to herbivory: advances and perspectives. Comments on Theoretical Biology 8:643-663.

Fornoni, J. J., J. Nunez-Farfan, P. L. Valverde, and M. D. Rausher. 2004. Evolution of mixed strategies of plant defense allocation against natural enemies. Evolution 58:1685-1695.

Fornoni, J. J., P. L. Valverde, and J. Nunez-Farfan. 2003b. Quantitative genetics of plant tolerance and resistance against natural enemies of two natural populations of *Datura stramonium*. Evolutionary Ecology Research 5:1049-1065.

Franks, S. J., S. Sim, and A.E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proceedings of the National Academy of Sciences of the United States of America 104:1278-1282.

Grant, V. 1950. Genetic and taxonomic studies in *Gilia*. I. *Gilia capitata.* Aliso 2:239-316.

Hall, M. C., C. J. Basten, and J. H. Willis. 2006. Pleiotropic quantitative trait loci contribute to population divergence in traits associated with life-history variation in *Mimulus guttatus*. Genetics 172:1829-1844.

Hall, M. C., and J. H. Willis. 2006. Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. Evolution 60

Hare, J. D., and E. Elle. 2002. Variable impact of diverse insect herbivores on dimorphic phenotypes of *Datura wrightii*. Ecology 83:2711-2720.

Havill, N. P., and K.F. Raffa. 1999. Effects of elicitation treatment and genotypic variation on induced resistance in *Populus*: impacts on gypsy moth (Lepidoptera: Lymantriidae) development and feeding behavior. Oecologia 120:295-303.
Jaenisch, R., and A. Bird. 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nature Genetics 33:245-254.
Karban, R., and J.S. Thaler. 1999. Plant phase change and resistance to herbivory. Ecology 80:510-517.
Kearsley, M. J., and T.G. Whitham. 1989. Developmental changes in resistance to herbivory: implications for individuals and populations. Ecology 70:422-434.
Kelly, J. K., and H. S. Arathi. 2003. Inbreeding and the genetic variance of floral traits in *Mimulus guttatus*. Heredity 90:77-83.
Larcher, W. 2001. Physiological Plant Ecology. Springer-Verlag, Berlin.
Leimu, R., and J. Koricheva. 2006. A meta-analysis of trade-offs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. Oikos 112:1-9.
Levin, D. A. 1973. The role of trichomes in plant defense. Quarterly Review of Biology 48:3-15.
Lewinsohn, E., M. Gijzen, and R. Croteau. 1991. Defense mechanisms of conifers: differences in constitutive and wound-induced monoterpene biosynthesis among species. Plant Physiol. 96:44-49.
Lynch, M., and B. Walsh. 1998. Genetics and Analysis of Quantitative Traits. Sinauer Associates, Inc., Sunderland, MA.
Marquis, R. J. 1991. Evolution of resistance in plants to herbivores. Evolutionary Trends in Plants 5:23-29.
Marquis, R. J. 1992. The selective impact of herbivory. Pp. 301-325 *in* R. S. Fritz and E. L. Simms, eds. Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics. University of Chicago Press, Chicago.
Mauricio, R. 2005. Ontogenetics of QTL: the genetic architecture of trichome density over time in *Arabidopsis thaliana*. Genetica 123:75-85.
McGuire, R. J., and M.T. Johnson. 2006. Plant genotype and induced responses affect resistance to herbivores in evening primrose (*Oenothera biennis)*. Ecological Entomology 31:20-31.
Mopper, S., J. B. Mitton, T. G. Whitham, N. S. Cobb, and K. M. Christensen. 1992. Genetic differentiation and heterozygosity in pinyon pine associated with resistance to herbivory and environmental stress. Evolution 45:989-999.
Moran, N. A., and T.G. Whitham. 1990. Interspecific competition between root feeding and leaf galling aphids mediated by host plant resistance. Ecology 71:1050-1058.
Morris, W. F., M. B. Traw, and J. Bergelson. 2006. On testing for a tradeoff between constitutive and induced resistance. Oikos 112:102-110.
Nunez-Farfan, J., and R. Dirzo. 1994. Evolutionary ecology of *Datura stramonium* L. in Central Mexico: natural selection for resistance to herbivorous insects. Evolution 48:423-436.
Orians, C. 2005. Herbivores, vascular pathways, and systemic induction: facts and artifacts. Journal of Chemical Ecology 31:2231-2242.

Poethig, R. S. 1997. Leaf morphogenesis in flowering plants. The Plant Cell 9:1077-1087.
Price, P. W., H. Roinenen, and J. Tahvanainen. 1987. Plant age and attack by the bud galler, *Euura mucronata*. Oecologia 73:334-337.
Pullin, A. S., and J. E. Gilbert. 1989. The stinging nettle, *Urtica dioica*, increases trichome density after herbivore and mechanical damage. Oikos 54:275-280.
Rakyan, V., and E. Whitelaw. 2003. Transgenerational epigenetic inheritance. Current Biology 31
Rausher, M. D., and E. L. Simms. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. I. Attempts to detect selection. Evolution 43:563-572.
Rehill, B. J., T.G. Whitham, G.D. Martinsen, J.A. Schweitzer, J.K. Bailey, R.L. Lindroth. 2006. Developmental trajectories in cottonwood phytochemistry. Journal of Chemical Ecology 32:2269-2285.
Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores. Pp. 4-55 *in* G. A. R. and D. H. Janzen, eds. Herbivores: their interaction with secondary plant metabolites. Academic Press, Orlando.
Romeis, J., T. G. Shanower, and A. J. Peter. 1999. Trichomes on Pigeonpea (*Cajanus cajan* (L.) Millsp) and two wild *Cajanus* spp. Crop Science 39:564-569.
Saji, F., Y. Samejima, S. Kamiura, and M. Koyama. 1999. Dynamics of immunoglobulins at the feto-maternal interface. Reviews of Reproduction 4:81-89.
Schlichting, C. D. 2004. The role of phenotype plasticity in diversification. Pp. 191-200 *in* T. Juenger and S. M. S. DeWitt, eds. Phenotypic plasticty: Functional and conceptual approaches. Oxford University Press, Oxford.
Schultz, J. C., H.M. Appel. 2004. Cross-kingdom talk: hormones shared by plants and their insect herbivores. Ecology 85:70-77.
Simms, E. L., and R. S. Fritz. 1990. The ecology and evolution of host-plant resistance to insects. Trends in Ecology and Evolution 5:356-360.
Simms, E. L., and M. D. Rausher. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. II. Natural selection by insects and costs of resistance. Evolution 43:573-585.
Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. Trends in Ecology and Evolution 14:179-185.
Sweigart, A. 1998. Microsatellite-Based Estimates on Individual Inbreeding and Relatedness for a Population of the Wildflower, *Mimulus guttatus*. Mathematics and Natural Sciences. Reed University.
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 83:625-632.
Thompson, J. N. 1994. The Coevolutionary Process. University of Chicago Press, Chicago.
Traw, M. B. 2002. Is induction response negatively correlated with constitutive resistance in black mustard? Evolution 56:2196-2205.
Traw, M. B., and T. E. Dawson. 2002. Differential induction of trichomes by three herbivores of black mustard. Oecologia 131:526-532.

Valverde, P. L., J. J. Fornoni, and J. Nunez-Farfan. 2001. Defensive role of leaf trichomes in resistance to herbivorous insects in *Datura stramonium*. Journal of Evolutionary Biology 14:424-432.

Valverde, P. L., J. J. Fornoni, and J. Nunez-Farfan. 2003. Evolutionary ecology of *Datura stramonium*: equal plant fitness benefits of growth and resistance against herbivory. Journal of Evolutionary Biology 16:127-137.

van Dam, N. M., and J. D. Hare. 1998. Differences in distribution and performance of two sap-sucking herbivores on glandular and non-glandular *Datura wrightii*. Ecological Entomology 23:22-32.

van Dam, N. M., J. D. Hare, and E. Elle. 1999. Inheritance and distribution of trichome phenotypes in *Datura wrightii*. The American Genetic Association 90:220-227.

Van Zandt, P. A., and A. A. Agrawal. 2004. Specificity of induced plant responses to specialist herbivores of the common milkweed *Asclepias syriaca*. Oikos 104:401-409.

Van Zandt, P. A., and A.A. Agrawal. 2004. Community-wide impacts of herbivore-induced plant responses in milkweed (*Asclepias syriaca*). Ecology 85:2630-2637.

Weinig, C., J. R. Stinchcombe, and J. Schmitt. 2003. Evolutionary genetics of resistance and tolerance to natural herbivory in *Arabidopsis thaliana.* . Evolution 57:1270-1280.

West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics 20:249-278.

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

Zangerl, A. R., and C.E. Rutledge. 1996. The probability of attack and patterns of constitutive and induced defense: A test of optimal defense theory. American Naturalist 147:599-608.

Zangerl, A. R., and F.A. Bazzaz. 1992. Theory and pattern in defense allocation. Pp. 363-392 *in* R. Fritz. and E. L. Simms, eds. Plant resistance to herbivores and pathogens. University of Chicago Press, Chicago.

Zangerl, A. R., and M.R. Berenbaum. 1990. Furanocoumarin induction in wild parsnip: genetics and population variation. Ecology 71:1933-1940.

Chapter 4. Glandular trichomes do not decrease meadow spittlebug feeding ability or preference in *Mimulus guttatus*.

Abstract

There is genetic variation in trichome density both within and among geographically distinct natural populations of *M. guttatus*. Here, no-choice and choice experiments using the generalist herbivore, meadow spittlebug (*Philaenus spumarius*) are used to examine how variation in trichome density affects herbivory by meadow spittlebugs and how this herbivory subsequently affects plant fitness. While trichomes confer resistance to some forms of herbivory, these experiments show that they do not deter meadow spittlebugs, either in their ability to feed or feeding preference. This is notable because (1) spittlebug herbivory did significantly reduce plant fitness, and (2) trichomes have been shown to deter spittlebugs from feeding on other plant species.

Introduction

Most plant species are consumed by insects and this interaction has been extensively studied (Berenbaum et al. 1986; Simms and Rausher 1989; Rausher and Simms 1989; Simms and Fritz 1990; Marquis 1991; Marquis 1992; Nunez-Farfan and Dirzo 1994; Hare and Elle 2002; Fornoni et al. 2003a). Geographical variation in plant-insect herbivore interactions is a widespread phenomenon, as distributions of particular insect and plant populations may overlap in only portions of their ranges (Thompson 1994). Differing severities and types of herbivory are likely to impose differing selection regimes among populations of a species for resistance and tolerance traits. The evolution of geographical trait variation is a precursor to speciation and a critical link between micro- and macro- evolutionary processes. It is thus surprising that there is relatively little data on the nature and extent of genetic differentiation among geographical populations of natural plant species in their responses to herbivory (Mopper et al. 1992; Valverde et al. 2001; Fornoni et al. 2003b; Valverde et al. 2003; Fornoni et al. 2004).

In response to natural selection imposed by herbivores, plants have evolved a wide range of defensive traits. 'Resistance characters' are mechanical or chemical features that prevent herbivory or limit its extent. These characters can be further subdivided depending on whether they affect insect preference (antixenosis) or performance (antibiosis). Defensive traits can be further classified as constitutive or induced. Constitutive resistance is always present, while induced resistance is triggered by herbivory (Morris et al. 2006). An alternative response to herbivory is through the

evolution of 'tolerance'. Tolerance characters reduce the effect of herbivory on fitness through mechanisms such as increased photosynthetic rates and/ or compensatory regrowth (Fineblum and Rausher 1995; Strauss and Agrawal 1999; Weinig et al. 2003a; Weinig et al. 2003b; Leimu and Koricheva 2006).

This paper describes experiments to determine the capacity of trichomes in the plant species *Mimulus guttatus* to resist herbivory by meadow spittlebug (*Philaenus spumarius*). Trichomes are hairlike structures that extend from the plant epidermis and occur in a variety of types including glandular or non-glandular, hooked or straight. Trichomes may serve a variety of defensive and physiological functions. Leaf trichomes have been shown to reduce insect herbivory in a number of plant species (Levin 1973; Marquis 1992; Agren and Schemske 1993; van Dam and Hare 1998; Romeis et al. 1999; Malakar and Tingey 2000; Valverde et al. 2001; Handley et al. 2005; Medeiros and Moreira 2005). They interfere with insect movement and feeding, and often secrete glandular fluids that may trap, poison, or repel herbivores (Levin 1973; Elle and Hare 2000). For plants in xeric habitats, trichomes reflect light and can reduce transpiration (Ehleringer 1984; Larcher 2001). Geographic and genetic variation in trichome density and type has been noted within a number of other plant species (Grant 1950; Levin 1973; van Dam et al. 2001; Valverde et al. 2001).

Trichome density is highly variable in *Mimulus guttatus* (yellow monkeyflower), a wildflower common throughout western North America. *M. guttatus* trichomes are straight and occur mostly in the glandular form, often secreting a sticky and potentially

noxious fluid. Substantial genetic variation exists in the trait, both within and among populations (Chapter 2). Trichome density is also environmentally sensitive in *M. guttatus*; higher trichome densities can be induced both within and across generations by simulated insect chewing damage on earlier leaves (Chapter 3).

In this study, I concentrate on two highly divergent populations to provide a more detailed analysis of trichome variation and its ecological and evolutionary implications. The inclusion of F2 or F6 hybrid individuals (in addition to the parentals) distinguishes the effects of trichome density from other differences between the two parental populations. Unless genetically correlated, trait differences among the parents will segregate more or less independently in the F2 and F6 populations. Thus, these individuals encompass a wide range of trichome densities within more similar genetic backgrounds than those of the parental plants (Hall and Willis 2006; Wright et al. 2006).

The low trichome density population is located on Iron Mountain in central Oregon (abbreviated IM) and has been extensively studied, both in terms of genetics and pollination biology (Willis 1993; Willis 1996; Sweigart et al. 1999; Kelly and Willis 2001; Kelly and Willis 2002). Most IM plants produce no trichomes on early leaves. In contrast, plants from the Point Reyes National Seashore population in California (abbreviated PR) have many trichomes: mean density of 1817/cm^2 (SD = 631/cm^2) on the second leaf pair, as well as high trichome densities on later leaves. In addition, PR plants produce copious quantities of sticky glandular exudate from these trichomes while IM plants produce little or none of this fluid. Physiological benefits (i.e. to reduce light

radiation or transpiration rates) are unlikely to explain the high trichome densities of PR plants as this population is located in the fog belt of coastal Northern California and should not suffer extreme drought or intense sunlight. The average amount of total solar radiation in the central Cascades of Oregon in May through July is higher than that in coastal Northern California (NOAA website). These observations reinforce the preliminary hypothesis that *M. guttatus* trichomes act to deter herbivores.

Meadow spittlebug nymphs feed in high densities within many *M. guttatus* populations (Carr and Eubanks 2002). Spittlebug herbivory has been shown to increase anther-stigma distance, increase rates of self-fertilization and reduce shoot elongation, flower number, flower size, and aboveground biomass in *M. guttatus* (Carr and Eubanks 2002; Eubanks et al. 2005; Ivey and Carr 2005). These results are consistent with studies of other plant species. In alfalfa, feeding by spittlebug nymphs has also been shown to reduce plant growth, yield, and rate of maturity (Wilson et al. 1979). Spittlebug herbivory in goldenrod (*Solidago altissima*) reduced photosynthetic rates, relative growth rates, total leaf mass, and root mass and did so more than herbivory by aphids (*Uroleucon caligatum*) and beetles (*Trirhabda* sp.) (Meyer and Whitlow 1992; Meyer 1993). Finally, spittlebug herbivory in *Rudbeckia hirta* reduced flower number and pollinator visitation rate, which reduced the number of viable seeds produced by the plants (Hamback 2001). Trichomes have been shown to deter feeding by meadow spittlebugs in pearly everlasting (*Anaphalis margaritacea)* and alfalfa (*Medicago sativa)* (Hoffman and McEvoy 1986).

Spittlebugs are common in the PR population of *M. guttatus*. Individual plants routinely host as many as 10 nymphs, in addition to a range of other herbivores (see Discussion). I have never observed meadow spittlebugs feeding at IM and densities of other insect herbivores are also low (Appendix D). The question that naturally follows from these observations is whether trichomes act to resist herbivory by spittlebugs in *M. guttatus*. I address this question using both 'choice' and 'no-choice' experiments. In a no-choice experiment, the herbivore is given access to a single plant. It either feeds on this plant or evacuates without feeding and dies. No-choice experiments are common methods to estimate absolute resistance (Gerard et al. 2005; Hare 2005; Poykko et al. 2005; Verdon et al 2007). In a choice experiment, the herbivore is given access to multiple plants and thus can chose the plant on which it feeds. This provides a measure of resistance for a particular plant relative to the plants it is grouped with. Choice experiments incorporate herbivore preference: while herbivores may be *able* to feed on a particular plant, as evidenced in a no-choice experiment, they may almost always prefer not to in nature (Capinera 1993; Siemann and Rogers 2003).

Here, the results of a no-choice and a choice experiment are used to address the following specific questions: **(1)** Do trichomes deter spittlebugs from feeding on *M. guttatus* plants? **(2)** What trichome phenotype do spittlebug nymphs prefer? **(3)** Is trichome production induced by herbivory of spittlebug nymphs, i.e. does feeding on early leaves increase higher trichome densities on later leaves?, **(4)** Are there fitness costs to this hypothesized resistance trait, i.e. is trichome production detrimental in the absence of herbivores?, and **(5)** Does spittlebug feeding affect fitness?

Materials and Methods

The plant— Yellow monkeyflower (Mimulus guttatus; Phrymaceae, (Beardsley and Olmstead 2002)) ranges from Mexico to Alaska in western North America and typically inhabits wet areas such as stream banks. It is a self-compatible, hermaphroditic plant that reproduces by a mixture of outcrossing and self-fertilization. Local populations differ extensively in morphology, life history, and selfing rate (Ritland 1989; Willis 1993; Fenster and Ritland 1994; Kelly and Arathi 2003; Hall et al. 2006; Hall and Willis 2006).

The herbivore— The meadow spittlebug (*Philaenus spumarius;* Cercopidae) ranges throughout much of North America and Europe and feeds on over 400 plant species. In the United States, it is found along the Pacific coast and from Louisiana to Minnesota and eastward (North Carolina State University Agricultural manual). The species is univoltine, overwintering as eggs and developing through five nymphal instars in the spring to emerge as adults in the early summer. Mating and oviposition occurs in early fall, and eggs are deposited on small plant tissue or on soil to overwinter (Weaver and King 1954; McEvoy 1986). Upon emergence, nymphs move to a nearby plant and establish a feeding position. Nymphs feed on xylem fluid and mix this fluid with gut contents to form conspicuous spittle masses that prevent desiccation. The necessity of forming a spittle mass quickly limits the period of searching for a host plant (Whitaker 1970; McEvoy 1986; Hamback 2001). Once a feeding site is established, host plant changes are uncommon; nymphs generally complete all five nymphal instars on the same plant (Eubanks et al. 2005).

Experiment 1: No-choice experiment

This common garden experiment had a factorial design: plants from the Iron Mountain and Point Reyes populations, along with F2 individuals, were cross-factored with the presence or absence of spittlebug. All plants from the PR population were full siblings of a single outbred family (field-collected seed). IM plants were all members of a highly inbred line (IM 767) extracted from the natural population (see Willis 1999 for details). F2 individuals were obtained by crossing of a PR plant (mother) with IM 767 (father). A single F1 individual from this cross was self-fertilized to generate an array of F2 individuals.

Plants were grown from seed (Day 0) in the greenhouse and on Day 14 were moved to a growth room (18 hour day length) at the University of Kansas. I collected *P. spumarius* nymphs while in their 2nd or 3rd instar from Point Reyes National Seashore, CA, on April 29, 2006. Nymphs were collected from non-*Mimulus guttatus* plants to minimize potential bias due to their prior feeding experiences (Egas and Sabelis 2001). They were transported back to the University of Kansas on the day after collection and refrigerated. Trichome counts were completed on all plants on one leaf from the 2nd leaf pair on a defined measuring area of 1 cm^2 on the basal central part of the adaxial side of the leaf, immediately after the leaves in the 3rd leaf pair were fully expanded, and prior to spittlebug treatment. A single spittlebug nymph was subsequently placed on all plants in the treatment group (2-7 days after nymph field collection; roughly day 24 for the plants). Because spittlebugs were placed on plants immediately after the 2nd leaf trichome count

was done, nymph response was measured at the same particular development stage across plants (Orians 2005; Morris et al. 2006). Nymphs were blotted dry and weighed prior to placement. After distributing the nymphs, I intermixed treatment and control plants within flats (each containing 32 pots) and these flats were rotated daily thereafter.

I monitored plants each day after the initiation of the spittlebug treatment; spittlebugs that failed to establish a feeding site were replaced with another weighed nymph and the plant ID noted. To minimize variation due to individual spittlebugs, I also noted plants with nymphs that evacuated after establishing a feeding site and replaced the nymph. All of the pots containing plants in the treatment category were lined around the edges with Pestick (Tanglefoot Industries), which prevented nymphs from moving from plant to plant. I continued second leaf trichome counts and placement of spittlebug nymphs until the supply of healthy nymphs was depleted, resulting in a total of 293 control plants (46 IM, 124 PR, 123 F2) and 320 treatment plants (81 IM, 108 PR, 131 F2). I also completed secondary trichome counts on all plants on a leaf from the fifth leaf pair of each plant immediately following expansion of the 6^{th} leaf pair. Spittlebugs remained on treatment plants for a maximum of 17 days. Many plants had spittlebug nymphs for fewer days due to nymphal emergence to adults or early evacuation. I caught and weighed spittlebugs emerging as adults in addition to all nymphs remaining at the end of the experiment.

I assayed all plants for pollen number and viability per flower, flower number, and aboveground biomass at harvest. Pollen was collected from one flower on each plant on the first day of flowering during the end of the treatment period. The number of viable

and inviable grains in this sample was estimated using a Coulter counter Model Z1 (see Kelly et al. 2002 for details of procedure). I calculated the Estimated Viable Pollen per flower (EVP) as the product of proportion of viable pollen grains and the total number of pollen grains from a flower. On Day 17 of treatment, after all nymphs had been removed from treatment plants, I counted the total number of flowers on each plant. Plants were then harvested, dried, and weighed. 'Integrated male fitness' is (flower number) x EVP. Aboveground biomass is positively correlated with lifetime seed production in the PR, IM, and F2 populations of *M. guttatus* (Appendix B) and I use it as a proxy for lifetime female fitness.

A procedural control experiment indicated that Pestick lined around the edge of plant pots decreases plant fitness (Appendix C), so comparisons in reproductive fitness between control plants (no Pestick and no spittlebug nymph) and treatment plants (Pestick and spittlebug nymph) are not reported here. Fitness data from only control plants (none of which had Pestick) was used in the "cost of trichome production?" analysis. Aboveground biomass measurements in the treatment plants (all of which had Pestick) were also used as a causal factor in some analyses.

Analysis: Trichome counts, flower numbers, EVP, aboveground biomass, and integrated male fitness were right-skewed and were log-transformed prior to statistical analysis. Logistic regression was used to determine the extent to which aboveground biomass (causal variable), 2nd leaf trichome density (causal variable), and/or population (discrete causal variable) affect whether a spittlebug nymph stays on a plant to feed or immediately

evacuates (feeds/evacuates, response variable). Linear regression was used to determine the extent to which aboveground biomass or 2nd leaf trichome density (causal variables) affect the number of days that a spittlebug remained on a plant (response variable). A General Linear Model ANOVA was performed to determine the effects of population and 2nd leaf trichome density on spittlebug weight gain after 17 days of feeding (population, fixed causal factor; 2nd leaf trichome density, covariate; final spittlebug mass – initial spittlebug mass, response variable). All statistics were done using Minitab 14 (SAS).

Experiment 2: Choice experiment

This experiment used plants from 4 'trichome phenotypic' categories: the two ancestral populations (IM and PR) and recombinant genotypes that exhibit either high or low trichome densitites. For the latter, I used F6 plants from a collection of recombinant inbred lines (RILs) from a cross between an IM 767 plant (father) and a PR plant (mother). These RILs have been characterized for 2nd leaf constitutive trichome density (Chapter 3). The 'low' group is a mixture of plants from 11 RILs with an average of fewer than 1 constitutive trichomes per cm^2 (category 3; referred to as "0"). The high group is a mixture from 12 RILs with an average constitutive trichome density of 634 per cm^2 (category 4; referred to as "+"). Use of F6 individuals allowed me to isolate the trait of interest, trichome density, amongst a relatively random background mixture of the PR and IM genomes. Use of multiple lines in each category allowed me to further randomize the genomic background of the plants, so that plants in the "0" and "+" categories were highly divergent in constitutive trichome density but similar in other phenotypic aspects.

I grew 150 plants from each category from seed (Day 0) in the greenhouse, and on Day 14, transplanted them into 4-inch pots. Each pot contained 2 plants, spaced 2 inches apart, of one of the following pairings: PR/IM, PR/+, PR/0, IM/+, IM/0, +/0. Each of these combinations had 50 replicates, resulting in a total of 300 pairs (600 total plants). After the transplant, the plants were placed in the same growth room as in Experiment 1, were randomized in position with respect to pair type, and were rotated in bench position daily. On Day 20, all of the 300 pots were lined with Pestick (Tanglefoot Industries) to prevent future spittlebug movement among plant pairs.

I collected spittlebug nymphs in their 2nd or 3rd instar from non-*M. guttatus* plants at Point Reyes National Seashore on April 18th, 2007 and transported them back to the University of Kansas. Two days after nymph collection (plant Day 24), I placed a single spittlebug nymph in the center of each pot, equidistant between the two plants of a particular pair. I monitored each nymph and recorded the plant that it initially selected. After the initial selection of the nymphs, I monitored plant pairs daily and recorded the plant that each nymph was feeding on. Plant pairs that had a first nymph evacuate (it was not feeding on either plant) were given a second nymph that was placed and monitored in the same manner. I tracked nymph feeding for 11 days, recording the plant in each pair that was being fed on in the 2nd, 3rd, 5th, 8th, and 11th day after the spittlebug placement. This resulted in a set of data for each plant pair that characterized initial spittlebug selection as well as if/for how long the nymph remained on its initial selection before moving to the other plant in the pair.

On the 11th day of spittlebug feeding, I removed the nymphs and harvested the plants for fitness measurements. I counted the total number of flowers on each plant. Plants were then harvested, dried, and weighed. Aboveground biomass is strongly positively correlated with lifetime seed production in the PR, IM, and F2 populations of *M. guttatus* (Holeski 2007) and I use it as a proxy for lifetime female fitness.

Analysis: For each plant within each of the 6 categories of plant pairings, I distilled the data regarding initial nymph plant preference as well as nymph preference on subsequent days into discrete counts. I used a series of binomials and Chi-square table analyses to evaluate this data. Flower number and aboveground biomass were right-skewed and I log-transformed this data prior to statistical analysis. I grouped plants into control and treatment categories to determine how spittlebug herbivory affected plant fitness. Control plants were those fed on by spittlebugs for a maximum of 1 day, while treatment plants were those fed on for 11 days. IM plants were excluded from the fitness analysis because only 1 IM plant remained in the treatment category after 11 days. Because herbivory was not always absolutely absent in the control plants, my results provide a conservative estimate of the effects of spittlebug herbivory on plant fitness. I used a series of ANOVAs to evaluate fitness data.

To determine if the plant that a particular focal plant was paired with differentially affected focal plant fitness across the treatment and control categories, I performed a

General Linear Model ANOVA including a treatment x plant pair interaction term. I calculated all statistics using Minitab 14 (SAS).

Results

Experiment 1: No-choice experiment

Average second leaf trichome density differed significantly among the populations ($F_{2,600}$ = 1879.47; $p < 0.001$). Average trichome counts per cm^2 prior to transformation were (average ± 1 standard deviation): PR (1320 ± 406), F2 (70.4 ± 221), IM (0.14 ± 1.57).

Factors affecting spittlebug herbivory

Trichomes did not deter spittlebug nymphs from feeding. In contrast, nymphs were more likely to initially feed on plants from the population with the highest trichome densities (PR, Figure 4-1). The differences in initial feeding were not due to differences in plant size between populations; the effect of aboveground biomass on initial feeding preference was not significant (constant, Z = 1.12, p-value = 0.262; 2^{nd} leaf trichome count, Z = 2.36, p-value = 0.018; aboveground biomass, Z = -0.96, p-value = 0.336). Population type also affected initial spittlebug feeding preference and length of feeding (constant, Z = 2.05, p-value = 0.04; F2, Z = -2.73, p-value = 0.006; IM, Z = -2.62, p-value = 0.009; aboveground biomass, Z = -1.59, p-value = 0.111). However, I was not able to disentangle the effects of 2^{nd} leaf trichome density from effect of population (constant, Z = 1.60, p-value = 0.109; 2^{nd} leaf trichome count, Z = 0.45, p-value = 0.651; F2, Z = -0.97, p-value = 0.334; IM, Z = -1.02, p-value = 0.306). The effects of 2^{nd} leaf trichome

density and aboveground biomass *within* each population on whether or not a bug fed were non-significant (logistic regression; p-values range from 0.13 to 0.46). Spittlebug nymphs were also more likely to remain on plants with higher trichome densities (Figure 4-2).

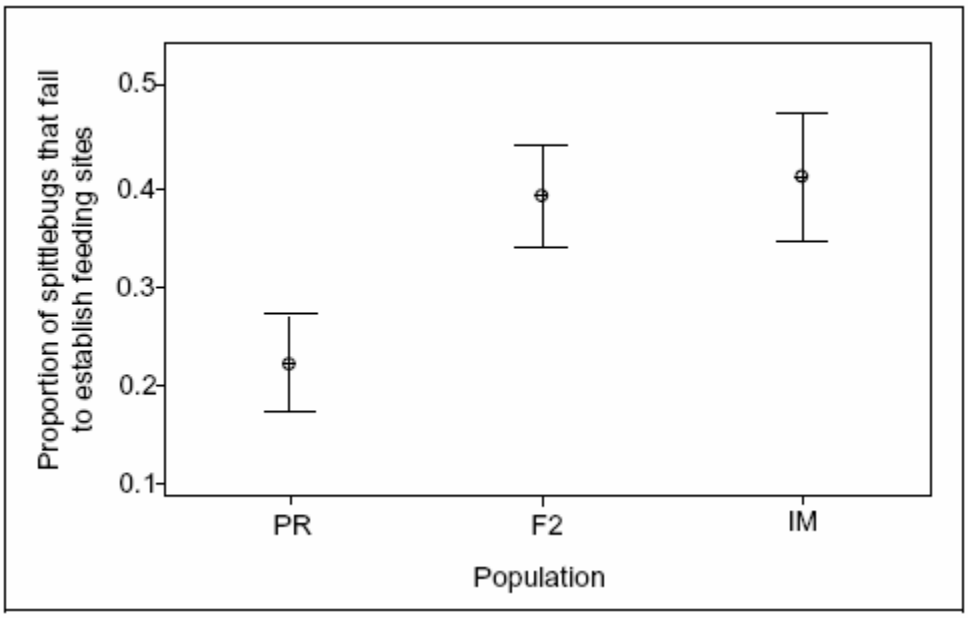


Figure 4-1. The proportion of spittlebug nymphs that **fail** to establish feeding sites on plants is given for each population in Experiment 1. Here, and in subsequent figures, error bars represent 1 standard error (SE) from the mean.

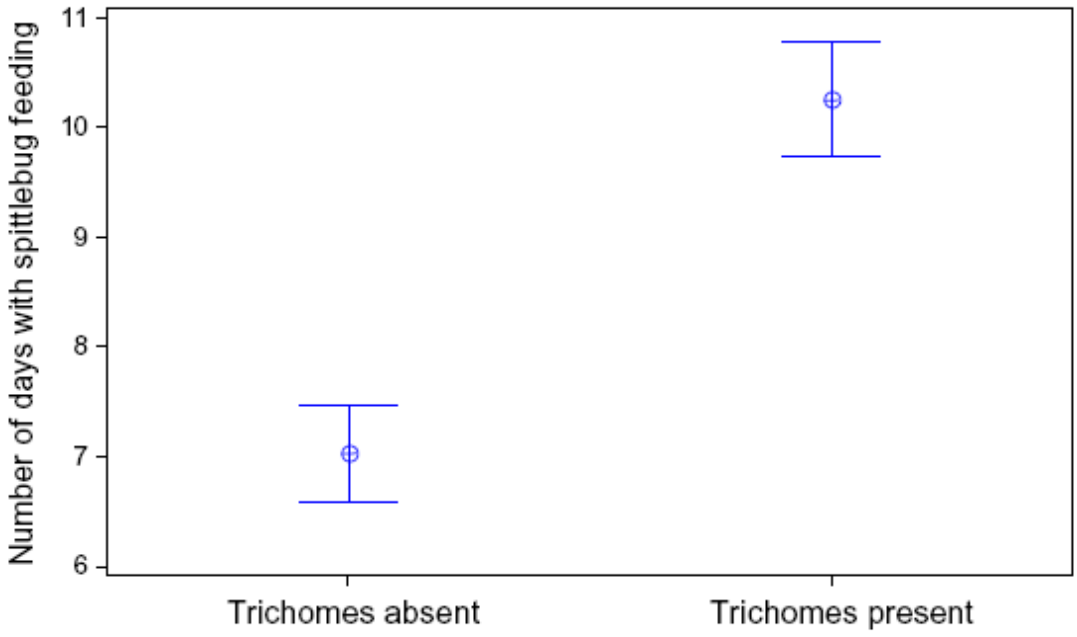


Figure 4-2. Plants in Experiment 1 that have constitutive 2nd leaf trichomes have spittlebugs remaining on plants to feed for longer than plants with no constitutive 2nd leaf trichomes.

There was no significant difference in weight gain for nymphs feeding on plants from different populations or with different 2nd leaf trichome densities (effect of population: $F_{2,80} = 0.18$, p-value = 0.837; effect of 2nd leaf trichome density as a covariate: $F_{1,80} = 0.58$, p-value = 0.447).

Trichome induction?

There was no significant difference in 5th leaf trichome counts between control plants and those that had been fed upon by spittlebugs. Mean 5th leaf trichome counts/ cm^2 were 1383 for control vs. 1401 for treatment for the PR population, 167 for control vs. 172 for

treatment in the F2 population, and 0 for control vs. 0.1 for treatment plants in the IM population.

Fitness cost of trichome production?

No cost of trichome production was detected: within each population of control plants, flower number, EVP, plant aboveground biomass, and integrated male fitness. 2^{nd} leaf trichome density (causal factor) was regressed separately onto 2^{nd} leaf trichome density. No significant relationship between 2^{nd} leaf trichome density and any of these fitness characters was detected (p-values range from 0.07 to 0.75).

Experiment 2: Choice experiment

Spittlebug feeding preferences:

Initially, spittlebug nymphs significantly preferred PR plants to 0 plants (Table 4-1). This was the only significant difference among the single pair-wise comparisons. There was a non-significant trend for + plants to be favored over 0 or IM plant types, and when plants were grouped into low (IM and or 0) and high trichome density (PR and or +) categories, plants with high trichome densities were significantly preferred to plants with low trichome densities (p-value = 0.002; Table 4-1). PR was the only type initially preferred no matter what plant type it was paired with (Table 4-1). Following the initial choice, spittlebugs were significantly more likely to stay for at least a day on plants with high trichome densities (PR or +; Table 4-2; Figure 4-3). However, this trend did not remain longer than the first 2 days of feeding, after which the PR population was

consistently the only population where significantly more spittlebugs remained to feed than evacuated (Figure 4-3, Table 4-3).

Comparison	Data	p-value
PR vs IM	30 vs. 18	0.111
PR vs 0	36 vs. 14	**0.002**
PR vs. +	28 vs. 21	0.390
IM vs 0	21 vs. 27	0.470
IM vs +	25 vs. 23	0.885
+ vs 0	30 vs. 19	0.152
Low vs. Low	27 0, 21 IM	0.470
High vs. High	21 +, 28 PR	0.392
Low vs. High	76 0 or IM, 119 + or PR	**0.002**
PR vs. other	94 vs. 53	**< 0.001**
IM vs. other	64 vs. 80	0.211
0 vs. other	60 vs. 87	**0.032**
+ vs. other	74 vs. 72	0.934

Table 4-1. Initial spittlebug nymph feeding choice in Experiment 2, distilled into several forms of comparison: pairwise comparisons of all plant types; plant types combined into "low" and "high" trichome density types; and each plant type compared against all others combined. Here and in subsequent tables, significant p-values are in bold print.

	Stay	Leave
PR	68	26
IM	32	30
0	31	28
+	47	29

Table 4-2. Number of spittlebug nymphs in Experiment 2 that remained feeding or evacuated each plant type by Day 2. $X^2_3 = 9.186$, p-value = **0.027**.

	Stay	Leave
PR	53	44
IM	1	62
0	20	48
+	21	56

Table 4-3. Number of spittlebug nymphs in Experiment 2 that remained feeding or evacuated each plant type by Day 11. $X^2_3 = 51.264$, p-value **< 0.001**.

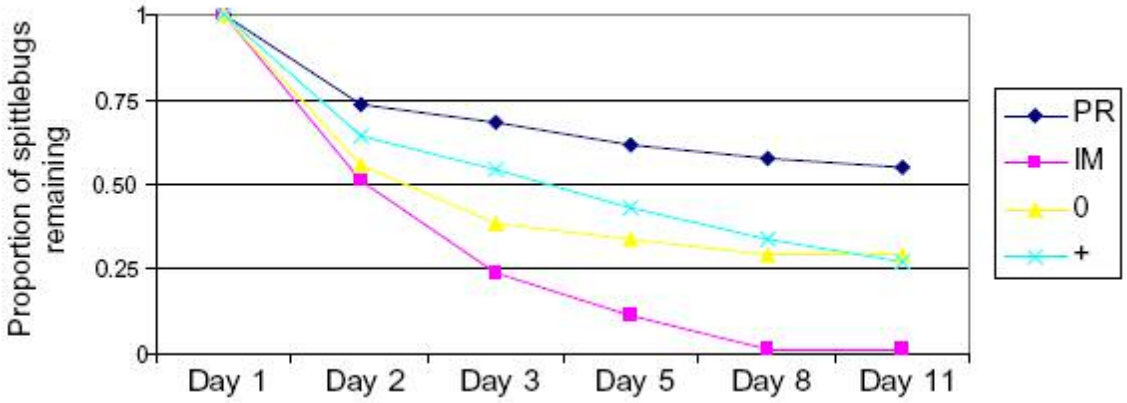


Figure 4-3. Proportion of initial spittlebugs feeding on each plant type that remain over time in Experiment 2.

Fitness effects of herbivory:

Spittlebug herbivory significantly reduced plant fitness relative to control plants. For aboveground biomass, there was a significant decrease in fitness due to herbivory across populations (p-value < 0.001, Table 4-4) although herbivory affected aboveground biomass differentially in each population (p-value = 0.005, Table 4-4). As is shown in Figure 4, there was a significant decrease in aboveground biomass with herbivory in the PR and + populations (p-values = 0.001 and 0.013, respectively), but a non-significant increase in fitness in the 0 population following herbivory (p-value = 0.304). There was no significant differential effect of the pair type that a particular focal plant was paired with within any of the populations across the treatment and control categories (Within the PR, 0, and + populations, the "treatment x pair type interaction" p-values ranged from 0.089 to 0.840).

Source	DF	SS	MS	F	P-value
Population	2	11.3257	5.6629	70.99	< **0.001**
Control/treatment	1	0.4600	0.4600	5.77	**0.017**
Population*Control/treatment	2	0.8595	0.4298	5.39	**0.005**
Error	180	14.3593	0.0798		
Total	185				

Table 4-4. The effects of population and/or treatment on aboveground biomass in Experiment 2.

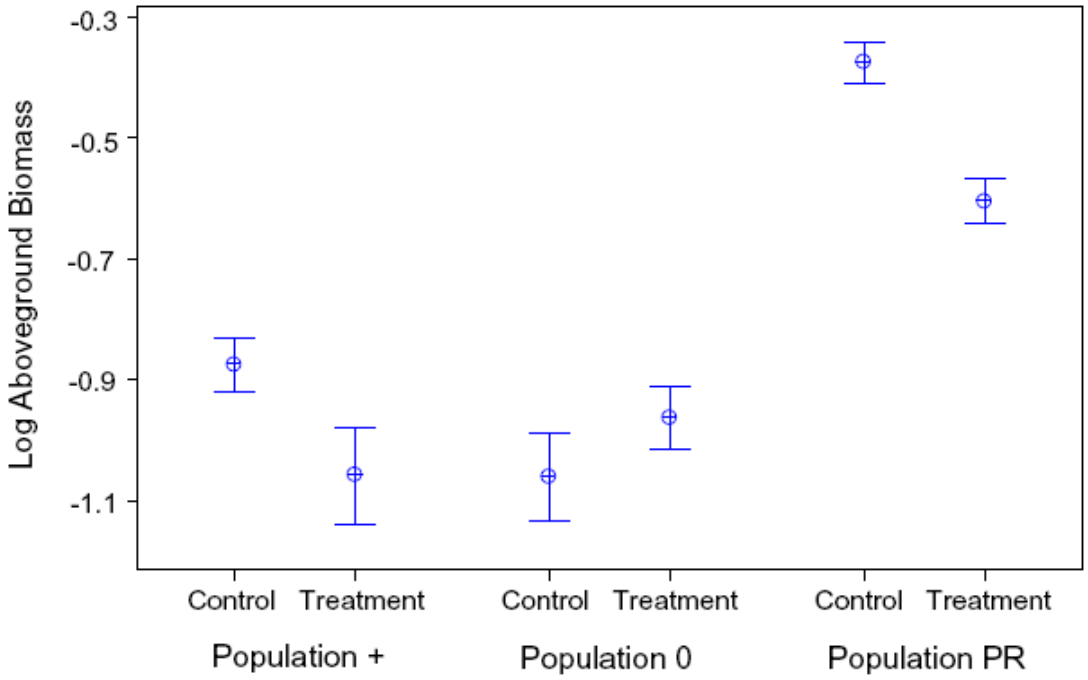


Figure 4-4. Aboveground biomass for control and treatment plants in Experiment 2.

Spittlebug herbivory significantly reduced flower number only in the PR population (p-value = 0.001; Figure 4-5). The + population and 0 population had a non-significant reduction (p-value = 0.53) and an increase (p-value = 0.075), respectively, in flower

number following herbivory (Figure 4-5). There was no significant differential effect of the pair type that a particular focal plant was paired with within either the PR or + populations across the treatment and control categories (treatment x pair type interaction p-values of 0.893 and 0.294). There was a significant differential effect of pair type within the 0 population: 0 focal plants paired with PR plant types had much lower fitness in the control group than in the treatment group with respect to flower number (treatment x pair type interaction p-value = 0.042). This interaction explains, in part, the non-significant increase in flower number following herbivory in the 0 population as mentioned above.

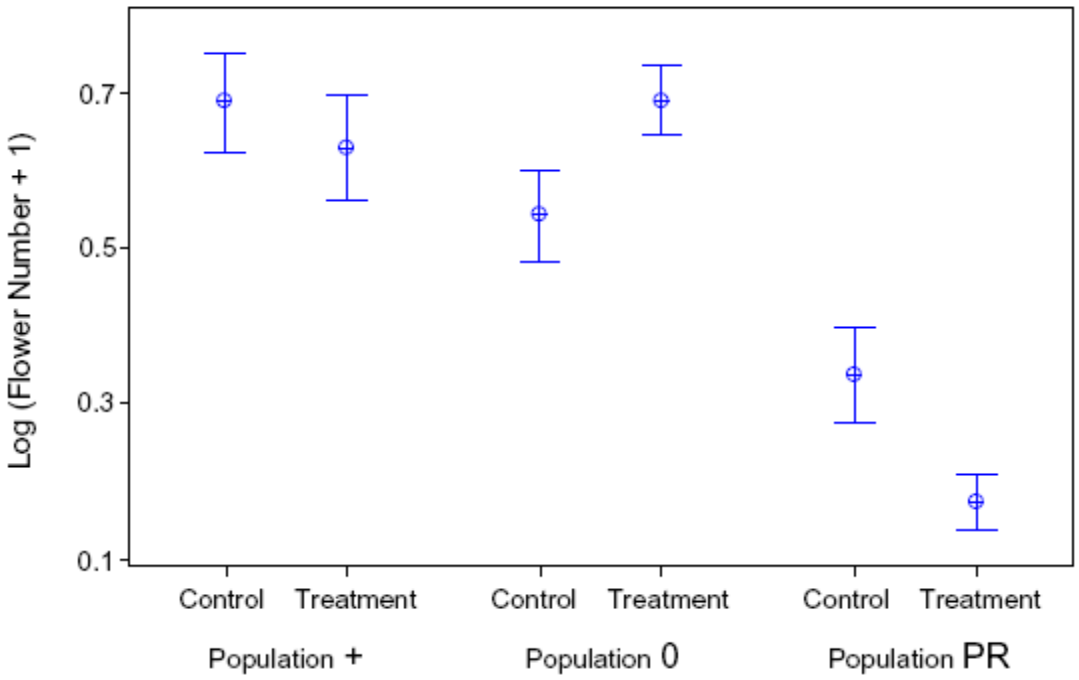


Figure 4-5. Flower number for control and treatment plants in Experiment 2.

Discussion

While *M. guttatus* trichomes may serve as a defense against some herbivores, trichomes do not deter meadow spittlebug nymph feeding ability or feeding preference, as I demonstrate in choice and no-choice experiments.

Factors affecting spittlebug feeding preference and performance

In Experiment 1, a no-choice experiment, I found that higher trichome densities did not reduce the likelihood of a spittlebug establishing a feeding position (Figure 4-1), the number of days it remained on the plant (Figure 4-2), or its weight gain after 17 days of feeding. I failed to find any cost to trichome production: plants with higher trichome densities did not suffer reduced fitness when spittlebugs were absent. In Experiment 2, a choice experiment, I found that spittlebug nymphs showed both an initial and lasting preference for PR plants, the plant type with the highest average trichome density (Tables 4-1, 4-2, and 4-3, Figure 4-3). In contrast, they were least likely to exhibit either an initial or a lasting preference for IM plants, the population type with the lowest average trichome density (Tables 4-1, 4-2, and 4-3; Figure 4-3). Finally, they were initially slightly more likely to prefer + plants then 0 plants (Table 4-1, Figure 4-3), and showed a greater lasting preference for + plants in comparison to 0 plants, although this preference disappeared over time (Table 4-2 and 4-3, Figure 4-3).

This result suggests that while spittlebug nymphs may initially prefer plants with high trichome densities, other factors may influence whether the chosen plant is a sufficient feeding site, i.e. whether the nymph remains on the initial host plant. By the end of Experiment 2, spittlebugs remained on the "0" and "+" RIL plants with almost equal numbers, and at a frequency intermediate to the two parental populations (Figure 4-3, Day 11). The factor(s) influencing spittlebug feeding tendencies by the end of the experiment thus seem to be closely associated with plant type (PR, RILs, or IM) but not with trichome density. This is consistent with the prediction that the RILs used in the experiment have similar genetic backgrounds for traits unlinked to trichome density (see Introduction). Factors such as stem diameter, stem toughness, and/or plant size could influence late feeding behavior (McEvoy 1986), although the results regarding aboveground biomass from Experiment 1 suggest that plant size is not a causal factor here.

The failure of trichomes to resist spittlebugs in Experiments 1 and 2 contrasts with previous studies of two other plant species, *Anaphalis margaritacea* and *Medicago sativa* (Hoffman and McEvoy 1986). In both species, trichomes deterred spittlebug nymphs from establishing feeding sites. Hoffman and McEvoy (1986) also demonstrate that nymphs in instars 1-3 are much less likely to establish feeding sites than nymphs in their 4^{th} or 5^{th} instar, suggesting that smaller insects are less able to overcome trichomes. In Experiment 1 of this study, smaller nymphs were *more likely* to establish feeding positions. Because trichomes did not deter even the smaller nymphs in my experiment, however, this conclusion may be a result of larger nymphs having a higher tendency to

roam before establishing a feeding position. Hoffman and McEvoy caged their nymphs on plants, perhaps giving larger nymphs less opportunity to roam if they initially reject a plant, whereas larger nymphs that roamed from my plants were captured in the Pest-Stick lining the pot sides and had no second chance at feeding.

The demonstrated initial preference of plants with high trichome densities by spittlebug nymphs could be a result of tritropic interactions in natural populations between host plant, spittlebug nymphs, and nymph predators. Details regarding natural predators of meadow spittlebugs are scarce but include reports of several species of spiders and nabids (Harper and Whittaker 1976) and prairie ants (*Formica montana*; (Henderson et al.1990)). Three species of ground beetles (Carabidae), and ants of the genera *Pheidole*, *Mycocepurus*, *Pachycondyla*, and *Ectatomma* have been documented as predators of two other spittlebug genera (Nachappa 2006; Sujii 2002). Previous studies have suggested that trichomes may hinder natural predators and parasitoids of herbivores (Obrycki 1986; Kauffman and Kennedy 1989; Farrar and Kennedy 1991; van Lenteren et al. 1995; Lovinger et al. 2000; Fordyce and Agrawal 2001; Mira and Bernays 2002). For example, trichomes on the vine Dutchman's pipe (*Aristolochia californica*) hinder the searching efficiency of the predacious green lacewing (Chrysopa carnea), and reduce its capture rate of caterpillar prey (Fordyce and Agrawal 2001). In contrast, Styrsky et al. (2006) found that the predator, red imported fire ant, actually had increased predation rates in pubescent soybean plants relative to glabrous plants. This resulted in fire ants more strongly reducing herbivore plant damage in plants with trichomes relative to those without (Styrsky et al. 2006).

The life-history strategy of the IM population, limited vegetative allocation relative to reproductive allocation and a short generation time, may act here as a method of herbivory escape. The natural IM population is an annual alpine population that must flower and reproduce in the short time (6-10 weeks) between snow melt and soil desiccation. It is thus most likely that this particular life-history evolved as a result of natural selection to escape drought (Franks et al. 2007). However, increased rate of development with reduced biomass allocation has previously been demonstrated to evolve in response to predation in vertebrates (Reznick et al. 1990; Remes 2007). This particular manner of herbivory escape has not previously been demonstrated in plants, neither as a direct product of herbivore pressures, nor as a byproduct of other selection pressures.

Trichomes and other insect herbivores or predators

Although trichomes do not apparently confer resistance to spittlebugs, they may deter other insect herbivores of *Mimulus guttatus*. The species is attacked by a variety of insects including Homoptera such as aphids (Aphididae), Lepidoptera larvae (Geometridae), a number of beetle larvae and adults including leaf beetles (Chrysomelidae), weevils (Curculionidae), and click beetles (Elateridae), Hemiptera such as stink bugs (Pentatomidae) and leaf bugs (Miridae), fly larvae (Diptera), thrips (Thysanoptera: Thripidae) and Orthoptera such as grasshoppers. The species is also eaten by slugs (*Deroceras reticulatum*, Gastropoda: Limacidae) and deer (*Odocoileus hemionus*, Artiodactyla: Cervidae) (M. Eubanks, D. Lowry, N. Martin, personal

communication; Carr and Eubanks 2002; Appendix D). Spittlebug nymphs feed on xylem fluid and usually establish only one feeding position. In contrast, herbivores causing direct foliar damage to plant leaves or stems rely on more extensive movement across the plant surface to establish multiple feeding positions. Leaf feeders that are smaller or less tenacious than spittlebug nymphs might be affected to a greater extent by the trichomes and glandular exudate.

Trichome induction

If trichomes do function in plant defense, it is natural to ask whether they are inducible. Inducible physical and/ or chemical defenses, including trichomes, have been demonstrated in a number of other plant species (Karban and Baldwin 1997). Simulated foliar insect damage in the 2nd leaves of plants that are full-siblings of the PR plants and F2 plants used in this experiment did induce significantly higher 5th leaf trichome densities (Chapter 3). However, in this experiment, feeding by spittlebug nymphs initiated at the 2nd leaf stage did not induce higher trichome densities on later leaves. This suggests that phenotypic plasticity in trichome number is specific to certain types of herbivore damage. Specificity in plastic response to an environmental variable has been noted elsewhere (Stout et al. 1998; Agrawal 2000; Van Zandt and Agrawal 2004; Agrawal 2005).

Fitness cost of trichome production?

I found no fitness cost of trichome production, either in terms of male or female fitness.

The PR, IM, and F2 populations exhibit no significant correlation between trichome production and fitness. Because fitness costs are evaluated in the absence of herbivory, the failure to find a cost to trichome production in this experiment has more widespread relevance to the study of *M. guttatus* trichomes acting as a putative resistance trait to other herbivores.

Fitness effects of herbivory

Herbivory by meadow spittlebug nymphs significantly reduced plant fitness relative to control plants (Table 4-4, Figures 4-4 and 4-5). However, plants did not respond to damage by spittlebug nymphs in a consistent manner across populations. Plants from the PR population showed the greatest decrease in both flower number and aboveground biomass following herbivory. This is surprising given that one might expect herbivory to have a greater proportional effect on the fitness of the smaller RIL population than a spittlebug removing the same amount of xylem fluid from a large perennial PR plant might have. Xylem fluid is predominantly water and adaptation to drought stress could have a positive influence on plant response to spittlebug herbivory. Common plant responses to drought stress include reduced node lengths in plant stems and decreases in plant biomass relative to well-watered plants, as well as relative increases in root allocation (Larcher 2001). Reductions in aboveground biomass were demonstrated among treatment plants in this experiment, and substantially reduced stem inter-node lengths were also observed in the treatment plants. Results from a previous experiment examining plant response to drought stress and involving plants from the PR, IM, and F2 populations demonstrate that PR plants have larger absolute and proportional decreases in

aboveground biomass than do F2 and IM plants under drought conditions, indicating that F2 and IM plants are more drought-tolerant (Appendix F). I suggest that drought tolerance as such may also have lessened the impact of spittlebug herbivory on the RIL plants relative to the PR plants, although this hypothesis would require more evaluation.

Summary

Trichome density shows extensive, genetically based variation among natural populations of *M. guttatus*. Meadow spittlebug nymphs preferred *M. guttatus* plants with high trichome densities in both choice and no-choice experiments. This is notable given that trichomes have been shown to deter feeding herbivores including meadow spittlebugs in a number of other plant species. Information regarding the interaction of other insect herbivores with the trait in *M. guttatus*, as well as more knowledge regarding tritrophic interactions between *M. guttatus* plants, spittlebugs, and spittlebug predators would provide insight upon its function as a putative resistance trait. In addition, more information regarding the genetic control of the trait would allow insight to the evolutionary patterns that have led to the observed trait distribution, as well as how the trait might respond to future selection pressures. Genomic tools allow an in-depth examination of the genetics of trichome density. We are currently mapping genomic regions (QTL) that influence both constitutive and inducible trichome density in *M. guttatus* using recombinant inbred lines developed from the IM/PR cross.

References:

Agrawal, A. A. 2000. Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. Oikos 89:493-500.

Agrawal, A. A. 2005. Future directions in the study of induced plant responses to herbivory. Entomologia Experimentalis et Applicata 115:97-105.

Agren, J., and D. W. Schemske. 1993. The cost of defense against herbivores: an experimental study of trichome production in *Brassica rapa*. American Naturalist 141: 338-350.

Beardsley, P. M., and F. G. Olmstead. 2002. Redefining Phrymaceae: The placement of *Mimulus*, tribe Mimuleae and Phryma. American Journal of Botany 89:1093-1102.

Berenbaum, M. R., A. R. Zangerl, and J. K. Nitao. 1986. Constraints on chemical coevolution: wild parsnips and the parsnip webworm. Evolution 40:1215-1228.

Capinera, J.L. Host-plant selection by *Schistocerca americana* (Orthoptera, Acrididae). Environmental Entomology 22:127-133.

Carr, D. E., and M. D. Eubanks. 2002. Inbreeding alters resistance to insect herbivory and host plant quality in *Mimulus guttatus* (Scrophulariaceae). Evolution 56:22-30.

Egas, M. and M. W. Sabelis. 2001. Adaptive learning of host preference in a herbivorous arthropod. Ecology Letters 4:190-195.

Ehleringer, J. 1984. Ecology and ecophysiology of leaf pubescence in North American desert plants. *in* E. Rodregues, P. L. Healey and I. Mehta, eds. Biology and Chemistry of Plant Trichomes. Plenum Press, New York.

Elle, E., and J. D. Hare. 2000. No benefit of glandular trichome production in natural populations of *Datura wrightii*? Oecologia 123:57-63.

Eubanks, M. D., D. E. Carr, and J. F. Murphy. 2005. Variation in the response of *Mimulus guttatus* (Scrophulariaceae) to herbivore and virus attack. Evolutionary Ecology 19:15-27.

Farrar, R. R., and G. Kennedy. 1991. Inhibition of *Telenomus sphingus*, an egg parasitoid of *Manduca* spp. by trichome 2-tridecanone-based host plant resistance in tomato. Entomological Experiments and Applications 60: 157-166.

Fenster, C. B., and K. Ritland. 1994. Quantitative genetics of mating system divergence in the yellow monkeyflower species complex. Heredity 73:422-435.

Fineblum, W. L., and M. D. Rausher. 1995. Tradeoff between resistance and tolerance to herbivore damage in a morning glory. Nature 377:517-520.

Fordyce, J. A., and A. A. Agrawal. 2001. The role of plant trichomes and caterpillar group size on growth and defense of the pipevine swallowtail *Battus philenor*. Journal of Animal Ecology 70: 997-1005.

Fornoni, J. J., J. Nunez-Farfan, and P. L. Valverde. 2003a. Evolutionary aspects of tolerance to herbivory: advances and perspectives. Comments on Theoretical Biology 8:643-663.

Fornoni, J. J., J. Nunez-Farfan, P. L. Valverde, and M. D. Rausher. 2004. Evolution of mixed strategies of plant defense allocation against natural enemies. Evolution 58:1685-1695.

Fornoni, J. J., P. L. Valverde, and J. Nunez-Farfan. 2003b. Quantitative genetics of plant tolerance and resistance against natural enemies of two natural populations of *Datura stramonium*. Evolutionary Ecology Research 5:1049-1065.

Franks, S. J., S. Sim, and A.E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proceedings of the National Academy of Sciences of the United States of America 104:1278-1282.

Grant, V. 1950. Genetic and taxonomic studies in *Gilia*. I. *Gilia capitata.* Aliso 2:239-316.

Gerard, P. J., J.R. Crush, and D.L. Hackell. 2005. Interaction between *Sitona lepidus* and red clover lines selected for formononetin content. Annals of Applied Biology 147: 173-181.

Hall, M. C., C. J. Basten, and J. H. Willis. 2006. Pleiotropic quantitative trait loci contribute to population divergence in traits associated with life-history variation in *Mimulus guttatus*. Genetics 172:1829-1844.

Hall, M. C., and J. H. Willis. 2006. Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. Evolution 60

Hamback, P. A. 2001. Direct and indirect effects of herbivory: Feeding by spittlebugs affects pollinator visitation rates and seedset of *Rudbeckia hirta*. Ecoscience 8:45-50.

Handley, R., B. Ekbom, and J. Agren. 2005. Variation in trichome density and resistance against a specialist insect herbivore in natural populations of *Arabidopsis thaliana*. Ecological Entomology 30: 284-292.

Hare, J. D. 2005. Biological activity of acyl glucose esters from *Datura wrightii* glandular trichomes against three native insect herbivores. Journal of Chemical Ecology 31: 1475-1491.

Hare, J. D., and E. Elle. 2002. Variable impact of diverse insect herbivores on dimorphic phenotypes of *Datura wrightii*. Ecology 83:2711-2720.

Harper, G. and J.B. Whittaker. 1976. The role of natural enemies in the colour polymorphism of *Philaenus spumarius*. Journal of Animal Ecology 45: 91-104.

Henderson, G., G.D. Hoffman, and R.L. Jeanne. 1990. Predation on cercopids and material use of the spittle in aphid-tent construction by prairie ants. Psyche 97:43-53.

Hoffman, G. D., and P. B. McEvoy. 1986. Mechanical limitations on feeding by meadow spittlebugs *Philaenus spumarius* (Homoptera: Cercopidae) on wild and cultivated host plants. Ecological Entomology 11:415-426.

Holeski, L. M. 2007. Quantitative trait evolution in *Mimulus guttatus* (yellow monkeyflower). Doctoral Dissertation. Ecology and Evolutionary Biology. University of Kansas, Lawrence.

Ivey, C. T., and D. E. Carr. 2005. Effects of herbivory and inbreeding on the pollinators and mating system of *Mimulus guttatus* (Phrymaceae). American Journal of Botany 92:1641-1649.

Karban, R., and I. T. Baldwin. 1997. Induced Responses to Herbivory. University of Chicago Press, Chicago.

Kauffman, W. C., and G. C. Kennedy. 1989. Relationship between trichome density in tomato and parasitism of *Heliothis* spp. (Lepidoptera: Noctuidae) eggs by

Trichogramma spp. (Hymenoptera: Trichogrammatidae). Enivironmental Entomology 18: 698-784.
Kelly, J. K., and J. H. Willis. 2001. Deleterious mutations and genetic variation for flower size in *Mimulus guttatus*. Evolution 55:937-942.
Kelly, J. K., and J. H. Willis. 2002. A manipulative experiment to estimate biparental inbreeding in monkeyflowers. International Journal of Plant Sciences 163:575-579.
Kelly, J.K., A. Rasch, and S. Kalisz. 2002. A method to estimate pollen viability from pollen size variation. American Journal of Botany 89: 1021-1023.
Kelly, J. K., and H. S. Arathi. 2003. Inbreeding and the genetic variance in floral traits of Mimulus guttatus. Heredity 90:77-83.
Larcher, W. 2001. Physiological Plant Ecology. Springer-Verlag, Berlin.
Leimu, R., and J. Koricheva. 2006. A meta-analysis of trade-offs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. Oikos 112:1-9.
van Lenteren, J. C., L. Z. Hua, J. W. Kamerman, and X. Rumei. The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). XXVI. Leaf hairs reduce the capacity of *Encarsia* to control greenhouse whitefly on cucumber. Journal of Applied Entomology 119: 553-559.
Levin, D. A. 1973. The role of trichomes in plant defense. Quarterly Review of Biology 48:3-15.
Lovinger, A., D. Liewehr, and W.O. Lamp. 2000. Glandular trichomes on alfalfa impede searching behavior of the potato leafhopper parasitoid. Biological Control 18:187-192.
Malakar, R., and W. M. Tingey. 2000. Glandular trichomes of Solanum berthaultii and its hybrids with potato deter oviposition and impair growth of potato tuber moth. Entomoligical Experiments and Applications. 94: 249-257.
Marquis, R. J. 1991. Evolution of resistance in plants to herbivores. Evolutionary Trends in Plants 5:23-29.
Marquis, R. J. 1992. The selective impact of herbivory. Pp. 301-325 *in* R. S. Fritz and E. L. Simms, eds. Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics. University of Chicago Press, Chicago.
McEvoy, P. B. 1986. Niche partitioning in spittlebugs (Homoptera: Cercopidae) sharing shelters on host plants. Ecology 67:465-478.
Medeiros, L., and G. R. P. Moreira. 2005. Larval feeding behavior of *Gratiana spadicea* (Coleoptera: Chrysomelidae: Cassidinae) on its host plant, *Solanum sisymbrifolium* (Solanaceae): Interaction with trichomes. The Coleopterists Bullitin 59(2): 339-350.
Meyer, G. A. 1993. A comparison of the impacts of leaf- and sap-feeding insects on growth and allocation of goldenrod. Ecology 74:1101-1116.
Meyer, G. A., and T. H. Whitlow. 1992. Effects of leaf and sap feeding insects on photosynthetic rates of goldenrod. Oecologia 92:480-489.
Mira, A. and E. A. Bernays. 2002. Trade-offs in host use by *Manduca sexta*: plant characters vs. natural enemies. Oikos 97: 387-397.

Mopper, S., J. B. Mitton, T. G. Whitham, N. S. Cobb, and K. M. Christensen. 1992. Genetic differentiation and heterozygosity in pinyon pine associated with resistance to herbivory and environmental stress. Evolution 45:989-999.
Morris, W. F., M. B. Traw, and J. Bergelson. 2006. On testing for a tradeoff between constitutive and induced resistance. Oikos 112:102-110.
Nachappa, P., L.P. Guillebeau, S.K. Braman, and J.N. All. 2006. Susceptibility of Twolined Spittlebug (Hemiptera: Cercopidae) life stages to entomophagous arthropods in turfgrass. Journal of Economic Entomology 99:1711-1716.
Nunez-Farfan, J., and R. Dirzo. 1994. Evolutionary ecology of *Datura stramonium* L. in Central Mexico: natural selection for resistance to herbivorous insects. Evolution 48:423-436.
Obrycki, J. J. 1986. The influence of foliar pubescence in entomophagous species. D. J. Boethel and R. D. Eikenbary, eds. Interactions of Plant Resistance and Parasitoids and Predators of Insects, pp. 61-97. Ellis Horwood, Chichester, U.K.
Orians, C. 2005. Herbivores, vascular pathways, and systemic induction: facts and artifacts. Journal of Chemical Ecology 31:2231-2242.
Poykko, H., M. Hyvarinen, and M. Backor. 2005. Removal of lichen secondary metabolites affects food choice and survival of lichenivorous moth larvae. Ecology 86: 2623-2632.
Rausher, M. D., and E. L. Simms. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. I. Attempts to detect selection. Evolution 43:563-572.
Remes, V. 2007. Avian growth and development rates and age-specific mortality: the roles of nest predation and adult mortality. Journal of Evolutionary Biology 20: 320-325.
Reznick, D., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural population. Nature 346: 357-359.
Ritland, K. 1989. Genetic differentiation, diversity, and inbreeding in the mountain monkeyflower (*Mimulus caespitosus*) of the Washington Cascades. Canadian Journal of Botany 67:2017-2024.
Romeis, J., T. G. Shanower, and A. J. Peter. 1999. Trichomes on Pigeonpea (*Cajanus cajan* (L.) Millsp) and two wild *Cajanus* spp. Crop Science 39:564-569.
Siemann, E., and W. E. Rogers. 2003. Reduced resistance of invasive varieties of the alien tree *Sapium sebiferum* to a generalist herbivore. Oecologia 135: 451- 457.
Simms, E. L., and R. S. Fritz. 1990. The ecology and evolution of host-plant resistance to insects. Trends in Ecology and Evolution 5:356-360.
Simms, E. L., and M. D. Rausher. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. II. Natural selection by insects and costs of resistance. Evolution 43:573-585.
Stout, M. J., K. V. Workman, R. M. Bostock, and S. S. Duffey. 1998. Specificity of induced resistance in the tomato, *Lycopersicon esculentum*. Oecologia 113:74-81.
Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. Trends in Ecology and Evolution 14:179-185.
Styrsky, J. D., I. Kaplan, and M. D. Eubanks. 2006. Plant trichomes indirectly enhance tritrophic interactions involving a generalist predator, the red imported fire ant. Biological Control 36:375-384.

Sujii, E. R., M.A. Garcia, E.M.G. Fontes, and R. O'Neil. 2002. Predation as a mortality factor in populations of the spittlebug, *Deois flavopicta* (Homoptera: Cercopidae). Neotropical Entomology 31:581-588.

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 83:625-632.

Thompson, J. N. 1994. The Coevolutionary Process. University of Chicago Press, Chicago.

Valverde, P. L., J. J. Fornoni, and J. Nunez-Farfan. 2001. Defensive role of leaf trichomes in resistance to herbivorous insects in *Datura stramonium*. Journal of Evolutionary Biology 14:424-432.

Valverde, P. L., J. J. Fornoni, and J. Nunez-Farfan. 2003. Evolutionary ecology of *Datura stramonium*: equal plant fitness benefits of growth and resistance against herbivory. Journal of Evolutionary Biology 16:127-137.

van Dam, N. M., and J. D. Hare. 1998. Differences in distribution and performance of two sap-sucking herbivores on glandular and non-glandular *Datura wrightii*. Ecological Entomology 23:22-32.

van Dam, N. M., J. D. Hare, and E. Elle. 1999. Inheritance and distribution of trichome phenotypes in *Datura wrightii*. The American Genetic Association 90:220-227.

Van Zandt, P. A., and A. A. Agrawal. 2004. Specificity of induced plant responses to specialist herbivores of the common milkweed *Asclepias syriaca*. Oikos 104:401-409.

Verdon, A., N. Margraf, A.C. Davison, M. Rahier, and R.E. Naisbit. 2007. Conserved oviposition preferences in alpine leaf beetle populations despite host shifts and isolation. Ecological Entomology 32: 62-69.

Weaver, C. R., and D. R. King. 1954. Meadow spittlebug. Pp. 1-99. Ohio Agricultural Experiment Station Research Bulletin.

Weinig, C., J. R. Stinchcombe, and J. Schmitt. 2003a. Evolutionary genetics of resistance and tolerance to natural herbivory in *Arabidopsis thaliana.* . Evolution 57:1270-1280.

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

Whittaker, J. B. 1970. Cercopid spittle as a microhabitat. Oikos 21:59-64.

Willis, J. H. 1993. Partial self-fertilization and inbreeding depression in 2 populations of *Mimulus guttatus*. Heredity 71:145-154.

Willis, J. H. 1996. Measures of phenotypic selection are biased by partial inbreeding. Evolution 50:1501-1511.

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

Wilson, M. C., J. K. Stewart, and H. D. Vail. 1979. Full season impact of the alfalfa weevil, meadow spittlebug, and potato leafhopper in an alfalfa field. Journal of Economic Entomology 72:830-834.

Wright, J. L., M. L. Stanton, and R. Scherson. 2006. Local adaptation to serpentine and non-serpentine soils in *Collinsia sparsiflora*. Evolutionary Ecology Research 8:1-21.

Chapter 5: Future directions

The underlying genetic basis of constitutive and induced trichome density in *M. guttatus*

A mapping project of QTL (quantitative trait loci) that influence both constitutive and induced trichome density is currently underway. To pinpoint QTL which influence trichome density, I am using a bulk segregant approach (Figure 5-1).

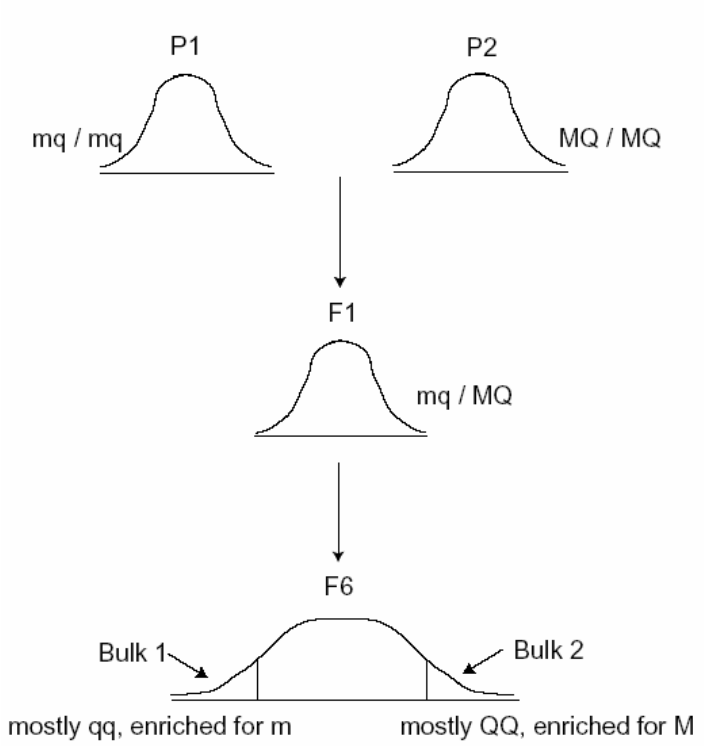


Figure 5-1. Schematic of bulk segregant approach, modified from Lynch and Walsh (1998, p. 402). P1 and P2 represent phenotype distributions from 2 parental populations with a divergent phenotype of interest (e.g. trichome density). Allele q is associated with low trichome density and is closely linked with marker allele m in Population P1, and allele Q is associated with high trichome density and is closely lined with marker allele M in Population P2.

As in Figure 5-1, individuals from each parental population are crossed to form F1 individuals, one of which is self-fertilized for several generations to form RILs in the F6 generation. These F6 RILs will have a phenotypes ranging from low to high trichome density. If RILs representing the extreme phenotypic categories (here, low trichome

density (Bulk 1) and high trichome density (Bulk 2)) are genotyped at a number of genetic markers, those markers closely linked to loci influencing trichome density should appear at a directional, non-random frequency. For example, 10 RILs in Bulk 1 and 10 RILs in Bulk 2 are genotyped at markers Mm and Gg. Five of the RILs in both Bulk 1 and Bulk 2 are G at the marker, while 5 of the RILs in each category are g. Since both alleles are equally represented in each bulk, marker Gg is not likely to be closely linked with loci controlling trichome density. In contrast, for marker Mm, in Bulk 1, 1 RIL is M at the marker while 9 RILs are m. In Bulk 2, 2 RILs are m at the marker, while 8 RILs are M. The asymetic distribution of Mm alleles indicates that this is a "promising initial screen" for marker Mm; it is likely to be closely linked with loci controlling trichome density and this marker will be screened against a greater number of RILs.

F6 RILs were phenotyped for the average constitutive 2nd leaf and average induced 5th leaf trichome density for each of the 450 lines using replicates. 154 gene-based markers (ESTs, or expressed sequence tags) that are polymorphic in the RIL parental populations (PR and IM) have been screened against the DNA from 46 RILs representing the most extreme phenotypes in 3 phenotype categories: 1) Many constitutive trichomes, many induced trichomes (12 RIL representatives), 2) No constitutive trichomes, no induced trichomes (17 RIL representatives), and 3) No constitutive trichomes, many induced trichomes (15 RIL representatives). Six of the 154 markers had promising initial screens among these 46 RILs and were then screened against 188 RILs representing a random phenotypic subset of the larger RIL group. Three of these 6 markers had non-significant

effects on trichome density after the larger screen, while 3 of the markers had significant but small effects explaining 3-5% of the variation in trichome density.

Screening of additional markers is continuing, both to provide better coverage of chromosomes and to delimit the boundaries of the current QTL with significant effects on trichome density. The relatively high heritability of trichome density in *M. guttatus*, combined with no QTL of major effect found thus far suggests that the trait is influenced by many loci of small effect. This is in striking contrast to QTL mapping results in *Arabidopsis*, where trichomes are influenced by a QTL of major effect (70-80%; Mauricio 2005).

Transgenerational induction of trichomes in *M. guttatus*

In collaboration with Lena Hileman, experiments are currently underway to investigate the mechanism of transgenerational trichome induction described in Chapter 2. In addition, a number of the tools necessary to examine the genetic basis of transgenerational trichome induction are already available. The same genetic markers that have been shown to be polymorphic in the RIL parental plants for QTL mapping of within-generation trichome influence can be used to map QTL influencing transgenerational induction, and a substantial subset of the F6 RILs have already been phenotyped for the trait.

Acknowledgements: I would like to thank my advisor, John Kelly, for his advice, support, and patience throughout the course of these experiments. My committee members, Maria Orive, Helen Alexander, Joy Ward, and Jennifer Roberts gave me helpful comments on my preliminary research proposal and/or manuscripts that became a part of this dissertation work. I would also like to thank Dan Crawford, Garrick Skalski, Jenny Gleason, Stuart Macdonald and the EEB Genetics discussion group who provided helpful comments on manuscript(s).

Undergraduates and other members of the Kelly lab, especially Scott Jablonski, Kim Milne, Karen Ganacias, Ronni Chase-Alone, Rose S. Kelly, Autumn McPherson, Tom Komp, and Julius Mojica helped with the plant work that made up these experiments. Two of my fellow graduate students associated with the Kelly lab, Tara Marriage and Young Wha Lee, were very generous with their time especially during the beginning of the QTL mapping project. Craig Martin, Joy Ward, Lena Hileman, Stuart MacDonald, Jenny Gleason, and Mark Mort kindly allowed me to use their labs for various parts of these experiments. Charles Michener identified the insect pollinators listed in Appendix E, Daniel Bennett helped me identify the insect herbivores listed in Appendix D, and Allen Smith-Pardo lent me the insect collecting equipment that I used.

I would like to thank my grandparents: Charlotte and Casimir Holeski, Woodrow and Martha Rankin, parents: Paul and Sue Holeski, and sisters: Carrie and Susie Holeski for their love and support.

Appendix A

The plants grown in generation 0 of Chapter 2 were part of a larger experiment investigating epistasis in *Mimulus guttatus*. Plants from the same families (F1 and inbred line) were measured in two additional grow-ups, one before and one after generation 0. Here, we combine these data to estimate variances within and among families. Inbred lines and F1 families are considered in separate analyses to estimate the variance among fully inbred and outbred genotypes, respectively. Across all 3 grow-ups, there were 1156 plants within F1 families and 1841 from inbred lines. A mixed model was fit to data from each response variable with grow-up (fixed) and family (random) as the factors. Using the statistical package JMP 5.1, we applied REML for estimation of variance components and assignment of standard errors. The estimates for genetic and environmental variances for corolla width (CW), days to flower (Days) and *I* are given for each analysis in Table A1.

Trait	V_G (SE)	V_E
Outbred plants		
Corolla width	2.54 (0.51)	3.40
Days to flower	1.83 (0.40)	6.08
I	0.53 (0.12)	1.34
CW + Days	5.07 (1.00)	9.09
CW + ***I***	5.02 (1.03)	7.77
Days + ***I***	1.51 (0.30)	3.25
Inbred plants		
Corolla width	3.11 (0.44)	3.48
Days to flower	4.44 (0.69)	8.68
I	0.95 (0.14)	1.59
CW + Days	8.31 (1.21)	12.02
CW + ***I***	6.52 (0.94)	8.10
Days + ***I***	2.80 (0.42)	4.52

Table A1. The variance among families estimates V_G while the intra-family variance estimates V_E (see text). Separate model fits were conducted for each trait within each dataset (outbred vs. inbred plants).

We also estimated variance components for sums that combine each pair of characters (Table A1). The variance estimates for sums can be to estimate genetic and environmental covariances by noting that for two traits (z1 and z2) the following relationship holds:

$$Var[z1 + z2] = Var[z1] + Var[z2] + 2\, Cov[z1, z2].$$

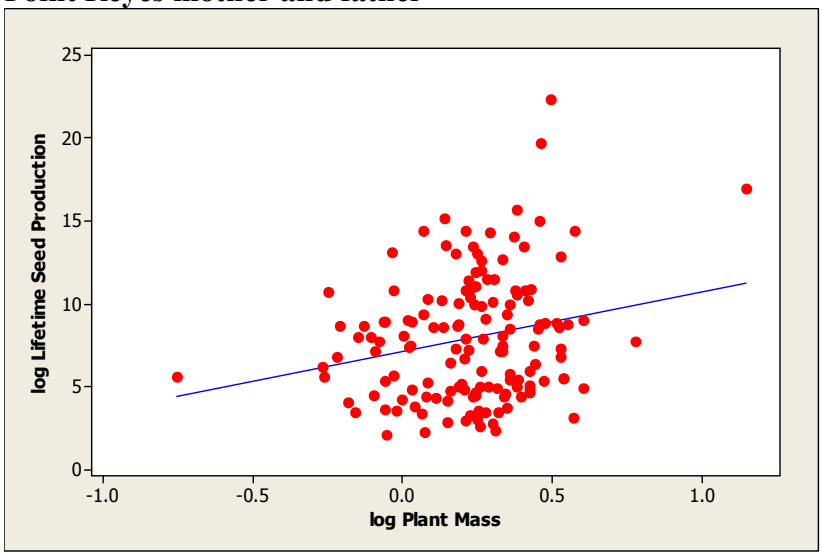
The genetic covariance can be obtained by substituting V_G estimates from Table A1 for the first three terms and solving for the fourth term. These estimates, in addition to the associated genetic correlation estimates, are given in Table A2.

Trait	Genetic covariance	Genetic correlation
Outbred plants		
CW / Days	0.35	0.16
CW / ***I***	0.98	0.84
Days / ***I***	-0.43	-0.43
Inbred plants		
CW / Days	0.38	0.10
CW / ***I***	1.23	0.72
Days / ***I***	-1.295	-0.63

Table A2. The genetic covariance and correlation between each pair of measurements are given for outbred and inbred plants, respectively.

Appendix B: Aboveground biomass as a predictor of lifetime seed production in the Point Reyes (PR), Iron Mountain (IM), and F2 populations of *Mimulus guttatus*.

Point Reyes mother and father

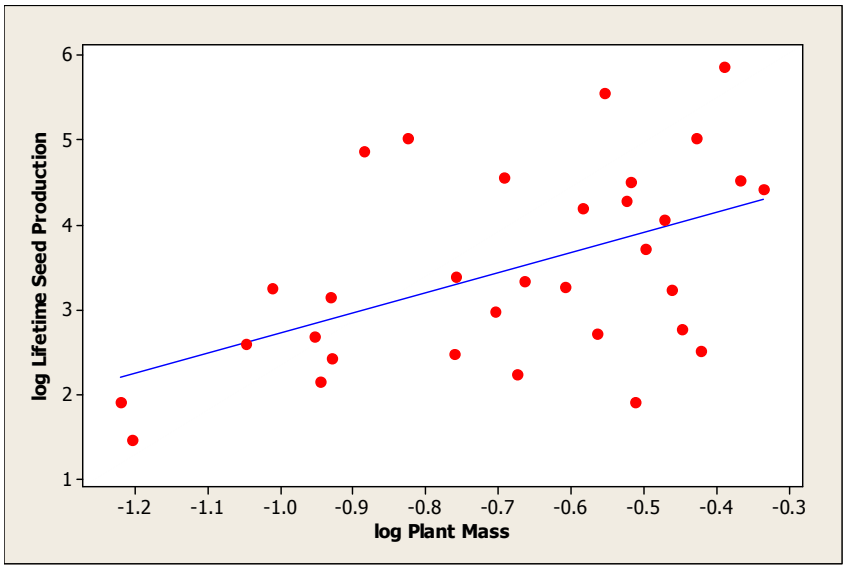


log Lifetime Seed Prodcution = 7.07 + 3.59 log Plant Mass

Predictor	Coef	SE Coef	T	P
Constant	7.0678	0.4270	16.55	0.000
log plant mass	3.587	1.310	2.74	**0.007**

S = 3.69155 R-Sq = 4.9% R-Sq(adj) = 4.3%

IM mother and father

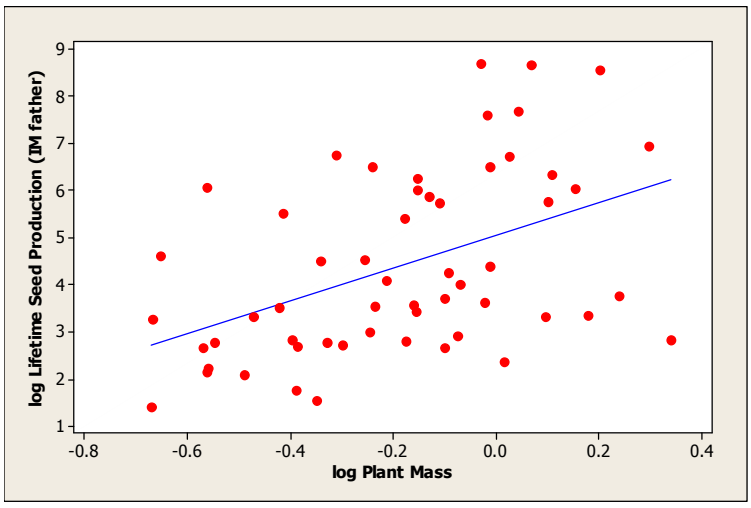


log Lifetime Seed Production = 5.10 + 2.38 log Plant Mass

Predictor	Coef	SE Coef	T	P
Constant	5.0976	0.5319	9.58	0.000
log plant mass	2.3771	0.7330	3.24	**0.003**

S = 1.00380 R-Sq = 26.0% R-Sq(adj) = 23.5%

F2 mother with IM father

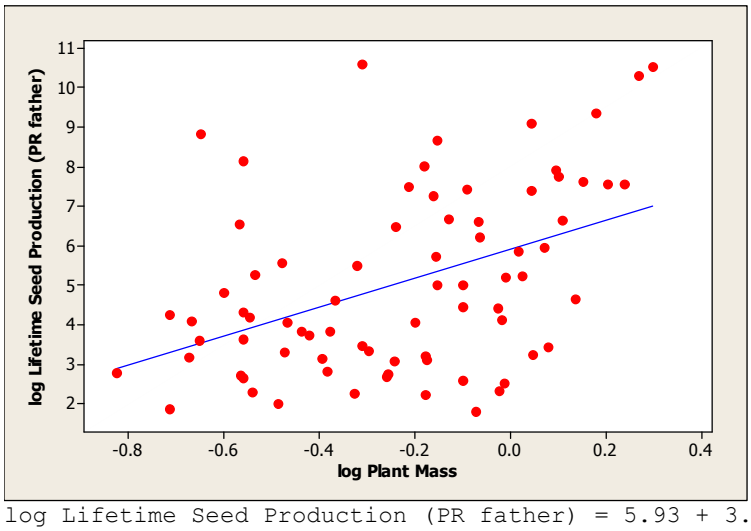


log Lifetime Seed Production (IM father) = 5.06 + 3.49 log Plant Mass

Predictor	Coef	SE Coef	T	P
Constant	5.0575	0.2869	17.63	0.000
log plant mass	3.4881	0.9086	3.84	**0.000**

S = 1.73401 R-Sq = 21.4% R-Sq(adj) = 20.0%

F2 mother with PR father



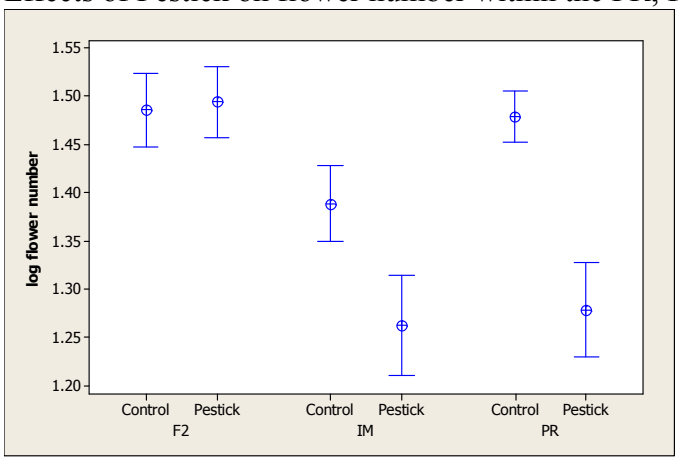
log Lifetime Seed Production (PR father) = 5.93 + 3.69 log Plant Mass

Predictor	Coef	SE Coef	T	P
Constant	5.9272	0.3125	18.97	0.000
log plant mass	3.6860	0.8634	4.27	**0.000**

S = 2.07509 R-Sq = 19.8% R-Sq(adj) = 18.7%

Appendix C: Effect of Pestick on plant fitness

Effects of Pestick on flower number within the PR, IM, and F2 populations



GLM ANOVA, Population and Treatment as fixed factors

Source	DF	SS	MS	F	P
Pop	2	0.83044	0.41522	8.24	**0.000**
Treatment	1	0.50518	0.50518	10.02	**0.002**
Pop*Treatment	2	0.33303	0.16651	3.30	**0.039**
Error	174	8.76894	0.05040		
Total	179				

One-way ANOVAs for each population:

PR

Source	DF	SS	MS	F	P
Treatment	1	0.6195	0.6195	13.18	**0.001**
Error	60	2.8208	0.0470		
Total	61	3.4402			

F2

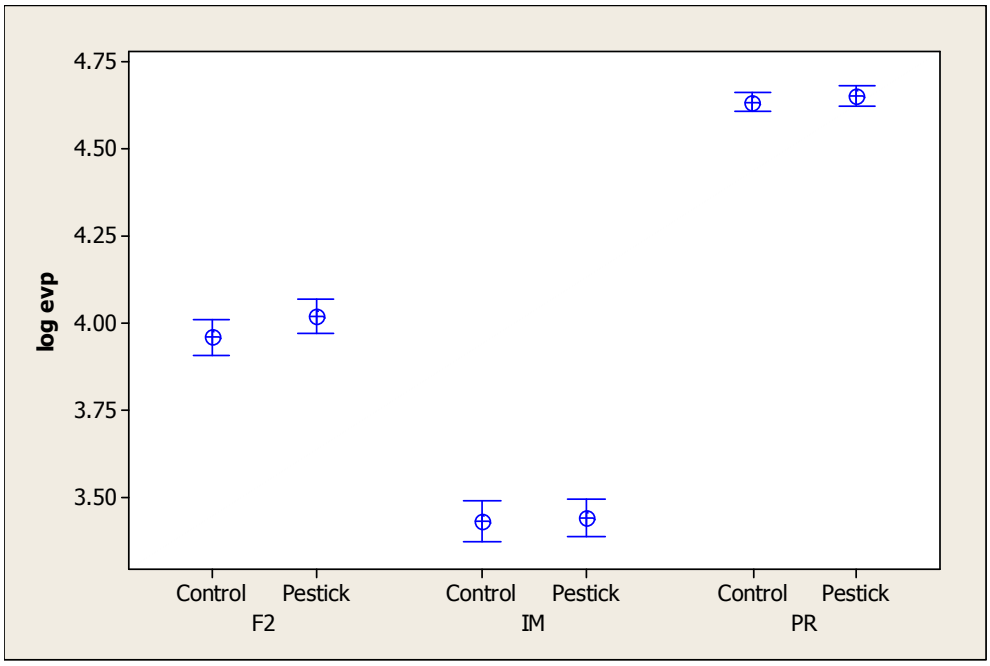
Source	DF	SS	MS	F	P
Treatment	1	0.0011	0.0011	0.03	0.873
Error	56	2.2996	0.0411		
Total	57	2.3007			

IM

Source	DF	SS	MS	F	P
Treatment	1	0.2405	0.2405	3.82	0.055
Error	58	3.6485	0.0629		
Total	59	3.8890			

Pestick significantly decreases flower number relative to control plants

Effects of Pestick on estimated viable pollen (EVP)

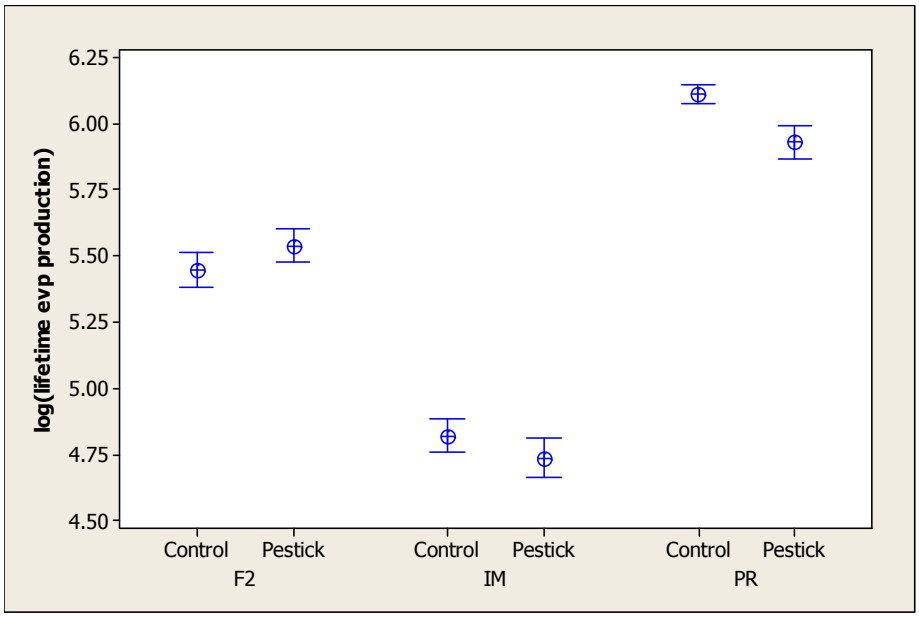


GLM ANOVA: Population and treatment as fixed factors

Source	DF	SS	MS	F	P
Pop	2	45.0858	22.5429	356.70	0.000
Treatment	1	0.0420	0.0420	0.66	0.416
Pop*Treatment	2	0.0223	0.0112	0.18	0.838
Error	176	11.1229	0.0632		
Total	181				

Pestick does not significantly affect EVP in a comparison of treatment and control plants

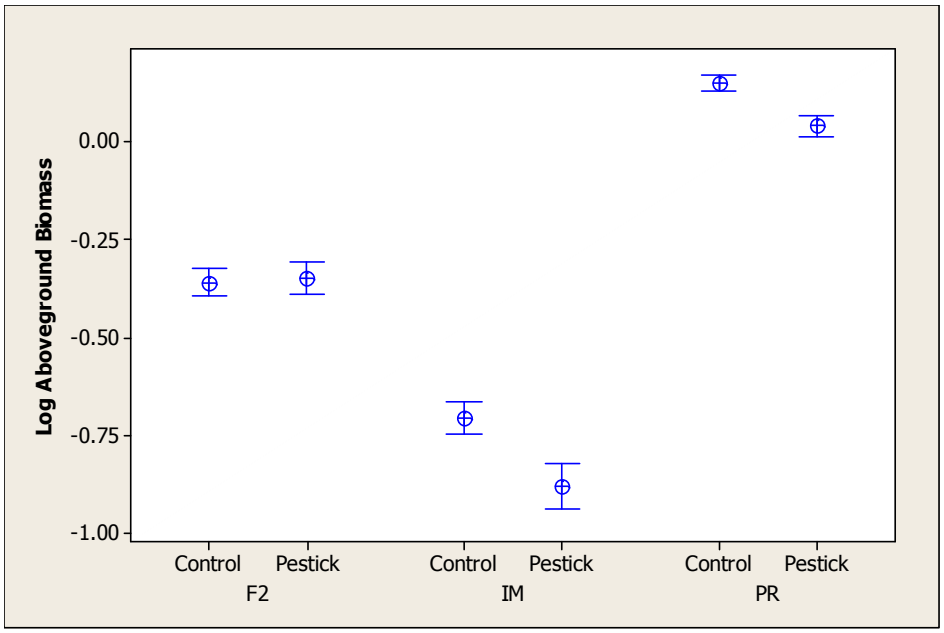
Effects of Pestick on lifetime EVP production (flower number x EVP)



GLM ANOVA: Population and treatment as fixed factors

Source	DF	SS	MS	F	P
Pop	2	47.0018	23.5009	215.25	0.000
Treatment	1	0.1428	0.1428	1.31	0.254
Pop*Treatment	2	0.5697	0.2848	2.61	0.077
Error	171	18.6699	0.1092		
Total	176				

Pestick does not significantly affect lifetime EVP in treatment vs. control plants



GLM ANOVA: Population and treatment as fixed factors

Source	DF	SS	MS	F	P
Population	2	24.3769	12.1885	265.22	0.000
Treatment	1	0.3759	0.3759	8.18	0.005
Population*Treatment	2	0.2678	0.1339	2.91	0.057
Error	178	8.1801	0.0460		
Total	183				

Pestick significantly reduces aboveground biomass

Appendix D: Insect herbivores in 6 natural *M. guttatus* populations

The herbivores listed were feeding on plants during 1-3 hour daytime survey periods in June 2004. All population locations are given in Figure 1 and Table 1 of Chapter 3 except Population IM 2. This population is approximately 1 mile east of the IM population along the Cone Peak trail, and is within 100m of the same elevation. No plants from this population were collected for experimental use. Populations D and F (Muir Beach and Point Reyes National Seashore, CA) were not surveyed, but a number of plants hosting multiple meadow spittlebug nymphs (Cercopidae) were observed in each of these populations in 2003 or 2005.

Population F, Oregon Dunes: Very little foliar damage

No herbivores collected during a 2 hour observation period

Population G, Metolius River: Very little foliar damage

multiple Aphididae (aphids)
single Buprestidae (metallic wood-boring beetle)

Population H, Cougar Reservoir: Foliar and corolla damage easily observed

multiple Cercopidae (adult froghoppers, adult leafhoppers, spittlebug nymphs)
multiple Orthoptera (grasshoppers)
multiple Aphididae (aphids)
multiple Thysanoptera (thrips)
single Miridae (leaf bug)

Population I, Dexter Reservoir: Foliar and corolla damage easily observed

multiple Curculionidae (weevils)
multiple Aphididae (aphids)
multiple Homoptera
multiple Cercopidae (froghoppers, leafhoppers, spittlebug nymphs)
multiple Chrysomelidae (leaf beetles)
single Orthoptera (grasshopper)
single Elateridae (click beetle)
single Pentatomidae (stink bug)

Population IM, Iron Mountain: Very little foliar damage

No herbivores collected during a 1 hour observation period

Population IM 2: Foliar and corolla damage easily observed

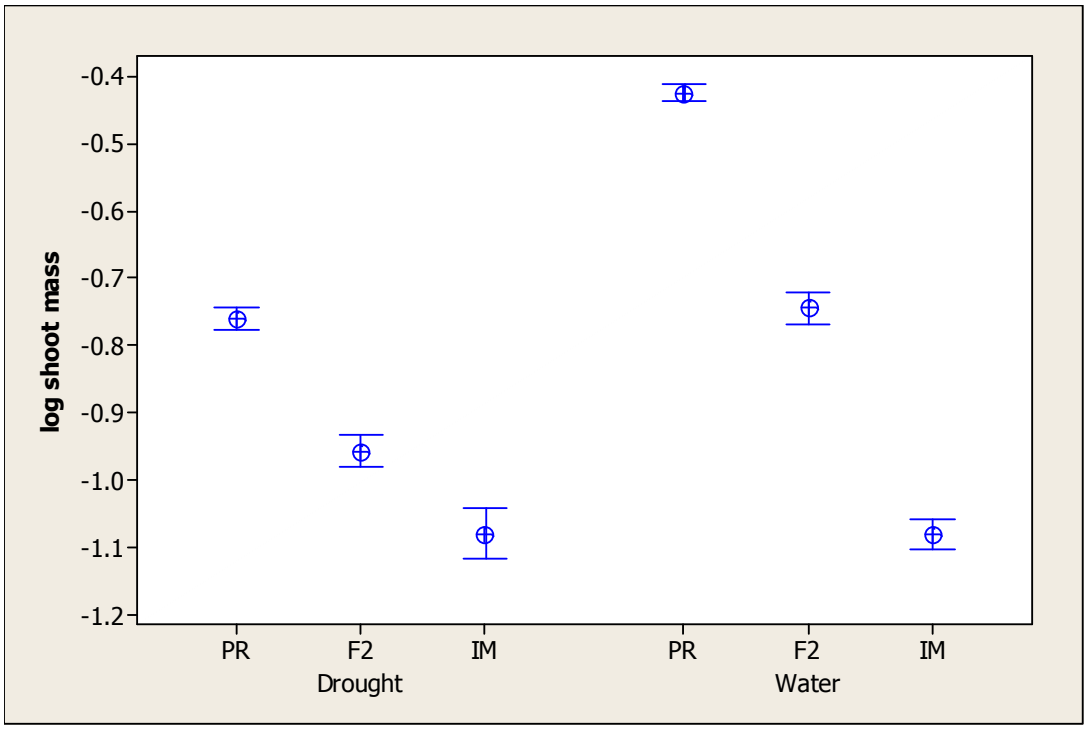
Multiple Aphididae (aphids)
Multiple Lepidoptera larvae
Multiple Curculionidae (weevils)
Multiple Thysanoptera (thrips)

Appendix E: Potential pollinators of 8 natural *M. guttatus* populations

Population	Insect pollinators
A	*Bombus californicus* (Smith)
B	*Apis mellifera* (Linneaus)
C	Mordellidae, Melyridae *(*Coleoptera*), Hylaeus* sp?, *Hoplitis producta* (Cresson)
E	Diabrotica (Chrysomelidae, Coleoptera)
F	Cercopidae (Homoptera), Lasioglossum s.str sp?, *Bombus vosnesenskii* (Rad.),Coccinellidae (Coleoptera), Empididae (Diptera), Ceratapogonidae (Diptera), *Bombus californicus* (Smith)
H	*Lasioglossum* (Evylaeus) sp?, *Bombus mixtus* (Cresson), *Bombus melanopygus* (Nylander), Elateridae (Coleoptera)
J	*Apis mellifera* (Linnaeus)
IM	*Bombus edwardsi* (Cresson), *Bombus melanopygus* (Nylander), *Bombus mixtus* (Cresson), Syrphidae

.

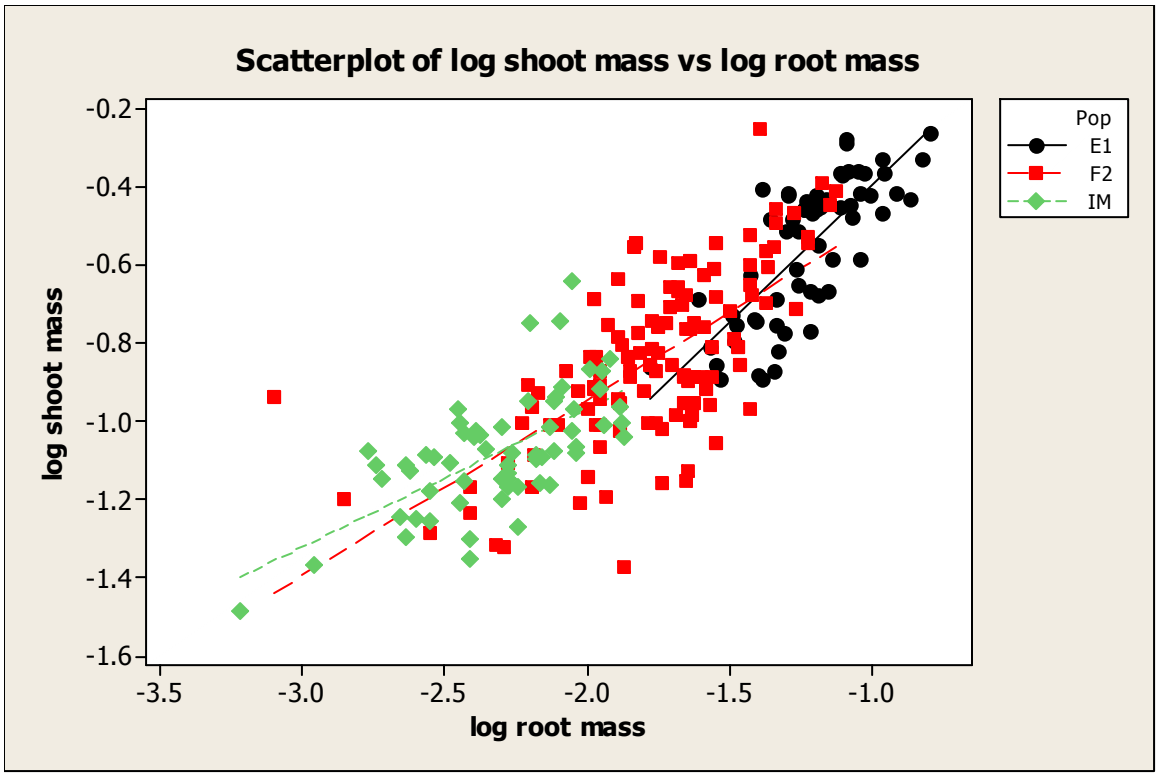
Appendix F: Effects of drought stress on root:shoot ratios and development time



GLM ANOVA

Source	DF	SS	MS	F	P
Water?	1	1.7400	1.7400	68.60	**<0.001**
Pop	2	6.8676	3.4338	135.38	**<0.001**
Water?*Pop	2	0.8133	0.4067	16.03	**<0.001**
Error	250	6.3411	0.0254		
Total	255				

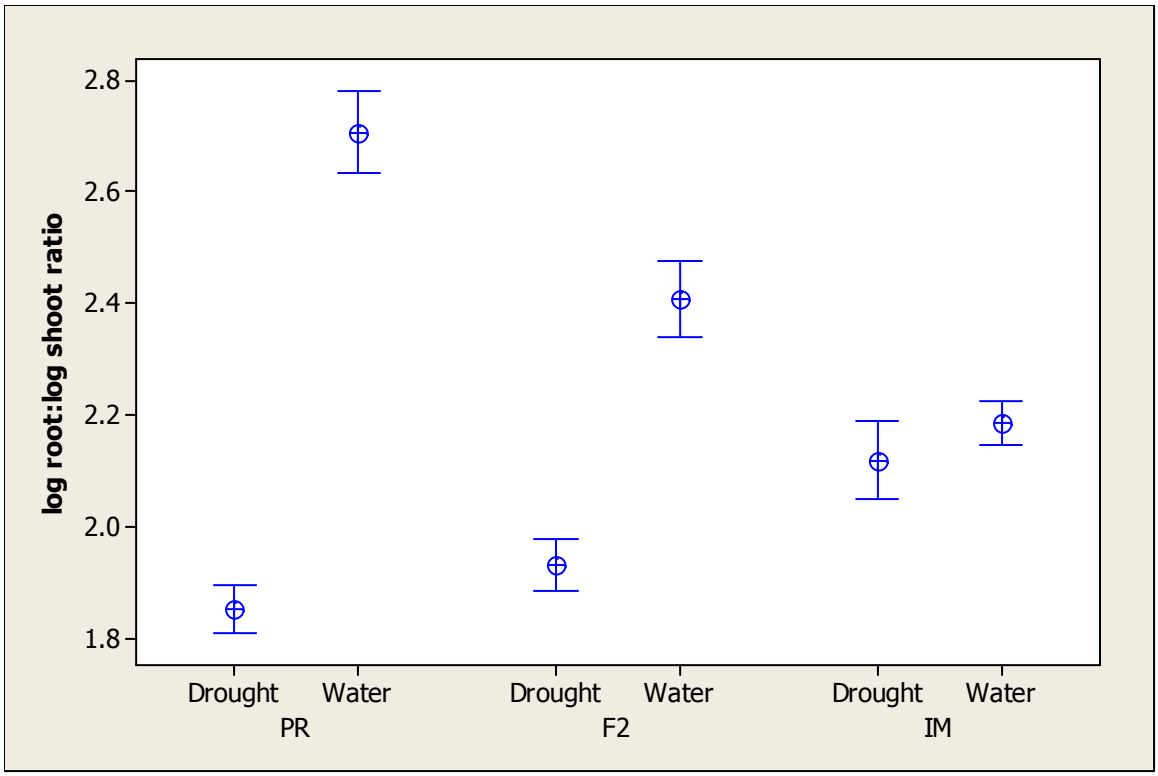
Clear direct effects of both population and treatment, plus differential response of populations to drought.



Source	DF	SS	MS	F	P
Pop	2	0.2117	0.1058	5.22	0.006
log root mass	1	3.1984	3.1984	157.65	0.000
Pop*log root mass	2	0.1931	0.0965	4.76	0.009
Error	238	4.8284	0.0203		
Total	243				

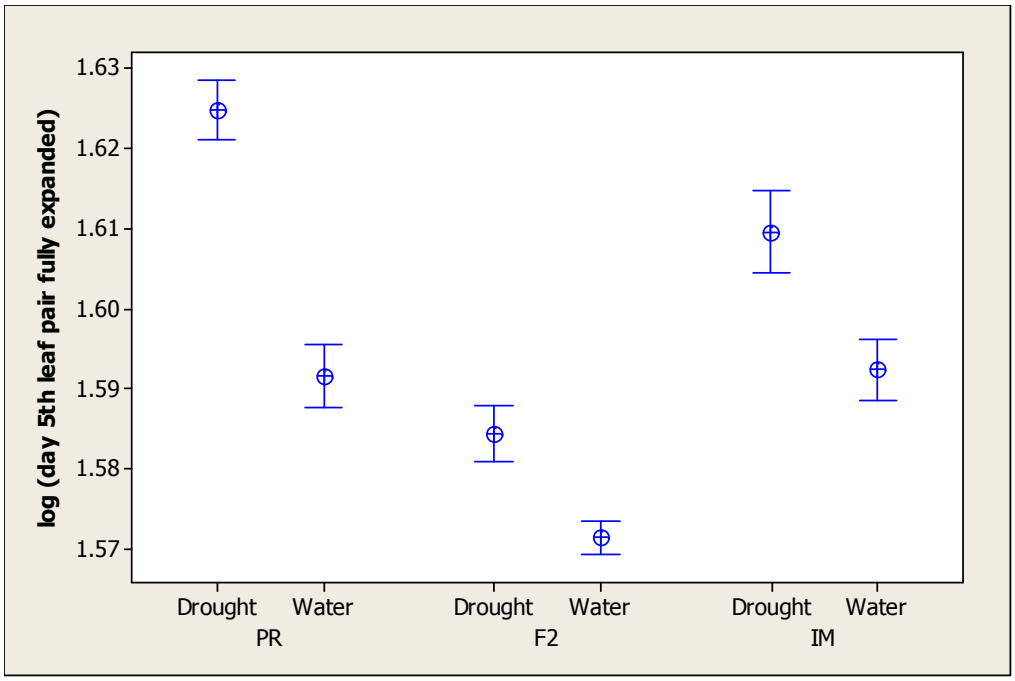
S = 0.142434 R-Sq = 72.74% R-Sq(adj) = 72.16%

Root:shoot ratio differs among populations in well-watered conditions
E1 in legend is PR population



Source	DF	SS	MS	F	P
Pop	2	0.5968	0.2984	1.82	0.164
Water?	1	10.8726	10.8726	66.32	0.000
Pop*Water?	2	4.2397	2.1199	12.93	0.000
Error	238	39.0191	0.1639		
Total	243				

Drought decreases root:shoot ratio, although not significantly within IM population



Source	DF	SS	MS	F	P
Water?	1	0.024013	0.024013	47.17	0.000
Pop	2	0.046211	0.023106	45.38	0.000
Water?*Pop	2	0.004424	0.002212	4.35	0.014
Error	253	0.128804	0.000509		
Total	258				

S = 0.0225634 R-Sq = 34.41% R-Sq(adj) = 33.11%

Drought stress delays shoot growth, and F2 plants demonstrate accelerated shoot development relative to PR and IM populations in normal and drought conditions