

The Genetic Basis of Local Adaptation to Serpentine Soils in *Mimulus guttatus*

by

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Dissertation submitted in partial fulfillment  
of the requirements for the degree of  
Doctor of Philosophy in the Department  
of Biology in the Graduate School of  
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ABSTRACT

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## Abstract

While local adaptation has been frequently demonstrated via reciprocal transplant experiments, our understanding of the genetic basis of it remains minimal. There is a notable lack of studies that identify naturally segregating variants, determine the traits controlled by these variants and characterize their fitness effects in the field. Such studies are critical for understanding how spatially varying selective pressures can drive population divergence and maintain genetic variation. The experiments presented here aim to characterize the genetic basis of local adaptation to serpentine soils in *Mimulus guttatus*. First, I show that serpentine and non-serpentine populations of *M. guttatus* are locally adapted to soil habitat wherein non-serpentine plants are unable to survive on serpentine soils. Serpentine tolerance appears to come at a cost as serpentine plants are smaller in the juvenile stage than non-serpentine plants when grown at non-serpentine field sites. These size differences may limit the competitive ability of serpentine tolerant plants in non-serpentine habitats which tend to be more heavily vegetated than serpentine habitats. Next I identify environmental variables that are important selective agents in the serpentine habitat. Using hydroponic assays to isolate an individual chemical variable of serpentine soils – low calcium levels to high magnesium levels (low Ca:Mg ratio) - I show that serpentine and non-serpentine populations of *M. guttatus* have significant differences in tolerance to low Ca:Mg. I then

characterize the genetic basis of these ecotypic differences in survival and tolerance using quantitative trait locus (QTL) mapping. I identify a single, major QTL that controls both the ability to survive on serpentine soils and tolerance to low Ca:Mg ratio which suggests that *M. guttatus* populations have adapted to serpentine soils through an ability to tolerate the low levels of Ca while simultaneously not suffering from Mg toxicity. Furthermore, I show that this same QTL controls ability to survive on serpentine soils in a second, geographically distant population. However, preliminary work suggests that the two populations are not equally tolerant to each other's soils indicating that either other loci also contribute to serpentine tolerance and these are not shared between the two serpentine populations or that there are different serpentine tolerance alleles at the major QTL are not functionally equivalent. This work addresses long-standing questions in evolutionary biology regarding the number and effect size of loci that underlie adaptive traits by identifying a large effect locus that contributes to adaptive differences between *M. guttatus* populations.

## **Dedication**

To Russell Jones.

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# 1. Local adaptation of *Mimulus guttatus* to serpentine soils

## 1.1 Introduction

Natural landscapes are highly heterogeneous, resulting in selection pressures that differ between habitats. Such divergent selection drives population differentiation, can maintain genetic variation and ultimately promote speciation (Hedrick 1986, Gillespie and Turelli 1989, Schluter and Conte 2009). Local adaptation is a type of genotype x environment (GxE) interaction where adaptation to one habitat reduces fitness in alternate habitats. However, spatially varying selection does not always lead to genetic differentiation of populations – phenotypic plasticity, limited genetic variation and the homogenizing effects of gene flow can all impede the formation of locally adapted populations. However, recent meta-analyses of studies testing for local adaptation using reciprocal transplant experiments find that nearly 70% of the studies demonstrate home-site fitness advantages (Leimu and Fischer 2008, Hereford 2009). Since the pioneering work of Clausen, Keck and Heisey, wild plant species have proved particularly attractive systems for studies of organism-environment interactions as they often exhibit local adaptation of populations to environmental heterogeneity across their geographic ranges (Clausen et al. 1940, Clausen et al. 1948, Leimu and Fischer 2008).

While local adaptation is frequently demonstrated in field-based reciprocal transplant experiments, often these studies do not enable identification of the traits that

underlie fitness differences in the alternate habitats nor the environmental variables that are important selective agents. Covariance between phenotypic traits and environmental variables may suggest that certain traits are involved in local adaptation; however, manipulative experiments are important for establishing a relationship between specific variables and adaptive traits. In order to understand how natural selection promotes and maintains population divergence it is necessary to identify the targets of selection. Hybrid-mapping populations can be used to disentangle correlations among traits as well as to measure selection against hybrids in alternate habitats in order to investigate the dynamics between the relative strength of selection versus the homogenizing effects of migration.

Some of the most striking examples of the power of natural selection to shape biological diversity involve local adaptation of plants to extreme soil environments such as mine tailings, saline, acidic and serpentine soils (Antonovics 1975, Linhart and Grant 1996, Brady et al. 2005). Due to their unique ionic compositions, these habitats are toxic to most plant species; however, it is rare to find soil that is completely devoid of plant life and there are species that have adapted to and thrive in all of these harsh soil habitats. Evolutionary ecologists have studied plant adaptation to extreme soils for decades and this classic work provides some of the best examples of “natural selection in action” (Antonovics et al. 1971) where local adaptation to mine tailings has been shown to evolve rapidly and plants can be locally adapted over a scale of meters despite

substantial gene flow (Antonovics and Bradshaw 1970, Macnair 1987, Al-Hiyaly et al. 1993). Adaptation to edaphic differences provides an ideal system for investigating patterns of local adaptation in the face of gene flow as well as for determining the environmental variables that are important selective agents and the traits that are under selection. Transitions between soil types are often abrupt, resulting in an ecological discontinuity with potentially strong selection over very short distances. Because of the small spatial scale, variables other than soil characteristics are likely to be minimally variable and the chemical composition of the different soil types is relatively easy to characterize.

Serpentine soils pose a unique set of ecological and physiological challenges to plants, resulting in highly distinctive and variable plant communities with high levels of endemism. These soils are formed by the weathering of ultramafic rocks that originate in the earth's mantle, break off at subduction zones and become exposed on the earth's surface. In western North America, serpentine soils are widely distributed, stretching from the Baja peninsula to Alaska, but typically occurring in relatively small and isolated patches (Kruckeberg 1984, Alexander et al. 2007). This patchy distribution means that populations on alternate soil habitats often occur in close proximity, and if populations are locally adapted to soil habitat this genetic differentiation has likely occurred in the face of gene flow. Serpentine soils are characterized by very low levels of the essential plant nutrients calcium (Ca), nitrogen (N), potassium (K), and

phosphorus (P) and have significantly elevated levels of magnesium (Mg) and heavy metals [nickel (Ni), cobalt (Co), chromium (Cr) and iron (Fe)]. However, there is substantial variation among serpentine soils in their chemical and physical properties due to differences in the primary mineralogical composition of parent materials, degree and conditions of metamorphic alteration and extent of weathering (Whittaker 1954, Proctor et al. 1975, Kruckeberg 1984, Alexander *et al.* 2007). Given the patchy distribution and variation in physiochemical properties of serpentine soils it is possible that widespread species have repeatedly adapted to different serpentine patches. Serpentine populations may be locally adapted to the soil conditions in their home patch rather than broadly tolerant of all serpentine soils; however, the effects of variation in soil properties between different serpentine patches on plant fitness has not been investigated.

The unique chemical composition of serpentine soils is toxic to most plant species resulting in depauperate habitats (Figure 1) which possess distinctive vegetative communities including a large number of endemic species. Serpentine endemism has long fascinated plant biologists and many of the studies of adaptation to serpentine soils have involved these endemic species. However, serpentine endemics may be reproductively isolated from their non-serpentine sister taxa, preventing genetic analysis of differences in the ability to tolerate serpentine soils. Furthermore, many endemic species have highly restricted ranges on just a few serpentine patches, making them ill-

suited for studies of parallel adaptation. In contrast, some plant species have populations that occur on both serpentine and non-serpentine soils and those with widespread distributions provide ideal models for studying local adaptation and parallel evolution.

Local adaptation of populations to serpentine habitats has frequently been demonstrated via reciprocal transplant and common garden experiments (reviewed in O'Dell and Rajakaruna 2011). Local adaptation is commonly evaluated by growing plants in controlled conditions in field-collected serpentine soils, and such experiments typically reveal that non-serpentine populations display higher mortality or greatly reduced growth compared to serpentine populations (e.g. *Achillea millefolium*, *Leptosiphon parviflorus*, *Gilia capitata*, *Phacelia californica*) (Kruckeberg 1950, O'Dell 2008, Kay et al. 2011). Field-based reciprocal transplant experiments between adjacent serpentine and non-serpentine populations are the best test for local adaptation, and the results from the modest number of such studies indicate strong genetic differentiation in the ability of plants to survive on serpentine soils (Kruckeberg 1950, Kruckeberg 1967, Jurjavcic et al. 2002, Sambatti and Rice 2006, Wright et al. 2006, Hufford et al. 2008). However, costs of serpentine tolerance in normal soil are not often found perhaps because if such costs involve a loss of competitive ability they may be difficult to detect. These studies demonstrate that plant species with serpentine and non-serpentine ecotypes are commonly locally adapted to soil habitat; however, in no instance have the

survival differences demonstrated in the field at serpentine sites been linked to individual traits that underlie these survival differences.

Many studies testing for intraspecific differences between serpentine and non-serpentine populations focus exclusively on identifying specific tolerance traits in hydroponic culture that are presumed to be adaptive but rarely tested in the field. Typically these studies use some measure of plant growth scored in a hydroponic culture that isolates a single chemical characteristic of serpentine soil (i.e. low Ca, high Mg, low Ca:Mg or high Ni). These experiments often find that serpentine populations are primarily adapted to low Ca and a low Ca:Mg ratio (reviewed in Brooks 1987, Brady et al. 2005). However, in some species serpentine adaptation clearly involves tolerance to high Mg (Proctor 1970) or Ni (Gabbrielli et al. 1990, Burrell et al. 2012). Caution is needed in interpreting hydroponic studies because differences in hydroponic “tolerance” can be difficult to assay (Baker 1987, Macnair 1993) and experiments may fail to replicate the complex interactions between different ions in the soil environment. Mg, Ca and Ni are all +2 cations and studies have revealed that differing concentrations of each affect the availability of the other ions to plants (Gabbrielli and Pandolfini 1984, Brooks 1987). Failure to detect differences in tolerance in hydroponic culture between serpentine and non-serpentine populations (Gardner 2000, Murren et al. 2006) does not necessarily indicate that populations are not locally adapted to serpentine habitats.

The experiments presented here aim to determine 1) whether *M. guttatus* is locally adapted to serpentine soils; 2) what environmental variables in serpentine soils are important selective agents; and 3) whether different populations of *M. guttatus* from distinct serpentine patches are equally tolerant to each other's soil or if they are locally adapted to the characteristics of their particular soil patch. In order to address these questions I initiated three separate reciprocal transplant studies in the field to test for local adaptation as well as used hydroponic assays to test for differences in tolerance to low Ca:Mg ratio between three focal serpentine and non-serpentine populations pairs. In a lab-based common-garden I measure survival differences between two different serpentine populations when grown in their home soil versus the alternate serpentine soil type. Finally, I include F1 and F2 hybrids from these population pairs in the field, hydroponic, and common-garden experiments in order to measure hybrid fitness and begin to characterize the genetic basis of serpentine tolerance.

## **1.2 Methods**

The wildflower genus *Mimulus* (Phrymaceae) has been the subject of intensive ecological and evolutionary genetic research for over 60 years. The *Mimulus guttatus* species complex is a group of closely related species that occur in western North America from the Pacific to the Rockies (Vickery 1978) and show tremendous ecological diversity along with attributes of a true genetic model system. *M. guttatus* is an outcrossing annual that is typically pollinated by *Apis mellifera*, *Bombus* spp. (Apidae)

and *Dialictus* spp. (Halictidae) (Gardner and Macnair, 2000). *M. guttatus* is well suited to both field and lab-based studies (Wu et al. 2007) because of its short generation time (2-3 months), small size (many grown in limited space), high fecundity (100-400 seeds per cross), and reproductive flexibility (clonal propagation and self-fertile). *M. guttatus* populations can be found from sea level to elevations over 10,000 feet, in grasslands, forests, desert streams and basalt cliffs, peat bogs, alpine meadows and seeps, coastal cliffs and sand dunes, and toxic copper mine tailings.

*M. guttatus* also occurs on serpentine soils across much of its range. The experiments described here focus on several on/off serpentine population pairs from two different regions in California: 1) the Northern Coast Range including Lake, Napa and Sonoma counties and 2) the Sierra foothills in Tuolumne and Mariposa counties (Figure 2). These two regions are located ~300km apart separated by the Central Valley of California where no serpentine soils occur (Alexander *et al.* 2007). I collected seeds or plants from 9 annual serpentine and 6 annual non-serpentine populations (Table 1). Each serpentine population is from a distinct serpentine patch except for REM and DHR which are both from the McLaughlin Reserve. All populations from the Sierra foothills region are located within ~40km of each other with the non-serpentine populations ranging from ~7km to 20km from the nearest serpentine population. The populations from the Northern Coast Range are spread out over a larger area (all within ~80km of

each other) and the non-serpentine populations ranging from ~4km to 40km distance from the nearest serpentine population.

### **1.2.1 Reciprocal transplant studies**

A total of three reciprocal transplant studies were conducted in order to test for local adaptation of *M. guttatus* populations to serpentine soils and to measure the fitness of hybrid individuals in field habitats. In 2010, two reciprocal transplants were carried out at sites in each of the focal regions of California – the Donald and Sylvia McLaughlin Natural Reserve (McL) in in the Northern Coast Range and at the Red Hills (RH) area managed by the Bureau of Land Management in the foothills of the Sierra Nevada (Figure 2). In 2012, a third reciprocal transplant study was conducted at the McL sites. By replicating the reciprocal transplants at different sites and across years as well as using multiple serpentine and non-serpentine populations in each experiment, this set-up allows me to test for local adaptation to the serpentine habitat as opposed to highly local characteristics of a particular site or year.

#### **1.2.1.1 Reciprocal transplant experiments 2010**

Seeds from two to four local (i.e. Northern Coast Range or Sierra foothills) serpentine and non-serpentine populations along with F1s and F2s were germinated on potting soil (Fafard 3B) outside at the McLaughlin Reserve in late January 2010. Seeds were derived from field collected lines that had been grown in the greenhouse one generation to reduce maternal effects and crossed with a second line from the same

population. One to two independent, outbred full-sibling families were planted from each population (Table 1 – all populations except DCM, OAE and STO). For two populations from the Northern Coast Range (REM and SOD) the only seed collections available were a single inbred line (5 generations inbred) from each population. Replicates from these inbred lines were included in the study because they are the parents of the F1s and F2s planted at the McL sites.

At McL I established four gardens – 2 serpentine and 2 non-serpentine – that were within ~8km of each other. However, one of the non-serpentine gardens was lost within a month of transplanting after many seedlings were washed away by heavy rains and is therefore not discussed further. At all McL field sites, 15 to 35 individuals from each family, 55 F1s and 500 F2s were planted (n=800 per site; see Table 4 for details on replication). F1s were produced by crossing inbred lines: the serpentine parent (REM) was derived from seeds collected from the *M. guttatus* population at one of the serpentine field sites and the non-serpentine parent (SOD) is from Napa county (~50km away). F2s were generated by selfing the F1. In the RH, I established three gardens – 2 serpentine and 1 non-serpentine (Figure 1) – where the serpentine sites were within 1km of each other and the non-serpentine site was ~20km away. At each RH site 20-25 individuals from each family, 40 F1s and 250 F2s were planted (n=600 per site; see Table 4 for details on replication). Two independent F1s were derived by crossing two different pairs of inbred lines from the local RH serpentine population (SLP) and a

nearby (~20km apart) non-serpentine population (KFY). These two F1s were then crossed in order to generate outbred F2s.

Seedlings were transplanted as cotyledons with bare roots over the course of 5 days in mid-Feb and marked with toothpicks. They were planted in small plots (4 x 3 seedlings, ~ 7.5cm x 5 cm) that were blocked together (6 blocks/site) with blocks were scattered throughout the native *M. guttatus* populations at each site. Native seedlings (primarily *M. guttatus*) within 2cm of transplants were removed with forceps.

Transplants were scored for survival 3 weeks after transplanting and then weekly thereafter. I measured rosette diameter at 7 weeks as well as scored flowering time and plant height and the length of the 1<sup>st</sup> true leaf at flowering. Due to restrictions in both regions, I was not allowed to let plants actually flower in the field. So, when the first plants in each region developed large buds, I began censusing plots every three days and recorded “flowering” as any plant with large buds that would likely flower before the next census. Any plants recorded as flowering were then dug up from field plots and measured for plant height and the length of the 1<sup>st</sup> true leaf (plants from the RH serpentine sites were not measured for these traits). F2 plants that were removed from field plots were re-potted in potting soil after measurement in order to collect tissue for DNA (results presented in Chapter 2). One of the serpentine sites at McL dried out rapidly and in an attempt to rescue as many F2s as possible for tissue collection they were removed from field plots in mid-May but all other classes of plants remained in the

plots. The experiments were terminated at the end of May (RH sites) and early June (McL) when nearly all plants had flowered or died (>90% at all sites except for one where nearly 50% of plants still alive had not flowered). All plants that had not flowered but were still alive were included in the survival analysis. Differences in survival between serpentine, non-serpentine and hybrid classes of plants in each planting habitat were analyzed using G-tests for independence. To determine whether there is overall multivariate morphological divergence between serpentine and non-serpentine plants growing at non-serpentine sites, a nested multivariate analysis of variance (MANOVA) was implemented in JMP 10.0 with habitat of origin, population (nested within habitat of origin), block and plot (nested within block) as factors and rosette diameter, plant height and leaf length as the dependent variables. Rosette diameter was also analyzed separately using one-way analysis of variance (ANOVA) as not all individuals flowered and were therefore measured for height and leaf length.

#### **1.2.1.2 Reciprocal transplant experiment 2012**

A third reciprocal transplant study was conducted in the spring of 2012 at the McLaughlin Reserve. Seeds from local serpentine and non-serpentine populations (DCM, GUA, GUG, REM, SOD and STO; Table 1) were germinated outside at McL in late January. The seeds planted represented pools of equal numbers of seeds collected from 20 field plants/population. F3s derived by reciprocally crossing two F2s from the same McL mapping population (serpentine parent = REM; non-serpentine parent = SOD)

as used above were also randomized within field plots. Seeds resulting from crosses between 120 pairs of F2s were pooled in equal numbers (~100 seeds/F2) and planted in trays filled with potting soil. Cotyledons were transplanted bare root into plots cleared of native vegetation at the same field sites (2 serpentine, 1 non-serpentine) used in 2010 over a week in mid-February. Due to low germination rates, the number of replicates transplanted for each population was highly variable (9-56 seedlings transplanted/population/site; F3: n=870/site; see Table 4 for details on replication). Thirty plots of 10x8 seedlings (~35x45cm) were haphazardly scattered throughout the native *M. guttatus* populations. 2012 was a dramatically drier year than 2010 and all of the seedlings at one of the serpentine sites died between transplanting and the first census 3 weeks later. For the remaining two sites, survival was recorded at 3 and 9 weeks post transplanting and rosette diameter was measured at both census dates; however, as some plants had begun to bolt by the week 9 census so only rosette measurements from March are included for analysis. Survival and rosette diameter differences between serpentine, non-serpentine and F3 plant classes were analyzed as above. Plants were removed from the field in mid-April after the 9 week census when most transplants were either rosettes or had recently bolted. This experiment was concluded nearly 6 weeks earlier than the experiments conducted in 2010 due to travel logistics as well as the fact the 2012 was a drier season than 2010 and I hoped to rescue as

many F3s as possible for genotyping. F3 plants were shipped back to Duke, transplanted to potting soil (Fafard 4p) and placed in the Duke University Greenhouse.

### **1.2.2 Common garden experiments on native serpentine soil**

In order to test the effects of serpentine soil in isolation, I conducted a lab-based common-garden experiment with inbred lines from a single on/off population pair from each focal region (Northern Coast Range = REM/SOD and Sierra foothills = SLP/TUL; Table 1) planted on field collected serpentine soil. In both pairs the serpentine population was native to the location of the reciprocal transplant experiments described above (McL in the Northern Coast Range and RH in the Sierras). I also included F2s derived by crossing each population pair and selfing the F1s. The F1s for the McL pair were also included in the study but there was not enough F1 seed for the RH pair to include. All classes of plants (parents, F1s and F2s) were planted on serpentine soil from the home sites of each of the serpentine populations (REM from the McLaughlin Reserve and SLP from the Red Hills) to test whether the serpentine populations are tolerant of soils from different serpentine localities. Serpentine soil was collected in the field, sifted through a 2mm metal screen to remove rocks, and autoclaved. The RH soil samples were collected from the rhizosphere of *M. guttatus* plants and represent pooled samples from several collection points throughout the population. The McL soil was collected from a road cut ~0.8km away from the home site of the serpentine parent. Soil was collected from this location as it has minimal vegetative cover and few native *Mimulus*

seeds. I bulked soil from several collection locations throughout the site. The same soil was used by Palm et al. (2012) and they show that it is not significantly different in chemical composition from the soil at the parental site.

Seeds from inbred lines of each population – McL (REM/SOD) and RH (SLP/TUL) – as well as the F1s and F2s were planted on 60x15mm petri plates, covered with ultrapure water (Nanopure Diamond purified) and stratified in the dark at 4°C for 5 days. Plates were then moved to a growth chamber and as soon as radicles were visible (~2days), germinants were transplanted using a pipette with a cut tip to petri plates filled with serpentine soil. Twelve seeds were planted per plate with 4 – 40 replicate plates per plant class (see Table 7 for details on replication). For the RH serpentine parental line (SLP) I had a small number of seeds and was only able to plant two plates (24 seeds) on RH soil at the same time as the F2s and non-serpentine line; however, I had previously planted the SLP line on RH soil using an identical set-up and the results from both experiments are combined and presented here. Plates were moved to a growth chamber and grown under short days (12 hours light) with daytime temperatures of 22°C and nighttime temperatures of 18°C. Seedlings were watered daily with ultrapure H<sub>2</sub>O and survival was scored weekly for five weeks. To test whether the proportions surviving between serpentine and non-serpentine lines from each population pair were the same I used Fisher's exact test of independence on the overall counts of "dead" versus "alive." To determine if parentals, F1s and F2s differ in

mean percent survival (using percent survival per plate as the unit of replication) on each of the two treatment soils and to test whether there is interaction between plant class and soil treatment, I conducted a two-way ANOVA. The non-serpentine lines were excluded from these analyses as they had zero survivors and no variance in this trait.

### **1.2.3 Soil analysis**

I collected soil samples from several serpentine (n=6) and non-serpentine (n=5) populations in both the Northern Coast Range and Sierra foothills regions. The serpentine samples included both McL and RH field locations as well as other populations planted in the field experiments. I only had a limited number of soil samples from non-serpentine populations; however, other lab members have analyzed soil from nearby non-serpentine populations using identical methodology and that data is included here. Soil samples were collected from the rhizosphere of *M. guttatus* plants in 2008. Soil samples represented bulks of equal volumes of soil from haphazardly chosen collection sites throughout *M. guttatus* populations. One to two bulk samples per population were air dried, sifted with a 2mm sieve and sent to the Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory where they were analyzed using the Mehlich III weak acid extraction for exchangeable base cations (Ca, Mg, Na, K, P) and some samples were also analyzed for micronutrient levels (Cu, Zn, Mn, Fe) (Sen Tran and Simard 1993). To summarize potentially important axes of soil variation, soil

data from the different populations were subjected to principal component analysis in JMP 10.0 on the eight soil variables that were characterized for all samples (pH, N, P, K, Ca, Mg, S and Na).

#### **1.2.4 Hydroponic assays for differences in tolerance between serpentine and non-serpentine plants to low Ca:Mg ratios**

In order to determine which characteristics of serpentine soils are important selective agents, our lab developed a high-throughput hydroponic platform to assay plant tolerance to individual soil chemical variables (in this case low Ca:Mg ratio). Tolerance is not a trait such as flower size which can be measured against an objective standard; rather, it is manifested as a differential response to the treatment solution. In other words, tolerant genotypes will be less affected by the treatment solution than non-tolerant genotypes. However, this differential response needs to be detected in some other trait which is itself subject to genetic and environmental variation. Typically some plant growth parameter (height, biomass, etc.) is used but one must control for inherent differences in growth. A common approach is to score plants for their tolerance index (TI):

$$TI = \frac{\textit{Growth in treatment environment}}{\textit{Growth in control environment}}$$

A limitation of this method is that it requires one to grow genetically identical individuals in both treatment and control solutions. Typically clones have been used for

this purpose; however, using clones taken from large, robust plants which have already acclimated to a benign growth environment fails to mimic how plants would experience these soils with altered nutrient profiles in the field. The method our lab developed is performed at the seedling stage and is based on the sequential testing method of Shat and ten Bookum (1992). Our approach tests single genotypes in increasingly severe treatments and scores root growth rate in each treatment level. For each individual the treatment concentration that stops root growth or “Effective Concentration 100%” (EC100) is scored.

We built watertight boxes out of PVC board (11.5” x 5” x 7.5”) with removable lids that have holes (4 rows of 17) in them through which drinking straws are suspended down into the solution (Figure 3). Seeds are sown on an inert rockwool media stuffed into PCR tubes with the tips clipped off. The tubes are placed into the holes in the box lids, seeds germinate and grow in a nutrient solution ( $\frac{1}{4}$  strength Hoagland’s, prepared as described by Epstein (1972)) until most seedlings have roots visible out the bottom of the rockwool (~7days). The position of the root tip is marked by sliding a small rubber band around the straw and treatments are initiated. Every two days, the position of the root tip is marked and the treatment solution is changed (Table 2). At the end of the series of treatments, the distance between the rubber bands is measured providing root growth rates in each treatment level from which EC100 is calculated for each individual.

Using this set-up, I conducted two separate experiments on two different on/off serpentine population pairs – the REM/SOD pair from McL and STO/OAE from Sonoma (Table 1) – to test for differences in degree of tolerance to a low Ca:Mg ratio growth environment between serpentine and non-serpentine plants. Replicates of a single inbred line from each population along with F1s and F2s (see Table 8 for sample sizes) from each population pair were planted on rockwool media in PCR tubes, stratified at 4°C for 5 days then moved to a growth room, saturated with ¼ strength Hoagland’s and allowed to germinate. The McL experiment was conducted over two separate temporal blocks. Multiple seeds were planted on each rockwool plug and seedlings were thinned to one per plug after germination and then randomized within the boxes which were filled with a ¼ strength Hoagland’s solution. Once root tips were visible out the bottom of the rockwool plugs low Ca:Mg treatments were started. All solutions were prepared with deionized water as described in Bradshaw (2005). In order to vary the concentrations of calcium and magnesium ions while keeping nitrate concentration and osmotic strength constant, calcium nitrate and magnesium nitrate were mixed in different proportions to sum to 4 mmol l<sup>-1</sup> (Table 2). Plants were censused every two days, the position of the root tip marked and then the treatment solution changed. After the final treatment all plants were put back into ¼ strength Hoagland’s solution and later transplanted to potting soil and moved to the greenhouse in order to collect tissue for DNA. To see whether there were differences in tolerance to low Ca:Mg ratio

between the serpentine and non-serpentine parental lines, I conducted one-way ANOVA using EC100 as the dependent variable and controlling for box effects.

The Red Hills population pair (SLP/KFY) was assayed for tolerance to low Ca:Mg ratio during the development of the hydroponic set-up described above. Only 5 replicates from each line were tested for tolerance to low Ca:Mg ratio in a set-up that was slightly different from above. Seeds were planted on rockwool stuffed into the wells of 96-well PCR plates which had the bottoms trimmed off. The plates floated in Tupperware containers which were filled with the same series of treatments described above. Plates were removed from solution and the roots were photographed at each census date. Root length in each treatment level was measured in ImageJ (Abràmoff et al. 2004) and EC100 was calculated for each individual. This set-up required alteration because during censusing the roots became entangled when removed from the solution, resulting in not only an overly lengthy process but one that could cause damage to the roots during separation.

### **1.2.5 Shoot elemental composition analysis**

To test for constitutive differences in calcium and magnesium uptake among *M. guttatus* serpentine and non-serpentine populations, I analyzed leaf elemental composition of plants grown in a nutrient solution. Seeds from inbred lines of six populations (3 serpentine – STO, GUG and SLP; 3 non-serpentine – OAE, MCC, MED) from both regions were planted on Fafard 4p potting soil, stratified for 5 days at 4°C,

allowed to germinate and then 10 individuals per line were transplanted as cotyledons to 2.5 inch pots filled with perlite. Pots were randomized across trays (32 pots/tray) and flooded with a ¼ strength Hoagland's solution (Ca:Mg =4; Table 2) such that the level of solution was ~1cm below the top surface of the perlite. The solution was changed every three days to maintain constant nutrient availability. To prevent plants from flowering, which could alter their physiology, they were grown under short days (8 hours light) in a growth chamber with daytime temperatures of 22°C and 16 hour periods of dark at 18°C. After 4 weeks, the second pair of true leaves from each plant was collected with plastic forceps, briefly submerged in 0.05% Triton, and rinsed in DI water. Leaves from two replicates per line were bulked into a single 15 mL plastic tube (VWR International) and dried in an incubator at 90°C for 24 hours. Samples were shipped to the Purdue Ionomics lab (<http://www.ionomicshub.org/home/PiiMS>) for elemental profiling using inductively coupled plasma mass spectrometry (ICP-MS) (Baxter 2007). Briefly, about 10 mg of dried leaf tissue is digested with concentrated HNO<sub>3</sub> (Mallinckrodt, AR Select grade) at 110°C for 4 hours. Each sample was diluted to 10.0 ml with 18 MΩ water and analyzed on a PerkinElmer Elan DRCe ICP-MS using a concentric nebulizer and cyclonic spray chamber (Glass Expansion). Differences in leaf Ca and Mg levels (measured in μg g<sup>-1</sup> or parts per million (ppm)) between serpentine and non-serpentine plants were analyzed using a nested ANOVA with population nested within habitat of origin. Leaf

Ca ppm was log transformed to fit assumptions of normality. All analyses were conducted in JMP 10.0.

## **1.3 Results**

### **1.3.1 Reciprocal transplant studies**

In both regions (McLaughlin and Red Hills) and in both years (2010 and 2012), there were significant survival differences between serpentine and non-serpentine plants at the serpentine field sites (Figure 4, Table 3). Non-serpentine plants exhibited signs of stress when planted at the serpentine sites (Figure 1) and had extremely low survivorship (~1.5% all experiments) while the serpentine plants enjoyed high survival rates (McL2010 = 28.7%; RH2010 = 20.8%; McL2012 = 44.8%; Figure 4, Table 3). There were no significant differences in survival between serpentine and non-serpentine plants at the non-serpentine field sites (Figure 4, Table 3).

Within each habitat of origin there was variation between the different populations in percent survival in each of the field habitats (Table 4). For nearly all populations there were significant differences in their percent survival in the serpentine versus non-serpentine field plots (Table 4). The exceptions were several serpentine populations (GUG, REM and SLP in 2010) that had similar survival rates in both habitats. At McL in 2010 there were significant differences in survival between the serpentine populations at the serpentine field sites ( $G_3 = 8.089$ ,  $p = 0.044$ ) where the GUG population from Napa county enjoyed the highest survival of the four populations. The

REM population which is native to one of the serpentine field sites also enjoyed high survival at the McL serpentine field sites; however, the DHR population which is from the same serpentine patch as REM (~0.5km away) had the lowest survival. At the RH serpentine sites there were significant differences between the serpentine populations ( $G_2 = 7.856$ ,  $p = 0.02$ ) where the SLP population which is native to the RH serpentine locality had the highest survival. Similarly there were significant differences between the serpentine populations at the serpentine sites at McL in 2012 ( $G_3 = 31.093$ ,  $p < 0.0001$ ) where the STO population from Sonoma county had no survivors while the GUG population from Napa county had 100% survival. However, these populations had low numbers of replicates ( $n = 11$  and  $9$  respectively). There were no significant differences in survival between the non-serpentine populations planted at the serpentine sites for any of the three experiments (**McL2010**:  $G_1 = 1.581$ ,  $p = 0.209$ ; **RH2010**:  $G_2 = 0.342$ ,  $p = 0.843$ ; **McL2012**:  $G_1 = 0.638$ ,  $p = 0.409$ ). At the non-serpentine sites there were no differences in survival between the populations within each habitat of origin nor between habitats of origin (Tables 3 and 4, Figure 4) except at McL in 2012 where there were significant survival differences between the serpentine populations at the non-serpentine site ( $G_2 = 11.998$ ,  $p = 0.0025$ ) where the GUG population had no survivors though again it had very few replicates ( $n = 11$ ).

The 2010 reciprocal transplant experiments at both McL and RH included F1s derived from crosses between a local on/off serpentine population pair (McL =

REM/SOD; RH = SLP/KFY) and in both experiments the F1s had survival rates that were not significantly different from the serpentine populations (**McL2010**:  $G_1=0.117$ ,  $p=0.732$ ; **RH2010**:  $G_1=2.09$ ,  $p=0.148$ ). In all three reciprocal transplant experiments, the F2/F3 generation of hybrids had survival rates that were significantly lower (~50% lower in all cases) than the serpentine plants (**McL2010**:  $G_1=45.89$ ,  $p<0.001$ ; **RH2010**:  $G_1=23.71$ ,  $p<0.001$ ; **McL2012**:  $G_1=13.63$ ,  $p<0.001$ ). There were no significant differences in survival between any of the plant classes at the non-serpentine sites in either experiment in 2010 (Figure 4) however in 2012 the F3s had higher survival than the serpentine and non-serpentine plants ( $G_2=17.99$ ,  $p<0.001$ ).

While there were no survival differences between serpentine and non-serpentine plants when growing at the non-serpentine sites, there were significant differences in plant size at the RH site (Table 5) wherein plants from non-serpentine populations were larger than plants from serpentine populations. The MANOVA analysis show significant differences between serpentine and non-serpentine plants for rosette diameter, plant height and leaf length at the RH sites but no significant differences in these morphological traits at the McL non-serpentine site (Table 6). Separate one-way ANOVAs were conducted on rosette diameter because not all individuals flowered in the field and therefore were not measured for height or leaf length. These analyses again show that non-serpentine plants were larger in the juvenile stage than serpentine plants at the RH non-serpentine site ( $F_{1,190}=1.69$ ,  $p<0.006$ ; Table 5). There were no

significant differences in rosette diameter at the McL non-serpentine sites in either year (**McL2010**:  $F_{1,178}=0.57$ ,  $p=0.45$ ; **McL2012**:  $F_{1,73}=0.1712$ ,  $p=0.681$ ; Table 5). There were no significant differences in days to flower between serpentine and non-serpentine plants at any sites in any year nor were there differences between the planting habitats (Table 5). The manner in which flowering time was binned due to limitations of the censusing schedule as well as imprecision of declaring a plant about to flower may have limited my power to detect any differences in this trait.

### **1.3.2 Common garden experiments in native serpentine soil.**

To test the effect of soil properties in isolation on plant survival, I planted inbred lines from two separate on/off serpentine population pairs (one from McL and one from RH) on their home serpentine soil. In both cases the serpentine population (McL = REM; RH = SLP) had high survival (McL:  $70\% \pm 4.3\%$ ; RH:  $94.5\% \pm 3.1\%$ ) while the non-serpentine line (McL = SOD; RH = TUL) had no survivors (Table 7). I also planted lines for each population pair on the alternate serpentine soil type. The McL non-serpentine line when planted on RH soil had zero survivors while the serpentine line enjoyed higher survival ( $32\% \pm 4.2\%$ ). However, the McL serpentine line has significantly lower survival on the RH soil than on its home serpentine soil ( $t=-4.012$ ,  $p=0.0016$ ) and mean percent survival at each weekly census (Figure 6) reveals variation in time to death for this line on the RH soil. Given that these were replicates of an inbred line, this suggests that there is significant environmental variance in time to death. Such differences could

be due to variation in water availability both within and between plates which would affect the rate at which plants are acquiring water and solutes from the soil matrix.

In contrast, the RH serpentine line has high survival on both serpentine soil types ( $t=0.908$ ,  $p=0.8058$ ). The RH non-serpentine line had very low survival on the McL soil (1.4%); however, an interesting difference in the degree of non-tolerance of this line on the each soil type emerged. When planted on its home RH soil, all seedlings die by the week 1 census. However, when planted on the McL soil, the RH non-serpentine line takes much longer to die. At week 1 census percent survival was 45.8% ( $\pm 11.9\%$ ) and only by week 5 had nearly all individuals died.

The survival of F1 and F2 individuals was also assessed in order to explore the genetic basis of these survival differences between the parental lines. The McL F1s and F2s had high survival on their home soil (F1 = 85.7%  $\pm 4.7\%$ ; F2 = 83.6%  $\pm 1.9\%$ ) but very low survival on the RH soil (F1 = 2.1%  $\pm 5.7\%$ ; F2 = 4.4%  $\pm 1.8\%$ ). There was a significant interaction between plant class and soil type ( $F_{2, 106}=18.89$ ,  $P<0.001$ ) where on McL soil the F1s and F2s had higher survival than the serpentine parent but lower survival than the serpentine parent when grown on RH soil (Figure 5). The F2s had a slower rate of mortality than the non-serpentine parent. All non-serpentine parental plants were dead at the week 1 census; however, only 6.5% of the F2s were dead at week one. This percentage of F2s that exhibit a similar non-tolerance phenotype to the non-serpentine parent is not significantly different from 1/16 ( $\chi^2=0.142$ ,  $p=0.706$ ) expected under a two

locus model where the double non-serpentine homozygotes are completely non-tolerant. The RH F2s had 51.8%  $\pm$ 1.8% survival on the RH soil and very high survival (94.1%  $\pm$ 1.6%) on the McL soil in contrast to the RH serpentine parent that had high survival on both soil types (Figure 5); the interaction term between plant class and soil type in the ANOVA was significant  $F_{1,3} = 67.429$ ,  $p < 0.0001$ .

### 1.3.3 Soil analysis

Principal components analysis of eight different soil variables show that soil from serpentine and non-serpentine populations differ for a number of attributes. Serpentine and non-serpentine sites segregate with respect to principal component 1 (PC1) which explains 47.9% of the sampled variation in soil variables (Figure 7). Non-serpentine soils have positive values for PC1 (indicating soils with high levels of calcium and other soil nutrients and low levels of magnesium) while serpentine soils have negative values for PC1. These results support the classification of populations by soil type and show that serpentine soils have Ca:Mg ratios much less than 1 while non-serpentine sites have Ca:Mg ratios of 1 or greater (Table 8). PC2 with heaving loadings of potassium, pH and phosphorus explains an additional 24.6% of the variation among soil samples. Despite substantial variation for PC2 among locations, serpentine and non-serpentine sites did not segregate consistently along this second axis of soil heterogeneity.

### **1.3.4 Hydroponics**

In each of the three on/off serpentine population pairs tested for differences in tolerance to low Ca:Mg ratio, the serpentine line continues growing roots in more severe (i.e. lower Ca:Mg ratios) than the non-serpentine line (Table 9, Figure 8). One-way analysis of variance reveals that these differences in tolerance between serpentine and non-serpentine lines in the McL and Sonoma population are significant (McL:  $F_{1,218}=1049.2$ ,  $p<0.001$ ; Sonoma:  $F_{1,111}=722.02$ ,  $p<0.001$ ). The Red Hills populations had extremely low replication ( $n=5$ /population) and mean differences in tolerance between the lines was not analyzed for statistical significance; however, the same pattern of tolerance differences is observed in this population pair as well. The experiments conducted with the McL and Sonoma population pairs also included F1s and F2s. In both experiments the F1s and F2s had high tolerance to low Ca:Mg ratio (Figure 8) and their means are greater than the mid-parent value (Table 9). The F2s recapture the parental phenotypes at both extremes (Figure 8).

### **1.3.5 Constitutive differences in leaf calcium and magnesium composition**

There were significant differences between the serpentine and non-serpentine plants in log of ppm Ca in leaves wherein serpentine plants have higher leaf Ca levels than non-serpentine plants ( $F_{1,21}=13.68$ ;  $p=0.0013$ ; Table 10); however, leaf Mg levels were not significantly different between serpentine and non-serpentine plants ( $F_{1,21}=0.178$ ,  $p=0.677$ ; Table 10). The nested term of population (habitat of origin) was significant ( $F_4$ ,

$t_{21} = 18.20, p < 0.001$ ) and to investigate differences between populations, I performed a one-way ANOVA using log ppm leaf Ca as the dependent variable. Post-hoc Tukey-Kramer comparisons of all pair-wise population means corrected for multiple testing show that the SLP serpentine population from the RH has significantly lower leaf Ca levels than either of the two other serpentine populations, both of which are from the Northern Coast Range (Table 10, Figure 9). The STO serpentine population from Sonoma county maintains significantly higher levels of leaf Ca than all other populations analyzed (Figure 9, Table 10).

## **1.4 Discussion**

This study demonstrates that populations of *M. guttatus* living on serpentine and non-serpentine habitats are genetically differentiated in their ability to tolerate the unique chemical composition of serpentine soils. *M. guttatus* plants from non-serpentine populations are unable to survive on serpentine soils in both field reciprocal transplant studies and lab-based common-garden experiments. Hydroponic assays demonstrate differential tolerance of serpentine and non-serpentine populations to low Ca:Mg ratios, suggesting that this defining feature of serpentine soils is likely an important selective agent in these habitats. The differing abilities of *M. guttatus* populations to cope with Ca:Mg ratios lower than one may contribute to the survival differences observed in the field. Finally, performance of F1 and F2 hybrids in both field and lab-based studies provides an initial characterization of the genetic basis of these survival and hydroponic

tolerance differences showing serpentine tolerance is dominant and likely under major gene control.

#### **1.4.1 Local adaptation of *M. guttatus* to serpentine soils**

Three separate field-based reciprocal transplant studies demonstrate that serpentine and non-serpentine populations of *M. guttatus* are locally adapted to serpentine soils. Previous studies involving only lab-based assays have shown mixed evidence for local adaptation of *M. guttatus* to serpentine habitats. Gardner (2000) grew plants from serpentine and non-serpentine populations of *M. guttatus* in solutions of varying Ca:Mg ratios. They found no significant differences in growth between serpentine and non-serpentine plants. However, the non-serpentine population they used came from copper mine tailings and it is possible that the copper tolerance mechanisms in this population (Macnair 1993) provides cross-tolerance to high Mg levels. A second study by Murren *et al.* (2006) looked at response of *M. guttatus* populations from serpentine and non-serpentine sites at the McLaughlin Reserve for tolerance to drought and low Ca:Mg ratio. They grew plants from both habitats in potting mix in the greenhouse and simultaneously altered Ca:Mg ratio and water availability. They did not find that plants from a particular field-habitat type performed best in the analogous greenhouse treatment leading the authors to conclude that *M. guttatus* populations do not show differentiation into serpentine and non-serpentine ecotypes. However, these results contrast with the findings of Palm *et al.* (2012) who,

working with the same REM/SOD population pair from the McLaughlin Reserve used in this study, demonstrate that seedlings from the non-serpentine population do not survive past the juvenile stage when planted on native serpentine soil in the lab. The differences between the results from these studies highlight the challenges of replicating the chemical and physical properties of serpentine soils in the lab.

Field-based reciprocal transplant studies are the best test for local adaptation and by conducting multiple reciprocal transplant studies across regions and years and including multiple serpentine and non-serpentine populations in each study, the work presented here clearly demonstrates that *M. guttatus* is locally adapted to serpentine soils. In all three experiments, plants from serpentine *M. guttatus* populations have high survival at serpentine field sites while non-serpentine plants die as rosettes. In lab-based common garden experiments conducted on native serpentine soils, I demonstrate similar patterns of survival differences between the ecotypes suggesting that survival differences are due to soil variables as opposed to some other environmental variable that may differ between serpentine and non-serpentine habitats.

#### **1.4.2 Cost to tolerance**

I found limited evidence for a cost to serpentine tolerance. There were no survival differences between serpentine and non-serpentine plants at the non-serpentine field sites; however, at the Red Hills non-serpentine site, plants from non-serpentine populations were larger in both the juvenile and adult stages. Palm et al (2012) found a

difference in plant biomass between an on/off pair of inbred lines from the McLaughlin Reserve when grown in potting soil. Failure to detect a similar difference in the field in this study may be due to limited sample sizes and significant environmental variance. In other species some serpentine-tolerant plants have been found to possess a slower intrinsic growth rate than non-serpentine plants (O'Dell and Claassen 2006, Sambatti and Rice 2006). It has also been shown that serpentine-tolerant plants do not grow as well as non-serpentine plants when grown together on non-serpentine soils (Kruckeberg 1954, Proctor *et al.* 1975, Jurjavcic *et al.* 2002) suggesting that there may be a trade-off between competitive ability and tolerance to serpentine.

The non-serpentine populations used in these studies are located from 4km to 40km away from the serpentine populations and the extremely low frequency of tolerant individuals in these populations (~1.5% in the populations tested) may be due to limited gene flow. It would be interesting to examine the frequency of tolerance alleles present in non-serpentine populations that occur at varying distances from serpentine populations. Nonetheless, based on the populations sampled in this study there were no differences in the frequency of tolerant individuals segregating in non-serpentine populations whether they were only 4km away from a serpentine population or much farther removed (40km). This lack of tolerant individuals found in non-serpentine populations suggests that there is likely a cost to serpentine tolerance. The smaller sizes of serpentine plants shown in the Red Hills populations may reduce their competitive

ability in non-serpentine sites which typically are much more heavily vegetated than serpentine habitats. Testing the fitness of serpentine and non-serpentine populations of *M. guttatus* under competitive treatments would help to clarify the potential cost to tolerance in this species.

### **1.4.3 Potential mechanisms of adaptation to serpentine soils in *M. guttatus***

Since the early 1900's many studies have tested isolated chemical features of serpentine soil for their effects on plant growth in an effort to determine the specific variables that may be most important for plant adaptation to these habitats. Much of the evidence points to low levels of Ca and high levels of Mg (Loew O. 1901, Vlamis 1949, Walker et al. 1955, McMillan 1956, Madhok and Walker 1969). Experiments testing for differential growth between serpentine and non-serpentine plants in response to other serpentine soil variables such as low nutrient levels and high heavy metal concentrations have shown mixed results (reviewed in Brady *et al.* 2005, Alexander *et al.* 2007, Kazakou et al. 2008).

Plants that are able to grow on serpentine soils must be able to cope with the dual challenges of maintaining adequate internal levels of Ca, while not suffering toxicity from elevated Mg. Calcium performs critical structural roles in cell walls and membranes, acts as an important counter-cation for anions in the vacuole, and serves in signal transduction. Ca deficiency ultimately leads to tissue necrosis due to disintegration of cell walls. I show here that *M. guttatus* populations from serpentine

and non-serpentine soils have differential tolerance to low Ca:Mg ratios. These differences in tolerance may underlie the survival differences observed in the field. In three on/off serpentine population pairs tested, the serpentine population is able to continue growing roots in treatment solutions with significantly lower levels of Ca and higher levels of Mg than the non-serpentine populations. Numerous studies looking at root and shoot elemental composition differences between serpentine and non-serpentine plants point to several whole-plant mechanisms of tolerance: selective uptake and/or translocation of Ca; exclusion or sequestration of Mg; or having a higher Mg requirement for proper growth (reviewed in Brady *et al.* 2005). The results from leaf elemental compositional analysis show that when grown in a high nutrient environment, serpentine *M. guttatus* populations maintain higher leaf Ca levels than non-serpentine populations but there is no significant difference in Mg levels. This difference in leaf Ca levels suggests that serpentine *M. guttatus* populations may selectively uptake or translocate Ca to shoot tissue at higher levels than non-serpentine plants. However, there are significant differences in leaf Ca concentrations among the different populations tested and the fact that the SLP and STO serpentine populations maintain significantly different leaf Ca:Mg ratios suggests they may have evolved serpentine tolerance via different underlying physiological mechanisms. However, it will be important to test these patterns of Ca and Mg acquisition when plants are grown on serpentine soils.

#### **1.4.4 Preliminary characterization of the genetic basis of serpentine tolerance in *M. guttatus***

This is the first study to investigate the genetic basis of tolerance to serpentine soils in *M. guttatus*. The performance of F1 and F2 hybrids in both field and lab-based studies show that serpentine tolerance is dominant and likely controlled by a relatively small number of loci. When planted on serpentine soils in both the field and the lab, F1s have high survival rates that are not significantly different from those of the serpentine parent in both RH and McL populations. Furthermore, the hydroponic assays show that F1s between both the McL and Sonoma populations have high tolerance to low Ca:Mg ratios similar to the serpentine lines.

The survival patterns of the F2s on serpentine soils suggest that one or a few genes likely control serpentine tolerance in *M. guttatus*. In the serpentine field plots, the F2/F3s from both McL and RH populations had survival rates that were ~50% lower than the serpentine parents. If serpentine tolerance was highly polygenic only a small proportion of individuals would be expected to have the capability to survive on serpentine soils. This intermediate survival rate suggests that serpentine tolerance likely has a simple genetic basis but is not controlled by a single locus. The common-garden experiments support these findings where the RH F2s again have a survival rate that is ~50% lower than that of the serpentine parent. In the McL population pair we see a different pattern where the F2s have high percent survival (83.6%) which is significantly different from a 3:1 ratio expected if serpentine tolerance is controlled by a single locus

and the tolerance allele is dominant ( $\chi^2 = 19.2$ , d.f. = 1,  $p < 0.001$ ). While the fact that the experiment was terminated after 5 weeks when seedlings were still small may explain this high survival rate, the slow death rate of the F2s suggests that there may be multiple loci controlling survival on serpentine soils with different dominance patterns than in the Red Hills. The non-serpentine McL parental line dies almost immediately after planting on serpentine soil; however, only a small proportion of F2s (6.5%) show a similar non-tolerance phenotype suggesting that several loci may contribute to this trait and that some combinations of genotypes confer a degree of tolerance though ultimately not enough to survive.

#### **1.4.5 Preliminary evidence for local adaptation of serpentine *M. guttatus* populations to their home serpentine soil patch**

This is the first study to examine the effects of variation between different serpentine soil types on plant fitness. The reciprocal transplant experiments measured the survival of serpentine populations collected from different serpentine localities at serpentine field sites at the McLaughlin Reserve and Red Hills. Some interesting differences in percent survival between the serpentine populations emerged. At the Red Hills, the native serpentine population had significantly higher survival than the two other serpentine populations that were collected from nearby but disjunct serpentine patches. At the McL serpentine field sites, both the native serpentine population and a population from Napa county had the highest survival rates in both 2010 and 2012. Unfortunately, I do not have soil data for all of these serpentine populations so it is not

possible to correlate differences in survival between the populations with similarities between their native serpentine soil and that of the field planting habitat. Nonetheless, these results suggest that not all serpentine *M. guttatus* populations are equally tolerant to all serpentine soils but may be locally adapted to the specific characteristics of their home serpentine patch.

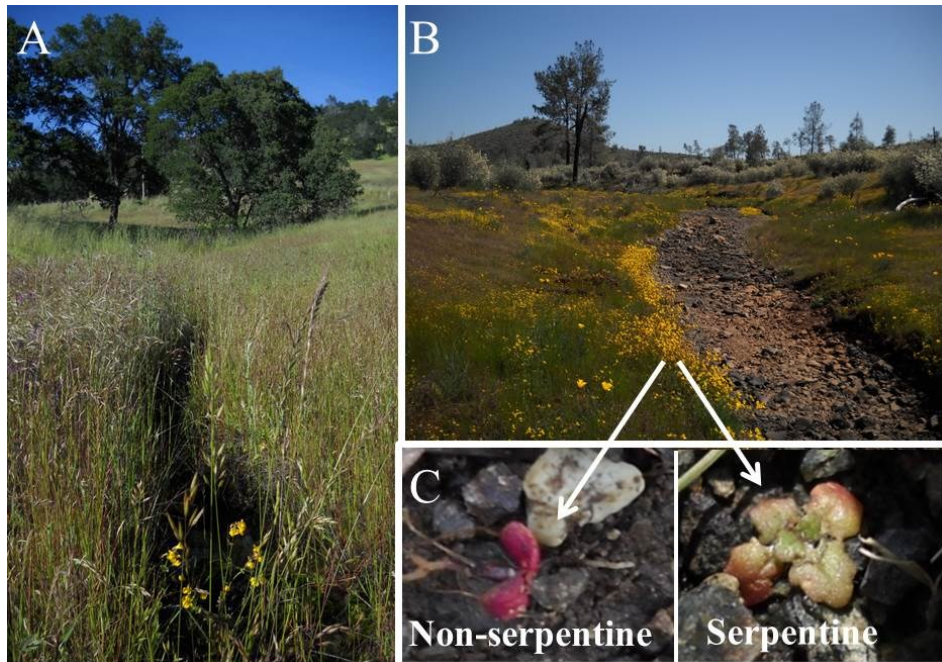
The lab-based common-garden experiments directly tested how plants from different serpentine soil patches perform on their home soil versus foreign serpentine soil. In this experiment, the RH serpentine population had high survival on both serpentine soil types while the McL serpentine line had significantly lower survival on the RH soil than on its home soil. This difference in the McL population's survival on the two different serpentine soils suggests that soil variables differ between these two sites and again that not all serpentine populations are broadly tolerant of all serpentine soils. The principal component analysis of soil attributes did not show much variation between the different serpentine soil samples. However, these samples were not analyzed for heavy metal concentrations which are known to vary significantly between different serpentine patches (Oze et al. 2004, Alexander *et al.* 2007, Morrison et al. 2009). The McL soil has a lower Ca:Mg ratio (0.056) than the RH soil (0.093) though the McL soil has higher absolute levels of both Ca and Mg the RH (McL: Ca = 257 ppm, Mg = 4745 ppm; RH: Ca = 156 ppm, Mg = 1690 ppm). It is possible that these differences in Ca and Mg between the two soil types could contribute to the survival differences observed

between the populations. However, it will be necessary to collect and analyze more soil samples from each population in order to test the significance of these differences.

#### **1.4.6 Conclusions**

This study demonstrates that populations of *M. guttatus* occurring on serpentine and non-serpentine habitats are genetically differentiated in their ability to tolerate the unique chemical composition of serpentine soils. *M. guttatus* plants from non-serpentine populations are unable to survive on serpentine soils in both field reciprocal transplant studies and lab-based common-garden experiments. Hydroponic assays demonstrate differential tolerance of serpentine and non-serpentine populations to low Ca:Mg ratios suggesting that this defining feature of serpentine soils is likely an important selective agent in these habitats. The differing abilities of *M. guttatus* populations to cope with Ca:Mg ratios lower than 1 may contribute to the survival differences observed in the field. The performance of F1 and F2 hybrids in both field and lab-based studies provides an initial characterization of the genetic basis of these survival and hydroponic tolerance differences and suggests that serpentine tolerance is dominant and likely under major gene control. Finally, the preliminary results showing differences between serpentine *M. guttatus* populations in their tolerance to home versus foreign serpentine soils represents an exciting avenue for future research. *M. guttatus* has colonized serpentine soils across much of its range and it would be interesting to

examine whether there is evidence for multiple origins of serpentine tolerance within this species.



**Figure 1: Photos of serpentine and non-serpentine field sites. A) Non-serpentine field site at RH; B) One of the serpentine field sites at the RH; C) Seedlings from non-serpentine versus serpentine populations growing at the serpentine site ~3 weeks after transplanting in 2010.**

**Table 1: Habitat and location information for populations used in this study.**

<b>Region</b>	<b>Population ID</b>	<b>Habitat</b>	<b>County</b>	<b>Latitude</b>	<b>Longitude</b>
<b>Sierra foothills</b>	COL	Serpentine	Mariposa	N 37°45'11"	W 120°14'52"
	HWY-49	Serpentine	Tuolumne	N 37°36'40"	W 120°8'13"
	KFY	Off	Stanislaus	N 37°49'10"	W 120°39'44"
	MCC	Off	Tuolumne	N 37°47'57"	W 120°14'15"
	SLP	Serpentine	Tuolumne	N 37°51'24"	W 120°27'13"
	TUL	Off	Tuolumne	N 37°50'24"	W 120°37'35"
<b>Northern Coast Range</b>	BSC	Serpentine	Napa	N 38°45'5"	W 122°30'34"
	DCM	Serpentine	Yolo	N 38°51'27"	W 122°22'07"
	DHR	Serpentine	Lake	N 38°30'36"	W 122°14'24"
	GUA	Off	Sonoma	N 38°40'5"	W 122°18'41"
	GUG	Serpentine	Napa	N 38°43'18"	W 122°30'23"
	OAE	Off	Sonoma	N 38°24'40"	W 122°57' 34"
	REM	Serpentine	Lake	N 38°51'27"	W 122°24'48"
	SOD	Off	Napa	N 38°25'6"	W 122°17'44"
STO	Serpentine	Sonoma	N 38°26'19"	W 122°56'25"	



Figure 2: Map of focal regions and locations of reciprocal transplant studies. Dots represent each of the populations listed in Table 1.

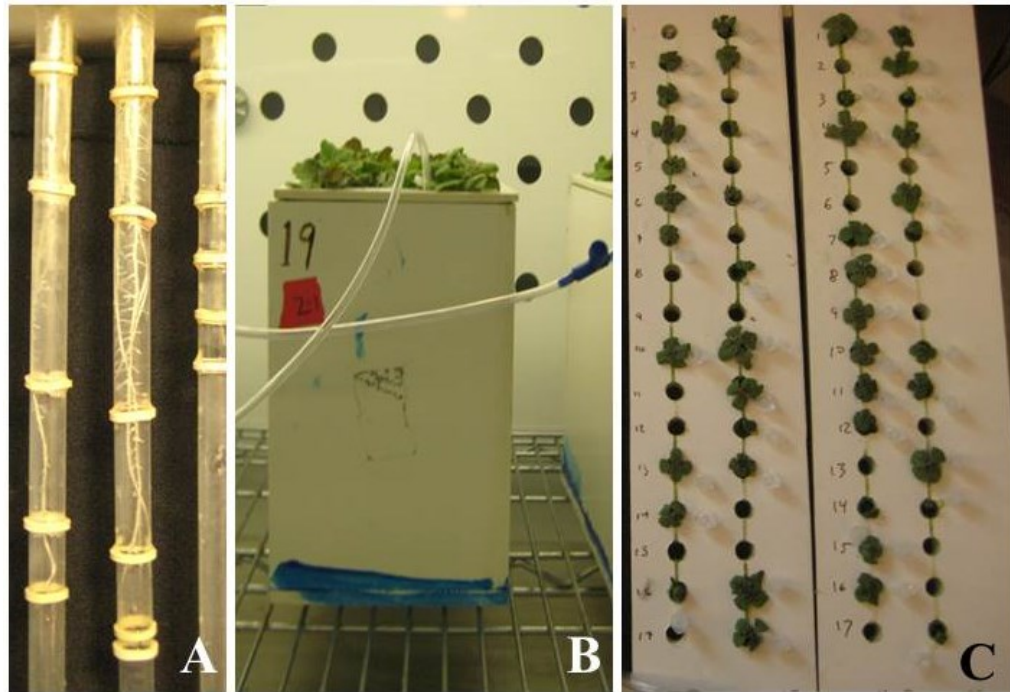


Figure 3: Photos of high-throughput hydroponic growth platform. A) Roots growing in straws with rubber bands marking positions of root tips in each treatment level. B) Front view of box at end of experiment; tubes are connected to an air pump. C) Top of box showing *M. guttatus* seedlings at start of experiment.

**Table 2: Sources of calcium and magnesium ions in series of hydroponic treatment solutions. Solutions were a standard ¼ strength Hoagland's with the following alterations of Ca(NO<sub>3</sub>)<sub>2</sub> and Mg(NO<sub>3</sub>)<sub>2</sub>. All solutions had pH = 5.7.**

Treatment	Ca(NO <sub>3</sub> ) <sub>2</sub> mmol l <sup>-1</sup>	Mg(NO <sub>3</sub> ) <sub>2</sub> mmol l <sup>-1</sup>	MgSO <sub>4</sub> mmol l <sup>-1</sup>	Ca:Mg mol : mol
¼ strength Hoagland's	4	0	1	4
1	0.2	3.8	1	0.05
2	0.1	3.9	1	0.025
3	0.05	3.95	1	0.0125
4	0.025	3.975	1	0.005
5	0.0125	3.9875	1	0.0025
6	0.00625	3.99375	1	0.00125
7	0.003125	3.996875	1	0.000625
8	0.0015625	3.9984375	1	0.0003125

**Table 3: Percent survival at field sites by plant class for the three reciprocal transplant experiments. Percent survival is given for each class of plants planted in alternative habitat types. Standard errors given in parentheses are based on the variation between populations within the parental classes. \*\*\*indicates p<0.0001 in G-test of independence on counts of dead versus alive compared between serpentine and non-serpentine habitats of origin.**

Experiment	Serpentine sites	Non-serpentine sites
McL 2010		
Serpentine	28.68% (±3.8)***	61.71% (±6.56)
Non-serpentine	1.10% (±0.03)	60.98% (±1.84)
F1	27.03%	53.70%
F2	15.21%	62.50%
RH 2010		
Serpentine	20.78% (±7.36)***	49.65% (±2.31)
Non-serpentine	1.60% (±0.33)	53.38% (±3.86)
F1	29.10%	48.84%
F2	13.96%	57.99%
McL 2012		
Serpentine	44.83% (±22.83)***	29.73% (±14.48)
Non-serpentine	1.27% (±0.89)	30.43% (±6.34)
F3	22.06%	48.99%

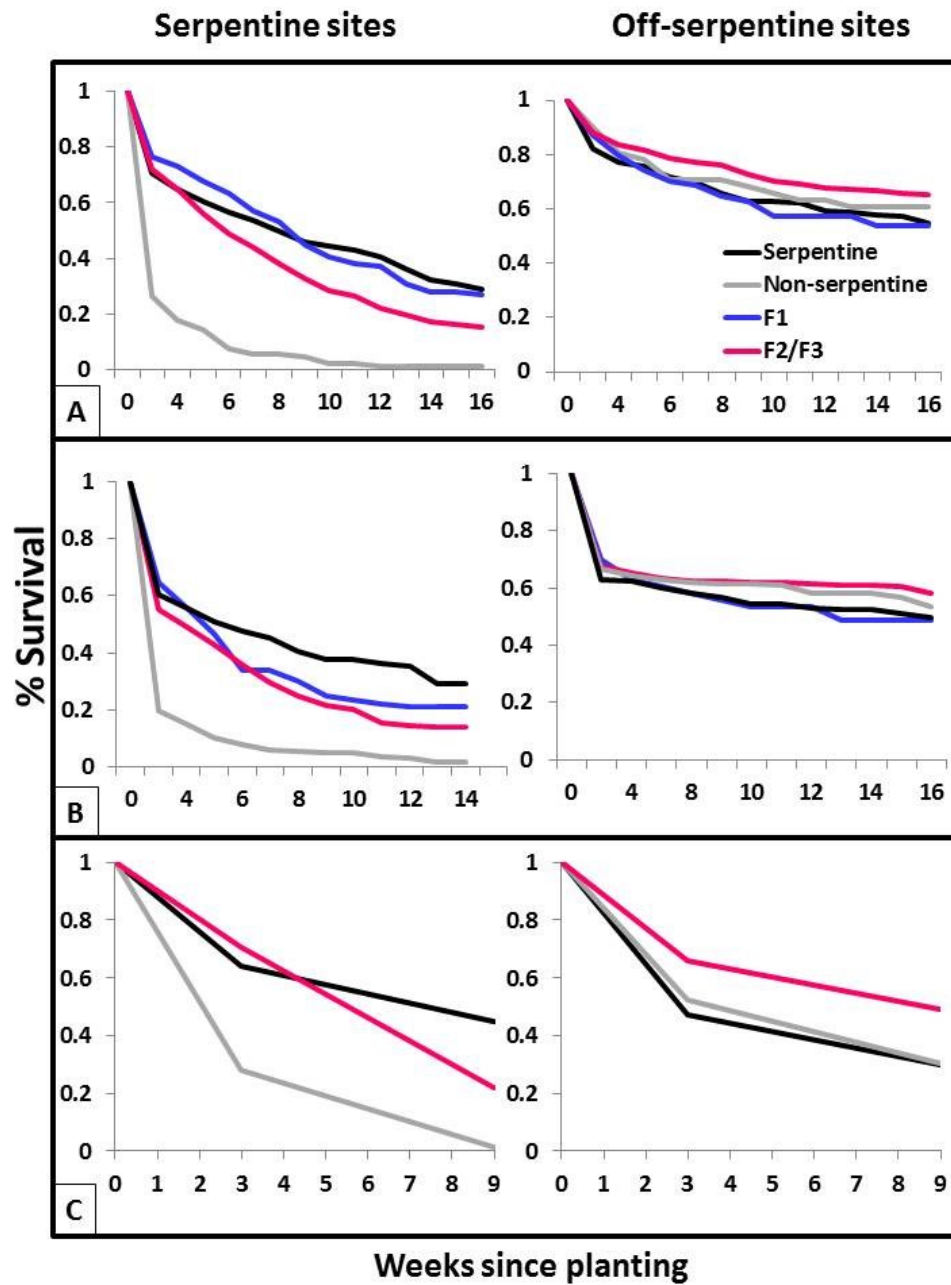


Figure 4: Survival curves at serpentine versus non-serpentine field sites for each of the reciprocal transplant experiments. A) McLaughlin sites in 2010; B) Red Hills sites in 2010; C) McLaughlin sites in 2012. X-axes have difference scales due to differences in duration of the experiments.

**Table 4: Percent survival at field sites by population for the three reciprocal transplant experiments. Population abbreviations given. Details on population locations provided in Table 1. Habitat of origin given in parentheses – S = serpentine; NS = non-serpentine. Significance of chi-square test for independence on counts of “alive” versus “dead” between serpentine and non-serpentine sites provided. \*p<0.05; \*\*p<0.01; \*\*\*p<0.0001.**

<b>Experiment</b>	<b>Population (habitat of origin)</b>	<b>% survival serpentine sites (n)</b>	<b>% survival non-serpentine sites (n)</b>
McLaughlin 2010	BSC (S)***	25.5% (145)	61.1% (72)
	DHR (S)**	21.65 (97)	52.2% (46)
	GUG (S)	38.8% (98)	54.3% (46)
	RES (S)	32.5% (40)	30.8% (13)
	GUA (NS)***	0% (50)	62.5% (24)
	SOD (NS)***	2.4% (41)	58.8% (17)
Red Hills 2010	COL (S)***	15.6% (77)	54.5% (44)
	HWY 49 (S)**	27.4% (62)	52.2% (46)
	SLP (S)	41.1% (95)	47.2% (53)
	KFY (NS)***	2.25 (90)	49.0% (51)
	MCC (NS)***	1.2% (82)	57.1% (51)
	TUL (NS)***	1.2% (81)	64.6% (48)
McLaughlin 2012	DCM (S)*	11.1% (9)	50% (24)
	GUG (S)***	100% (9)	0% (11)
	REM (S)*	55.2% (29)	28.6% (35)
	STO (S)	0% (11)	--
	GUA (NS)***	0% (23)	35.9% (39)
	SOD (NS)**	1.8% (56)	23.3% (30)

**Table 5: Morphological measurements on plants at the non-serpentine field sites.**  
**Units for rosette diameter, plant height and length of 1st leaf are cm. Standard errors given in parentheses.**

<b>Experiment</b>	<b>Site</b>	<b>Rosette diameter</b>	<b>Days to flower</b>	<b>Plant height</b>	<b>Length of 1<sup>st</sup> leaf</b>
<b>McL 2010</b>	<b>Serpentine</b>				
	Serpentine	0.43 (0.012)	102.4 (1.6)	3.52 (0.040)	0.26 (0.03)
	Non-serpentine	0.31 (0.054)	--	--	--
	F1	0.44 (0.018)	99.6 (2.3)	4.90 (0.45)	0.27 (0.034)
	F2	0.37 (0.007)	99.1 (1.6)	3.08 (0.32)	0.22 (0.024)
	<b>Non-serpentine</b>				
	Serpentine	0.82 (0.044)	103.4 (1.2)	7.69 (0.54)	0.46 (0.053)
	Non-serpentine	0.84 (0.078)	102.9 (2.1)	6.04 (1.01)	0.38 (0.070)
	F1	1.04 (0.063)	99.4 (1.6)	9.77 (0.82)	0.62 (0.060)
	F2	0.91 (0.020)	100.6 (0.5)	8.06 (0.24)	0.56 (0.019)
<b>RH 2010</b>	<b>Serpentine</b>				
	Serpentine	0.61 (0.23)	78.0 (0.81)	--	--
	Non-serpentine	0.52 (0.47)	--	--	--
	F1	0.61 (0.41)	83.0 (2.0)	--	--
	F2	0.57 (0.17)	80.2 (1.6)	--	--
	<b>Non-serpentine</b>				
	Serpentine	0.61 (0.037)	80.2 (1.0)	5.56 (0.49)	0.33 (0.021)
	Non-serpentine	0.75 (0.056)	83.1 (1.3)	7.75 (0.56)	0.47 (0.027)
<b>McL 2012</b>	<b>Serpentine</b>				
	Serpentine	0.24 (0.035)	--	--	--
	Non-serpentine	0.29 (0.043)	--	--	--
	F3	0.32 (0.038)	--	--	--
	<b>Non-serpentine</b>				
	Serpentine	0.33 (0.042)	--	--	--
	Non-serpentine	0.33 (0.029)	--	--	--
	F3	0.42 (0.007)	--	--	--

Table 6: Results from MANOVA on size metrics at non-serpentine field sites in 2010. Dependent variables were rosette diameter, plant height and length of first true leaf.

Experiment	Source	df	Wilks' $\lambda$	F	p
McL non-serpentine site	Habitat of origin	2, 55	--	1.0762	0.348
	Population [Habitat of origin]	8, 110	0.7533	2.0920	0.0424
	Block	10, 110	0.5611	3.6845	0.0003
	Plot [Block]	110, 100	0.0628	2.9895	<0.0001
RH non-serpentine site	Habitat of origin	2, 74	--	4.2643	0.0177
	Population [Habitat of origin]	8, 148	0.8446	1.6299	0.1209
	Block	14, 148	0.5296	3.9547	<0.0001
	Plot [Block]	120, 148	0.1936	1.5698	0.0046

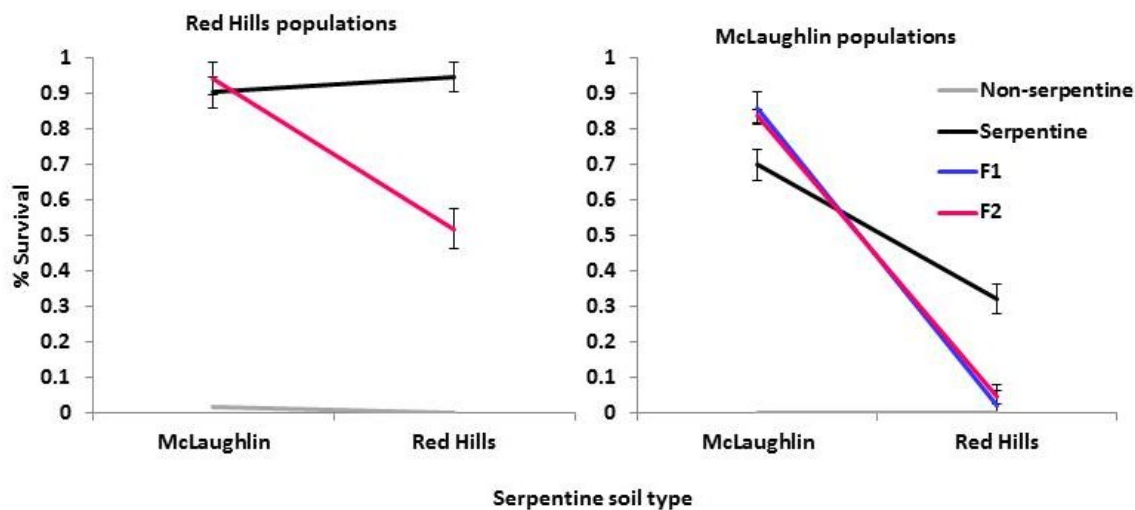


Figure 5: Percent survival of Red Hills and McLaughlin parents, F1s and F2s on home versus foreign serpentine soil treatments. Bars represent  $\pm$ SE.

Table 7: Percent survival of McLaughlin and Red Hills parents, F1s and F2s on two serpentine soil types in the lab.

Region/Plant class	Soil planting habitat					
	McLaughlin			Red Hills		
	% Survival	SE	n	% Survival	SE	n
McLaughlin						
Serpentine	69.8%	±4.3%	8	32.1%	±4.2%	7
Non-serpentine	0%	--	4	0%	--	4
F1	85.7%	±4.7%	7	2.1%	±5.7%	4
F2	83.6%	±1.9%	40	4.4%	±1.8%	40
Red Hills						
Serpentine	90.3%	±4.1%	6	94.5%	±3.1%	11
Non-serpentine	1.4%	--	6	0%	--	6
F1	--	--	--	--	--	--
F2	94.1%	±1.6%	40	51.8%	±1.8%	33

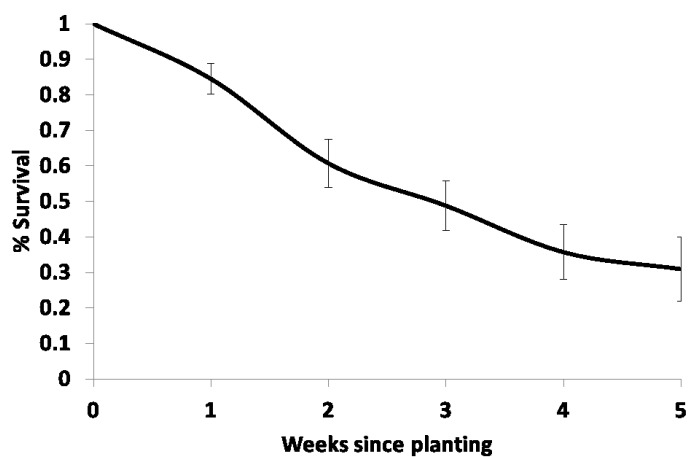


Figure 6: Survival of the McL serpentine inbred line (REM) on the RH soil at each census date.

Table 8: Analysis of soil variation between serpentine and non-serpentine populations. Mean and standard deviations (s) for soil attributes in six serpentine and five non-serpentine *M. guttatus* populations. Soil attribute loadings for the first two principal components together account for 72.5% of sampled soil variation are shown.

	PC1	PC2	Off-serpentine		Serpentine	
			Mean	s	Mean	s
pH	-0.5943	0.7443	6.63	0.82	7.76	0.7
Nitrate-N	0.4726	-0.4556	8.4	5.2	7.5	4.9
Phosphorus	0.4635	-0.6459	28	28.9	8.3	4.2
Potassium	0.4963	0.5199	68.8	15.7	54.3	14.3
Calcium	0.9079	0.3500	2345.1	1350.5	237.3	71.8
Magnesium	-0.7514	0.3315	785.8	315.0	3108.5	1964.5
Ca:Mg	--	--	3.42	2.0	0.11	0.11
Sulfur	0.8493	0.3158	43.5	43.7	9.4	5.9
Sodium	0.8293	0.4370	216.8	76.4	118.3	23.2
Iron	--	--	17.2	4.3	8.8	5.3
Zinc	--	--	1.92	1.3	0.9	1.0
Manganese	--	--	21.9	16.4	19.1	8.5
Copper	--	--	5.1	6.5	1.1	0.6

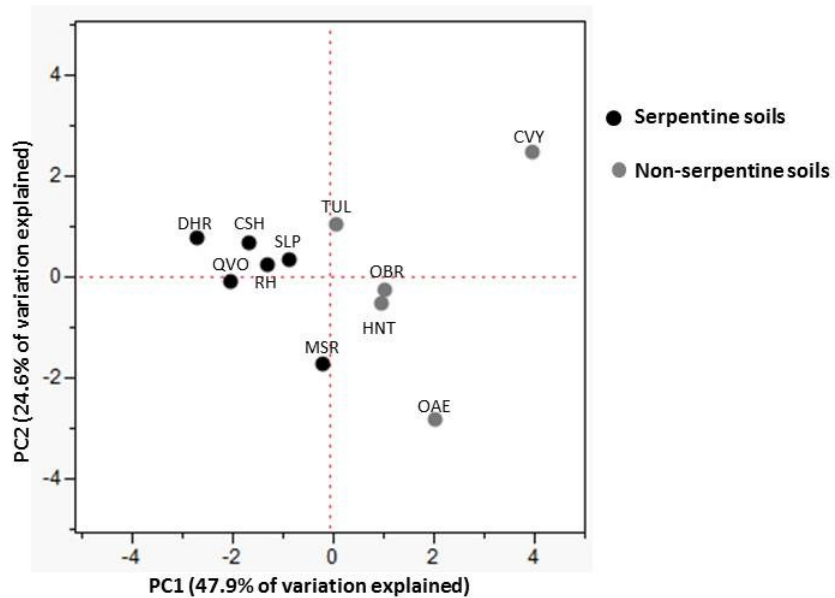


Figure 7: Principal component analysis of soil variables among six serpentine and five non-serpentine populations of *M. guttatus*. Populations by region: McLaughlin Reserve = DHR, CSH, QVO and CVY; Sonoma = MSR and OAE; Red Hills = RH, SLP, TUL, OBR and HNT.

**Table 9: Means of tolerance to low Ca:Mg ratio for serpentine, non-serpentine and hybrids from three populations pairs. Samples sizes, mean and standard deviation in EC100 given for all plant classes from McLaughlin, Sonoma and Red Hills hydroponic assays. EC100 is given as the ratio of Mg to Ca in the treatment solution that stopped root growth. Higher mean EC100 indicates greater tolerance to “serpentine-like” growth environment.**

<b>Experiment</b>				
	<b>Plant class</b>	<b>n=</b>	<b>Mean EC100</b>	<b>s</b>
McLaughlin				
	Serpentine	140	1440	361.6
	Non-serpentine	80	106.5	97.2
	F1	72	1270.4	419.3
	F2	1320	1077.9	526.0
Sonoma				
	Serpentine	53	1486.8	372.0
	Non-serpentine	71	155.5	226.9
	F1	35	1285.7	458.3
	F2	552	1047.8	590.1
Red Hills				
	Serpentine	5	768	770
	Non-serpentine	5	112	163

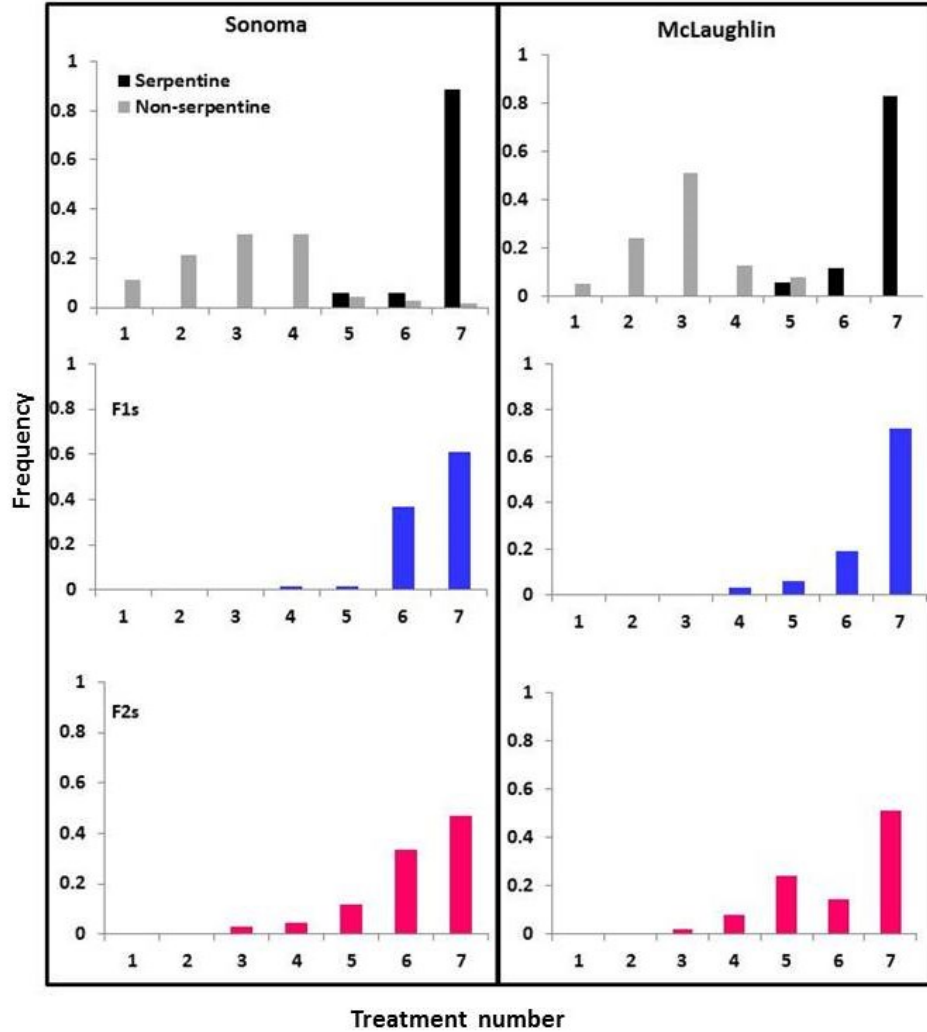


Figure 8: Frequency distributions of effective concentration 100 in low Ca:Mg ratio treatment solutions for serpentine and non-serpentine parental lines, F1s and F2s from two separate populations pairs. The proportion of plants that stopped growing roots in each treatment level is shown. Treatments are represented by their order in the series from least to most severe rather than the actual concentration for ease of interpretation. Treatment 1= Ca:Mg 0.05; Treatment 7 = Ca:Mg 0.000625. Actual treatments increased in severity geometrically not linearly (see Table 2).

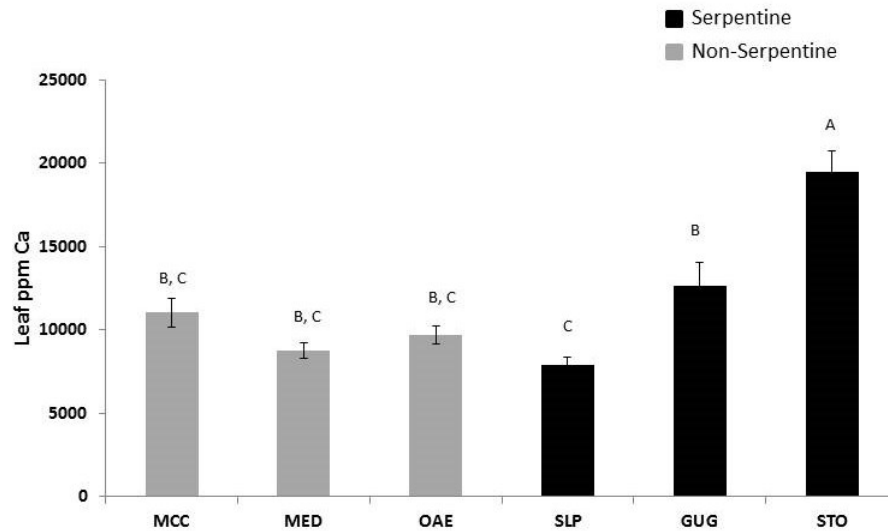


Figure 9: Variation in parts per million of calcium in leaf tissue of six different *M. guttatus* populations grown in nutrient solution. Populations not connected by the same letter are significantly different in Tukey-Kramer comparison of means with  $\alpha=0.05$ . Bars represent  $\pm$ SE.

Table 10: ICP-MS results for parts per million of calcium and magnesium in three serpentine and three non-serpentine *M. guttatus* populations. ( $\pm$ SE)

Population	Habitat of Origin	n=	Mg (ppm)	Ca (ppm)	Ca:Mg
MED	Non	3	3425 (336)	8751 (478)	2.6 (0.25)
OAE	Non	5	2013 (285)	9397 (619)	4.4 (0.40)
MCC	Non	5	1746 (233)	11051 (874)	6.5 (0.45)
SLP	Serp	5	2921 (105)	7924 (435)	2.7 (0.28)
STO	Serp	5	2523 (279)	19513 (1229)	7.9 (0.42)
GUG	Serp	5	1674 (169)	12654 (1430)	7.6 (0.74)

## **2. The genetic basis of adaptation to serpentine soils in *Mimulus guttatus***

### **2.1 Introduction**

While local adaptation has frequently been demonstrated via reciprocal transplant experiments (reviewed in Hereford 2009), our understanding of the genetic basis of it remains minimal. Many reciprocal transplant experiments demonstrate local adaptation but do not enable identification of individual traits that contribute to it. On the other hand, many laboratory studies identify loci that underlie putatively adaptive traits but the fitness effects of these loci are rarely tested in the field. There is a vital need for studies that bridge the divide between the ecology of plant adaptation and the molecular genetic control of traits often presumed to be adaptive. There is a notable lack of studies that identify naturally segregating variants, determine the traits controlled by these variants, and characterize their fitness effects in the field (but see Lexer et al. 2003, Weinig et al. 2003, Verhoeven et al. 2008, Lowry and Willis 2010, Ågren et al. 2013, Anderson et al. 2013). In order to understand the evolutionary dynamics of how spatially varying selective pressures create and maintain genetically differentiated populations it is necessary to identify the loci contributing to fitness differences between habitats and characterize how selection in the field is acting on these loci.

Local adaptation is a type of genotype x environment interaction where adaptation to one habitat reduces fitness in alternate habitats (Levene 1953, Kawecki and

Ebert 2004). This pattern of crossing reaction norms for fitness across two different habitat types could be maintained by a trade-off at the genetic level; however, it could also occur in the absence of a genetic trade-off. Antagonistic pleiotropy, wherein selection targets the same alleles in different environments but in opposite directions, results in a fitness trade-off and means that no single recombinant genotype can have high fitness in both environments. However, local adaptation could also be caused by multiple, independent loci where an allele increases fitness in one habitat but is selectively neutral in another (Kawecki and Ebert 2004). Trade-offs in fitness can occur if other loci show an opposite pattern across the habitats.

The handful of studies that have tested quantitative trait loci (QTLs) for their phenotypic effects across environments in natural populations (Lexer *et al.* 2003, Weinig *et al.* 2003, Verhoeven *et al.* 2008) support the idea that local adaptation has occurred in the absence of genetic trade-offs. However, two recent studies clearly demonstrate that antagonistic pleiotropic gene action contributes to local adaptation (Lowry and Willis 2010, Anderson *et al.* 2013). However, the generality of either model for the genetic basis of local adaptation requires more studies that test how selection is acting on individual loci in the field. Furthermore, the genetic basis of population differentiation will depend on the traits that contribute to high fitness in the alternate habitats, the strength of selection and the levels of gene flow. Oftentimes populations that are chosen for studies of local adaptation are geographically widespread in order to increase the power to

detect phenotypic differences. Studies of local adaptation in the face of gene flow are important for investigating the genetic basis of local adaptation under migration-selection balance (Savolainen et al. 2013).

### **2.1.1 The genetic basis of serpentine tolerance**

What are the most important evolutionary genetic changes that have enabled certain plant species to survive and reproduce on serpentine soils? Plants native to serpentine soils face numerous challenges: critically low levels of essential plant nutrients calcium (Ca), nitrogen (N), potassium (K), and phosphorus (P), low Ca:Mg, and elevated levels of magnesium (Mg), nickel (Ni), cobalt (Co), chromium (Cr), and iron (Fe). Despite this complex chemical environment, classic genetic and QTL mapping studies often reveal a simple, genetic basis of tolerance to mine or serpentine environments (Pollard et al. 2002, Bratteler et al. 2006, Clemens 2006, Burrell *et al.* 2012). In contrast, recent approaches using high-resolution population genomics can identify candidate genes or even SNPs contributing to local adaptation, but cannot distinguish between weakly or strongly selected loci. Recent population genomic studies of *Arabidopsis lyrata* suggest SNPs in dozens of genes may be involved in serpentine adaptation, but it is not known which of these genes are most critical for tolerance and which are subtle modifiers (Turner et al. 2008, Turner et al. 2010).

The handful of studies that have mapped QTLs for the genetic basis of serpentine tolerance (Bratteler *et al.* 2006, Burrell *et al.* 2012) exclusively use hydroponics to map

QTLs for tolerance to an isolated chemical feature of the soil that is presumed to be important for plant adaptation. While hydroponic approaches can offer powerful insights into which environmental variables are important selective agents and the potential mechanisms of serpentine tolerance, none of these hydroponic QTLs have been tested for their effects on plant fitness in native soil. Hydroponic approaches do not mimic the often complex interactions between ions that may occur in the native soils nor the physical properties of these soils. For example, Mg, Ca and Ni are all +2 cations and studies have revealed that differing concentrations of each affect the availability of the other ions to plants. The high levels of Mg typical of serpentine soils may limit the uptake of Ca (Brooks 1987) while addition of calcium reduces the effects of excess Mg (Gabbrielli and Pandolfini 1984). High levels of Ni may reduce uptake of Ca and Mg while higher levels of Ca and Mg reduce Ni toxicity (Gabbrielli and Pandolfini 1984, Gabbrielli *et al.* 1990, Yang *et al.* 1996, Nagy and Proctor 1997). In order to characterize the genetic basis of adaptation to serpentine soils mapping experiments should be conducted on these native soils. Hydroponics can then be used to determine what traits these fitness QTLs may control. Alternatively, if mapping is conducted on hydroponic tolerance these QTLs must be tested in the field in order to understand their effects on fitness.

### **2.1.2 The genetic basis of parallel adaptation to serpentine soils**

Serpentine soils are patchily distributed and vary substantially in their physiochemical properties (Whittaker 1954, Proctor *et al.* 1975, Kruckeberg 1984, Alexander *et al.* 2007), and it is not known whether widespread species, repeatedly adapt to different serpentine patches via the same or different molecular mechanisms. Are serpentine adapted alleles and pathways selectively equivalent on soils from different serpentine regions, or uniquely suited to each particular serpentine habitat? The genetic basis of such parallel serpentine adaptation is not known in most systems, but a recent study in *A. lyrata* offers the first data on this issue. Population genomic comparisons of two serpentine and two granitic populations of *A. lyrata* in North America identified ~96 candidate genes implicated in serpentine adaptation based on large SNP allele frequency differences between the two habitats (Turner *et al.* 2008, Turner *et al.* 2010). Follow-up on three strong candidate genes in a UK population pair uncovered different candidate SNP alleles than those in the US populations, suggesting independent mutational origins (Turner *et al.* 2010). These studies present only a preliminary view of parallel adaptation to serpentine, particularly since it is not known which of the candidates have measurable effects on serpentine adaptation.

### **2.1.3 Potential molecular mechanisms of serpentine tolerance**

None of the major loci contributing to serpentine adaptation have been resolved at the gene level in any plant system and therefore virtually nothing is known about the

underlying physiological, cellular, and molecular mechanisms. However, work on the molecular mechanisms of plant ion uptake and homeostasis, metal tolerance and stress response suggest possible mechanisms for dealing with the challenges presented by serpentine soils. There are several ways plants can maintain adequate internal Ca levels; for example, by altering the expression or activity of transporters involved in Ca homeostasis. A large-scale screen for *Arabidopsis thaliana* mutants able to survive in a serpentine-like medium (low Ca:Mg ratio) identified loss of function mutants in CAX1 (Bradshaw 2005), a vacuolar transporter that sequesters Ca in the vacuole (Hirschi et al. 1996). Another way of controlling ion uptake is through variation in suberin and Casparian strip deposition which control the flow of water and solutes to the xylem in roots. While Ca and Mg are generally positively correlated in plants (White and Broadley 2009), the *esb1 A. thaliana* mutant has a 50% decrease in total leaf Ca accumulation with little or no change in Mg accumulation (Baxter et al. 2009) suggesting that differences in suberisation and Casparian strip functionality at the endodermis could be a possible mechanism to alter Ca and Mg uptake. Finally, a major consequence of heavy metal accumulation is the production of reactive oxygen species (ROS) which can cause widespread cellular damage. There are several ways that plants may deal with high levels of heavy metals by chelation or vacuolar compartmentalization of these compounds. Numerous genes have been identified that carry out these functions and have been shown to affect Ni tolerance in the Ni hyperaccumulating species *Nocca*

*goesingense* (formally *Thlaspi goesingense*), *Noccea caerulescens* (Vacchina et al. 2003, Mari et al. 2006, Callahan et al. 2007) and Zn tolerance in *Arabidopsis halleri* (Deinlein et al. 2012). However, the fitness effects of naturally segregating variation at loci involved in these molecular processes has not been investigated.

In this study, I use QTL mapping approaches to identify loci that contribute to survival differences on serpentine soils between serpentine and non-serpentine populations of *M. guttatus*. I also map QTLs for tolerance to low Ca:Mg ratio in hydroponic culture and look for co-localization between survival and hydroponic QTLs to see whether differential ability to tolerate the low Ca:Mg ratios that typify serpentine soils may contribute to the survival differences observed in the field. Finally, I investigate whether serpentine tolerance has a shared genetic basis in geographically widespread *M. guttatus* populations by testing whether serpentine tolerance QTLs identified in one population are also linked to survival differences in an independent F2 mapping population.

## **2.2 Methods**

The incredible ecological diversity of *M. guttatus* is coupled with genetic resources that make it a model system for ecological genomics (Wu et al 2007). *M. guttatus* has a relatively small genome (~450 Mbp), but with 14 chromosome pairs a long genetic map length (~2000 cM). Genetic dissection of complex traits is enabled because on average 1 cM  $\approx$  215 kb, but in gene rich regions 1 cM  $\approx$  40-70 kb, or ~12 genes. The

Willis lab has led collaborative efforts to develop genomic tools for *M. guttatus*, including extensive EST and RNA-seq data, over 1,000 highly polymorphic PCR gene-based markers, fingerprinted BAC libraries, and integrated genetic and physical maps.

### **2.2.1 Mapping QTLs that control survival differences on serpentine soils**

In the spring of 2010, I conducted a reciprocal transplant experiment at the McLaughlin Reserve (McL) as described in Chapter 1. In that experiment, nearly all plants that originated from non-serpentine habitats died in the juvenile stage while plants from serpentine habitats enjoyed high survival. An F2 mapping population derived from a cross between a local serpentine/non-serpentine population pair (REM/SOD, Table 1) was also randomized within field plots at serpentine and non-serpentine field sites allowing me to characterize the genetic basis of these survival differences. Just prior to flowering, F2s were removed from the field plots and transplanted to 2.5-inch square pots filled with potting soil. These pots were kept outside at the McLaughlin Reserve and watered daily. At the end of the field experiment (early June 2010) the F2s were removed from the potting soil and shipped overnight to Duke University where they were re-transplanted to potting soil and placed in the greenhouse. I was only able to collect tissue from 44 out of 146 F2 survivors at the serpentine sites and 220 out of 303 survivors from the non-serpentine sites. The F2s that I collected tissue from in both habitats do not differ significantly from those that died prior to tissue collection. I used one-way analysis of variance (ANOVA)

to test for differences in rosette diameter, flowering time, plant height or leaf length between the F2s used for genetic analyses and the entire pool of F2 survivors; none of these variables were significantly different between the groups. There was a significant difference between the two serpentine sites with one site, QVO, only having 8 F2s that survived to tissue collection. The QVO site dried out rapidly compared to the other site, REM, and I removed all the F2s 2-3 weeks before the end of the experiment in hopes of rescuing many of these in order to collect tissue but many were too stressed and died after transplantation to potting soil.

In order to rapidly map QTLs controlling the survival differences on serpentine soil between serpentine and non-serpentine plants I used bulk segregant analysis (BSA; Michelmore et al. 1991). BSA has frequently been used to quickly but crudely map major QTLs using traditional markers and with next-generation sequencing techniques that rapidly quantify allele frequencies at densely spaced markers, QTLs can be mapped with greater precision (Magwene et al. 2011). I sequenced two pools (bulks) of individuals: those F2s that survived at the McL serpentine sites (n=44) and those that survived at the McL non-serpentine site (n=220). For the serpentine survivors I collected 4 samples containing 0.1g of bud tissue from each of 11 individuals in 15-ml tubes and froze it at -80°C. For the non-serpentine survivors I collected one sample containing a single bud from each of the F2 survivors. DNA was then extracted from the samples following a urea extraction protocol modified from Shure et al. (1983). The

concentration of each of the four serpentine samples was measured using a Qubit flourometer (Invitrogen, Grand Island, NY, USA) and the samples were then combined in equimolar quantities. Each bulk of genomic DNA was submitted to the Duke Genome Sequencing and Analysis Core Resource for library preparation and then sequenced on two lanes of an Illumina GAII machine for 75 base-pair, single-end reads.

Raw reads were checked for quality using FastQC (Andrews 2010) and then aligned to the *M. guttatus* reference genome v2.0 (<http://www.phytozome.net>) using BWA v.6.2 (Li and Durbin 2010) with all settings left at default and the single-end alignment option (*samse*). Three open-source packages were used for downstream processing and variant calling: Genome analysis toolkit (GATK; McKenna et al. 2010), SAMtools (Li et al. 2009), and Picard Tools. The alignment files generated by BWA were sorted using Samtools. Picard (*CleanSam*) was then used to soft-clip an alignment that hangs off the end of the reference sequence and to set MAPQ to 0 if a read is unmapped. Local realignment of reads around indels was performed with GATK (IndelRealigner) as indels relative to the reference can result in many base mismatches which can be mistaken for SNPs. Finally, SNPs were called on the combined read files from both pools using the UnifiedGenotyper in GATK. Using custom R scripts written by Chenling Xu, SNPs were then filtered to only retain those with a mapping quality  $\geq 30$  and at least 3 $\times$  but no greater than 30 $\times$  coverage. The frequency of each allele was then calculated in both pools by dividing the allele counts for each SNP by the total coverage

at that site. To account for low coverage and low read counts for any given SNP, a sliding window analysis was used that calculated the difference in allele frequency between the two pools averaged across 2000 SNPs with a step size of 500 SNPs. Differences in allele frequencies are expected to be close to zero at unlinked markers; however, at markers closely linked to a survival QTL allele frequency differences should increase between the pools.

Bulk segregant analysis does not provide information on the phenotypic effect sizes of QTLs as it is based only on differences in allele frequencies. In order to confirm putative QTLs identified via BSA as well as estimate the effect sizes of these QTLs, I genotyped all of the individual F2 survivors from the McL field sites for markers in regions of elevated allele frequency difference identified in the BSA. DNA was extracted from all of the F2s using a modified CTAB protocol (Kelly and Willis 1998). I first screened the inbred parental lines for polymorphism using exon-primed intro-crossing (EPIC) markers derived from expressed sequence tags (ESTs). Polymorphism was evaluated in terms of variation in the length of PCR products which is typically caused by indel variation in the introns and the size of the amplified fragments was scored using the program Genemarker (SoftGenetics, State College, PA, USA). The development of these markers is outlined elsewhere (Fishman et al. 2008) and the primers can be found at <http://www.mimulusevolution.org>. The markers chosen (Table 12) to screen in the F2s were polymorphic and of different sizes which allowed for

multiplexing of the amplified products for capillary electrophoresis and fragment analysis on an ABI 3730xl DNA Analyser (Applied Biosystems, Foster City, CA, USA) by the Duke University Genome Sequencing and Analysis Core Resource.

All F2 survivors from the McL field sites were genotyped at multiple markers in putative QTL regions as well as a single marker outside of any QTLs. Markers were tested for goodness of fit with Mendelian (1:2:1) expectations using a  $\chi^2$  and significance of p values were confirmed using a Monte Carlo randomization test (10,000 trials) in R because of small sample sizes. We would predict that the genotypic ratio of the F2 survivors from the serpentine site will deviate from 1:2:1 at QTLs which control the ability to survive on serpentine soils. However, as there were no survival differences between serpentine and non-serpentine plants at the off-serpentine site (Chapter 1) we do not predict that genotype frequencies will deviate from Mendelian expectations. Based on the assumption that the F2 populations that were planted initially fit a 1:2:1 ratio of genotypes at the LG13 QTL marker (which is reasonable given the non-serpentine survivors do not show any segregation distortion in this genomic region), I calculated the survival rate for each of the three genotypes based on the genotypic frequencies of the F2 survivors at the serpentine sites. Assuming that the genotype frequencies of the F2s I was able to collect tissue from are representative of the overall genotypic frequencies of the total pool for F2 survivors (including those individuals who died prior to tissue collection), I calculated survival rate as the total

number of individuals of a given genotype that survived divided by the total number of individuals of that genotype that were planted. Using this survival rate as a fitness measure, selection coefficients were then calculated as  $1-w_{12}$  or  $w_{22}$  where  $w_{12}$  and  $w_{22}$  represent the relative fitness of the heterozygote and the non-serpentine homozygote respectively.

As described in Chapter 1, I conducted two other reciprocal transplant experiments to test for local adaptation of *M. guttatus* to serpentine soils. The first was simultaneous with the McL experiment above but was conducted at the Red Hills (RH) serpentine area in the Sierra Foothills (~300km away from McL) using local populations as well as F2s (derived from crosses between SLP and KFY parental lines, Table 1). In order to see whether QTLs identified in the BSA also control the ability to survive on serpentine soils in a geographically distant population, I genotyped individual F2 survivors from the serpentine (n=25) and non-serpentine (n=84) RH field sites. I also planted RH F2s (derived from a cross between SLP and TUL parental lines, Table 1) on native serpentine in the lab and I genotyped a random sample of survivors (n=119) from this experiment at QTLs as well. I conducted a second reciprocal transplant study at McL in 2012 which included F3s from the same parental lines (REM/SOD, Table 1) used for the BSA analysis above. The surviving F3s from both serpentine (n=116) and non-serpentine (n=260) field sites were genotyped at QTL. For all three experiments I calculated selection coefficients as above.

The reciprocal transplant experiments described in Chapter 1 found that non-serpentine plants grew larger than serpentine plants at the RH and this difference was evident in both the juvenile (rosette diameter) and adult (height and leaf length at flowering) stages. In order to see whether the survival QTL identified above also controls these differences in plant size, I conducted nested ANOVAs for each of the three field experiments to see if there were significant differences in rosette diameter between the three genotypes while controlling for block and plot effects.

## **2.2.2 Mapping QTLs for hydroponic tolerance to low Ca:Mg ratio**

I conducted a second BSA in order to identify QTLs that control differences in tolerance to low Ca:Mg ratio between serpentine and non-serpentine plants. I created two pools of F2s from the McL hydroponic growout described in Chapter 1 (parental lines = REM/SOD, Table 1). Tolerant (n=220) and non-tolerant (n=140) pools were determined based on effective concentration 100 (EC100, described in Chapter 1). I collected 0.1g of bud tissue from 10 individuals in a single 15mL-tube and then extracted each tube individually, quantified the DNA concentration using a Qubit and pooled the individual samples in equimolar concentrations into a “tolerant” and “non-tolerant” pool. Pools were submitted to the Genome Sequencing and Analysis Core Resource for library prep and sequencing on the Illumina HiSeq for 100bp single-end reads. Each pool was sequenced on a separate lane which provided 160 million raw reads for each pool (Table 10). These reads were then aligned to the *Mimulus guttatus* reference

genome and SNPs were called as described above. SNPs were parsed based on quality (>30) and coverage (between 6x and 70x inclusive) leaving 3.4 million SNPs that had a mean coverage of 18x. Allele frequency difference between the pools was analyzed as above using a sliding window with a 2000 SNP window size and 500 SNP step size.

Given the higher coverage provided by the HiSeq platform (compared to the GA II), the mean allele frequency difference between the pools outside of QTL regions should not be as high as in the survival BSA.

As above, putative QTLs (i.e. regions of large allele frequency difference) were confirmed using PCR-based markers screened in a random sample of 96 individual F2s. I conducted one-way ANOVA to test for an association between marker genotype and EC100. Newly identified QTLs were also tested for their effects on survival on serpentine soils by genotyping the survivors from the serpentine field sites and the plate experiment to see whether other these QTL may also affect survival but may not have been detected in the field BSA due to low power.

### **2.2.3 Fine mapping**

I used recombinant backcross progeny testing to narrow the survival QTL interval by scoring backcross progeny's ability to survive on native serpentine soil in the lab. I genotyped the F2s from the hydroponic assay above at 6 markers across the QTL region that spanned ~1600kb at the very end of LG13 (Figure 15). I identified individuals that had recombination events between each of these markers and

backcrossed them to the non-serpentine parent. I also backcrossed a single non-recombinant F2 for each genotypic class. The non-serpentine inbred parental line was used as the pollen parent for all crosses. Due to the dominance of serpentine tolerance we expect an F2 that is homozygous for the serpentine allele will result in all heterozygote progeny and these progeny should enjoy high survival rates on serpentine soil. An F2 that is heterozygous at the QTL region will result in 50% progeny who are homozygous non-serpentine and 50% that are heterozygous. The progeny that are homozygous non-serpentine will not survive while those that are heterozygous will survive, resulting in an overall survival rate of ~50%. F2 individuals who are homozygous non-serpentine will produce progeny who are all also homozygous non-serpentine and they will have low survival.

Seeds from all the backcrosses as well as both parental lines were planted on 50mm petri plates, covered with ultrapure water and stratified for 5 days at 4°C. Seeds were then moved to a growth room, allowed to germinate and then transplanted via pipettor to plug trays (288 cells/tray, each cell = 3/4" x 3/4" x 1.5" deep) filled with serpentine soil from the McLaughlin Reserve. The soil used for this study came from a road cut about ~0.8km away from the home site of the serpentine parent and is the same soil used for the common-garden experiments described in Chapter 1. Soil from the same collection location was also used by Palm *et al.* (2012) and they show that it is not significantly different from the serpentine soil at the parental site. For each line I

planted 96 replicates (n=64 for each parental line) and randomized lines within trays. The trays were watered with ultrapure water and survival was scored weekly for 4 weeks.

## **2.3 Results**

### **2.3.1 Field survival mapping**

The Illumina sequencing generated 55-60 million 75bp SE reads for each pool of the survivors from the two field habitats. Of these reads, ~60% were mapped to the reference genome which is not unexpected given the highly repetitive nature of the *M. guttatus* genome and the fact that the assembly only contains 320 of the 430 total megabases. GATK called ~3.6 million SNPs (Table 11) however many of these sites were filtered out due to low (<3x) or high (>30x) coverage. Furthermore, sites where both pools were fixed for an alternate allele from the reference sequence were common and were also removed leaving ~1.3million high quality SNPs which had a mean coverage of 10x and 8.5x in the serpentine versus non-serpentine pools respectively (Table 11). Due to the low coverage, the mean allele frequency difference between the pools averaged across the genome was 22.6%. Nonetheless, the sliding window analysis identified two regions of elevated allele frequency difference (Figure 10): one on linkage group (LG) 1 and another on LG13 that were the only regions in the genome that were outliers at the 99 percentile. The peak allele frequency difference between the pools in these regions is 27.5% (LG1) and 30.5% (LG13). The difference between the LG13 peak and the genome-

wide mean allele frequency difference is ~8%. Given that there were no survival differences between serpentine and non-serpentine plants at the non-serpentine site we expect the F2 survivors from this site to have both alleles present at roughly 50%. The survivors from the serpentine site should have an allele frequency that, given the dominance of the serpentine tolerance phenotype, is closer to 66.7% therefore the maximal allele frequency difference that we expect between these two pools is ~16.7%.

Genotyping individual F2 survivors from the McL serpentine site at markers within both of these putative QTL regions confirmed the QTL on LG13 only. There were significant deviations from the expected Mendelian ratio of 1:2:1 in the serpentine survivors at marker MgSTS419 in LG13 but not at MgSTS436 on LG1 (Figure 11 and Tables 12 and 13). The non-serpentine allele when homozygous is completely lethal on serpentine soils ( $s=1$ ; Table 13). The difference in survival rate between the alternate homozygotes at MgSTS419 explains 86% of the survival differences between the serpentine and non-serpentine parents. The overall frequency of the serpentine allele was 69.8% and the genotypes of the survivors were not significantly different from a 1:2 ratio of serpentine homozygotes to heterozygotes ( $\chi^2=0.74$ , 1d.f.,  $p=0.389$ ) which is consistent with the serpentine allele being dominant. However, serpentine tolerance is not completely dominant and the heterozygotes have a slightly lower survival rate compared to the serpentine homozygotes reflected in the selection coefficient ( $s=0.19$ , Table 14). I also genotyped two markers flanking 419– MgSTS117 which is ~750kb away

and MgSTS557 which is ~325kb to the other side of 419 (Table 12). Both of these markers show significant deviations from Mendelian expectations (117:  $\chi^2=12.2$ , 2 d.f.,  $p<0.002$  ; 557:  $\chi^2=15.3$ , 2 d.f.,  $p<0.001$ ). There was a single recombinant between 117 and 419 which was heterozygous at 419 and homozygous non-serpentine at 117 suggesting that the causal locus must be between 117 at 20,109,853bp and the end of LG13 ~1100kb away. The genotype ratios of all (n=220) the survivors from the non-serpentine site do not differ significantly from 1:2:1 at 419 (Figure 11, Table 13). I also genotyped a subset of these individuals (n=52) at 117 and 557 and similarly these markers do not show deviation from a 1:2:1 (117:  $\chi^2=3.2$ , 2 d.f.,  $p=0.20$ ; 557:  $\chi^2= 1.5$ , 2 d.f.,  $p>0.30$ ). The genotyping results from the McL 2012 reciprocal transplant experiment are similar to those described for 2010 (see Figure 11 and Table 13). However, selection coefficients were slightly smaller (Table 14) and 9.5% of the survivors at the serpentine site were homozygous for the non-serpentine allele. The difference between the two years is likely due to the fact that the experiment in 2012 was terminated nearly 5 weeks earlier than the 2010 experiment.

The genotype ratios of the survivors from the RH reciprocal transplant experiment show that the same genomic region on LG13 also controls differences in survival on serpentine soils in this geographically distant population. The genotypic ratio of the survivors from the serpentine field sites does not fit the expected 1:2:1 Mendelian ratio while the same marker (MgSTS310, Table 12) in the non-serpentine

survivors does not differ significantly from this expectation (Figure 11 and Table 13). Genotyping the survivors from the RH lab-based common-garden experiment also reveals that the survivors deviate from expected genotypic ratios (Figure 11 and Table 13). As at McL, selection is very strong against the non-serpentine homozygotes in both the field ( $s=0.89$ ) and the lab ( $s=0.9$ ; Table 14). Serpentine tolerance is only partially dominant as the heterozygotes are still selectively disadvantaged (field:  $s=0.17$ ; lab:  $s=0.36$ ; Table 13). The similarity between the field and the plate experiments clearly demonstrate that the LG13 QTL controls differences in tolerance to soil variables and not some other environmental variables that may differ between serpentine and non-serpentine habitats.

### **2.3.2 The effect of the LG13 QTL at non-serpentine field sites**

Genotyping the F2 survivors from the non-serpentine field sites for both regions (McL and RH) from 2010 and McL in 2012 did not reveal a cost to the serpentine allele in non-serpentine sites based on the traits measured. There were no significant differences in rosette diameter, flowering time or plant height or leaf length at flowering between the different LG13 marker genotypes in any of the three experiments (Table 15).

### **2.3.3 The genetic basis of tolerance to low Ca:Mg in hydroponic feeds**

The BSA for tolerance to low Ca:Mg differences between the McL population pair identified three regions of elevated allele frequency difference between the tolerant and non-tolerant pool. The BSA identified a putative QTL on the end of LG13 in the

same region as the survival QTL as well as two new putative QTL regions which show elevated allele frequency differences on LG6 and LG12 (Figure 12). Genotyping of a random sample of 96 individual F2s from the McL hydroponic screen of tolerance to low Ca:Mg ratio at MgSTS419 (the same marker used in the McL field survivors) confirms the LG13 QTL, where marker genotype has a significant effect on differences in tolerance to low Ca:Mg ratio ( $F_{2,82}=64.157$ ,  $p<0.0001$ , Figure 13). Genotype at this marker explains 31% of the total phenotypic variation ( $R^2=0.31$ ). I also genotyped a subset ( $n=96$ ) of these F2s at a marker (MgSTS 508, Table 12) in the putative QTL region on LG6 and find that this marker is also linked to tolerance to low Ca:Mg ratio ( $F_{2,82}=19.405$ ,  $p=0.017$ ) and explains 9% percent of the total variation in this trait ( $R^2=0.09$ ). I conducted a two-way ANOVA to investigate the main effects of genotype at both markers as well as the interaction between them on tolerance to low Ca:Mg. The interaction term is not significant ( $F_{4,76}=4.111$ ,  $p=0.652$ ) indicating that these two loci interact additively to control differences in tolerance to low Ca:Mg ratio (Figure 13). I have not verified the putative QTL region on LG12.

In order to see whether the LG6 QTL also contributed to survival differences on serpentine soil but may have been below the power of detection due to small sample sizes and low coverage in the BSA, I genotyped the survivors from the McL2010 field experiment and the RH plate experiment. The LG6 marker (MgSTS25, Table 12) does not differ from an expected 1:2:1 ratio in the McL field survivors ( $\chi^2=1.14$ , 2 d.f.,  $p=0.283$ );

however, it does in the RH plate survivors ( $\chi^2=7.1$ , 2 d.f.,  $p=0.026$ ; Table 13). The selection coefficient against the non-serpentine homozygotes is  $s=0.7$  and for the heterozygotes  $s=0.28$ . Furthermore, looking at the two locus genotypes for the LG13 and LG6 QTLs among these RH survivors shows that there are survivors that are homozygous for the non-serpentine allele at both loci (Table 16).

### **2.3.4 Fine mapping to narrow the QTL region**

The parental lines (REM and SOD), had significantly different survival rates on the serpentine soil (REM = 92%; SOD=0) while the backcross F3s that were non-recombinant throughout the LG13 QTL had percent survival not significantly different from the expectations described in the Methods (Figure 14). The non-recombinant backcross progeny from the F2 serpentine homozygote had ~80% survival while the progeny from the heterozygote had ~50% and the progeny from the non-serpentine homozygote 2.6%. The survival rates of the recombinant individuals suggest that the causal locus lies between markers MgSTS419 and MgSTS601 (Figure 14). These two markers are the most tightly linked to survival differences. Individuals who are homozygous serpentine at these markers have high survival while those who are heterozygous have an intermediate survival rate and those who are homozygous non-serpentine have a low survival rate. However, there is significant variation between genotypic classes suggesting that there may be other modifying loci that account for background effects. I have cytoplasm information for all the lines; however, I do not

have each genotype at the QTL represented on both cytoplasms. For individuals who are heterozygous at the QTL for which I did have both cytoplasm types represented there was no significant difference in survival due to cytoplasm ( $G=0.35$ ,  $p=0.56$ ).

The fine mapped region is ~190kb long and contains 41 genes based on the most recent *M. guttatus* annotation available on phytozome (Table 17). I used the *M. guttatus* annotation and looked at protein domain and gene ontology annotations for the 41 genes in the fine mapped region. I also blasted the *M. guttatus* coding sequence against the *Arabidopsis thaliana* genome in The Arabidopsis Information Resource database to identify *A. thaliana* homologs. David Salt searched the ionomics database ([www.ionomicshub.org](http://www.ionomicshub.org)) to see whether any of these *A. thaliana* homologs have known ionomic phenotypic affects in either *A. thaliana* or yeast. None of the loci in this region have known ionomic affects though many of the homologs have not been examined.

In continuing to work with my BSA sequencing data, I discovered that there is a gap in the genome assembly within the fine mapped interval (Figure 15). I had not noted this earlier because I only performed sliding window analyses on the SNP data and looked at coverage across the genome on a broad scale. However, when I plotted coverage zoomed in on the fine mapped region (Figure 15), I observed that a portion of this region had no reads that mapped to it. This region falls right where two small scaffolds from an earlier version of the genome assembly (v1.0) are joined together on LG13 in the current assembly. The old assembly was produced using shot-gun

sequencing methods but was highly fragmented due to the repetitive nature of the *M. guttatus* genome and consisted of over 2,000 sequence scaffolds. Despite the inclusion of paired end reads from ~70,000 BACs, the largest of these scaffolds was only about 5 Mb, so there was nothing approximating chromosome-length sequence assemblies. The current assembly used linkage in a recombinant inbred line population to genetically map and orient these scaffolds into chromosomal-scale “pseudo-molecules”(referred to linkage groups). The current assembly consists of ~320Mb (out of a total 430Mb) assembled into 14 linkage groups as well as several hundred small scaffolds which were not mapped based on linkage analysis.

At the gap within my QTL interval, two of these scaffolds (83 and 115) are joined on linkage group 13. The linkage analysis tells us that these two scaffolds are linked but the sequencing data has no BACs or pair-end reads that connect these two scaffolds. So we do not know the actual physical distance between the scaffolds on LG13. A standard number of “n’s” are inserted into the reference sequence between these scaffolds and that is why I see the drop in coverage in this region in my BSA data (Figure 15). Each of these old scaffolds were broken up in the middle and part of each of them assembles to LG13 (Figure 15). It is likely that there is some repetitive sequence that both scaffolds 83 and 115 share which is why they were initially put together in the old assembly but are now known to be linked on LG13. If this missing piece of LG13 is large and highly

repetitive that might explain why there are no BACs that connect the unique sequences in scaffolds 83 and 115.

In an effort to find identify the missing piece of LG13, I used BSA to compare two pools of F2 individuals that were homozygous for the alternate alleles at markers flanking the fine-mapped region. The expectation is that SNPs within the LG13 QTL region will show fixed allele frequency differences between the pools but should be roughly equivalent outside of this region. However, if the missing fragment of LG13 is in the genome assembly – either mis-assembled on one of the 14 linkage groups or on one of the small scaffolds – this region should also show fixed allele frequency differences between the pools. I created the DNA pools using F2 individuals from the hydroponic experiment above which had been genotyped at markers MgSTS419 and MgSTS601 that define the fine-mapped region (Figure 15). One pool contained only individuals homozygous for the serpentine allele at both flanking markers (n=48) and the other only individuals homozygous for the non-serpentine allele at these markers (n=36). The two pools were sequenced on an Illumina HiSeq RapidRun for 150bp paired-end reads. I conducted assembly and SNP calling as above and conducted sliding window analyses to look for highly differentiated regions of the genome. The allele frequency difference between the pools within the LG13 QTL region was ~1 as expected; however, there were no other regions of the genome that showed fixed or highly elevated allele frequency differences.

## **2.4 Discussion**

### **2.4.1 A large effect QTL controls the ability to survive on serpentine soils in *M. guttatus***

A long-standing question in evolutionary biology is the distribution of effect sizes that contribute to adaptive traits. The experiments presented here show that a major QTL contributes to local adaptation of *M. guttatus* populations to serpentine soils. The bulk segregant analysis identified a single region of the genome that had large allele frequency differences between F2 survivors at serpentine versus non-serpentine field sites in the McLaughlin 2010 reciprocal transplant study. This putative QTL on the end of chromosome 13 (LG13) was confirmed by genotyping individual F2s at markers within the interval of elevated allele frequency difference. The non-serpentine allele is completely lethal on serpentine soils as no individuals who are homozygous for this allele survived at the serpentine field sites. This QTL also controls survival differences across years and in a geographically distant (~300km away) serpentine population. In both populations and across years, selection coefficients indicate extremely strong selection against the non-serpentine allele at serpentine field sites. Selection coefficients against individuals who are homozygous for the non-serpentine allele range from 0.71 to 1 in the three different reciprocal transplant experiments. In all cases, while individuals who are heterozygous at this locus have significantly higher survival rates than the non-serpentine homozygotes, they still have lower relative fitness than individuals who are homozygous for the serpentine allele ( $s=0.17-0.19$ ). The genotype

frequencies of F2 individuals that survive on serpentine soils in the lab show similar deviations from Mendelian expectations as observed in the field, demonstrating that differences in tolerance to variables in the soil matrix alone explains much of the survival differences between serpentine and non-serpentine *M. guttatus* plants.

I also identified a second locus that controls serpentine tolerance in *M. guttatus* on chromosome 6 (LG6). This locus was identified via a bulk segregant analysis on difference tolerance to low Ca:Mg ratio in hydroponic feeds between the McLaughlin populations. This locus explains a smaller percentage of the variance in tolerance to low Ca:Mg ratio than the LG13 QTL (9% versus 31%) and the two loci interact additively to control tolerance differences. This locus does not contribute to survival on serpentine soils in the field in the McLaughlin population and genotype frequencies at this locus fit a 1:2:1 Mendelian expectation. However, I was only able to genotype a relatively small number (n=44) of F2s from the serpentine field sites and it is possible that the LG6 QTL may have more subtle, modifying effects which I would have limited power to detect.

This second QTL on LG6 was shown to contribute to survival differences on serpentine soils between the Red Hills populations. F2s grown on Red Hills soil in the lab show a deviation from Mendelian expectations at the locus and furthermore, the two locus genotypes of survivors at both QTLs reveal no survivors that are homozygous for the non-serpentine allele at both QTLs. In both the field and lab-based experiments presented in Chapter 1 for the Red Hills population, the F2s had survival rates that were

~50% lower than the serpentine parents which suggested that survival was likely controlled by more than a single locus. In order to understand the effects of both QTLs on survival, the common-garden experiment needs to be conducted to look at survival to flowering. It is not clear from the current genotypic results what combination of genotypes at the two QTLs confer the ability to survive on serpentine soils in the Red Hills, simply that both loci affect survival though based on the selection coefficients LG13 QTL has the largest effect.

The genetic basis of local adaptation is not well understood but theoretical models often assume antagonistic pleiotropic gene action (Levene 1953) because then no recombinant genotype can have high fitness in both habitats. When local adaptation occurs with migration, as is likely the case with the evolution of serpentine tolerance in *M. guttatus*, alleles that confer high fitness in one habitat must come at a cost in the alternate habitat (Kawecki and Ebert 2004). However, in the current study I did not find evidence for a genetic trade-off at the major QTL on LG13. I measured several plant size traits (rosette diameter, plant height and leaf length at flowering) at non-serpentine field sites. Differences between serpentine and non-serpentine plants for these traits were only observed in the Red Hills population; however, other work on the McLaughlin population pair has found that the non-serpentine population is bigger than the serpentine population when grown in potting soil in the greenhouse (Palm *et al.* 2012). However, there were no significant differences in any of these size traits between the

three genotypes at the LG13 QTL for F2s growing at the non-serpentine field sites.

These traits may not be correlated with fitness in the non-serpentine habitat and as I was not allowed to let the experimental plants flower in the field, I was limited as to fitness-related components that I could measure. Serpentine tolerance is not present at high frequency in the non-serpentine *M. guttatus* populations that I have tested (Chapter 1) suggesting that there is a cost to this allele in the non-serpentine environment.

#### **2.4.2 The genetic basis of parallel adaptation to serpentine soils**

Serpentine soils are patchily distributed and vary substantially in their physiochemical properties (Whittaker 1954, Proctor et al. 1975, Kruckeberg 1984, Alexander et al. 2007); however, and it is not known whether widespread species, such as *M. guttatus*, repeatedly adapt to different serpentine patches via the same or different molecular mechanisms. However, a recent study in *A. lyrata* offers the first data on the genetic basis of parallel adaptation to serpentine soils. Population genomic comparisons of two serpentine and two granitic populations of *A. lyrata* in North America identified ~96 candidate genes implicated in serpentine adaptation based on large SNP allele frequency differences between the two habitats (Turner *et al.* 2008, Turner *et al.* 2010). Follow-up on three strong candidate genes in a UK population pair uncovered different candidate SNP alleles within these genes than those in the US populations, suggesting independent mutational origins (Turner et al. 2010). However, the effects of these candidates on serpentine adaptation are not known.

I found that the same major QTL controls the ability to survive on serpentine soils in two geographically distant *M. guttatus* populations in California. While I do not know whether these two populations represent independent evolutions of serpentine adaptation, these populations have differential tolerance to each other's soil (Chapter 1). The serpentine population from the McLaughlin Reserve has significantly lower survival when planted on the Red Hills soil compared to its home soil. In contrast, the Red Hills population has high survival on both soil types. These results suggest that some soil variable differs between these two serpentine soil types (e.g. concentration of heavy metals such as Ni or the absolute concentrations of Ca and Mg) such that the McLaughlin population has lower fitness on the Red Hills soil. Despite the fact that these two population share the major LG13 QTL, these differences in tolerance between the populations suggests that either other loci are involved in serpentine tolerance which are not shared between the populations or that the populations have different alleles at this QTL which have different physiological effects.

There are significant differences in tolerance to low Ca:Mg ratio growth environment between serpentine and non-serpentine populations from both McLaughlin and the Red Hills (Chapter 1). The bulk segregant analysis and subsequent genotyping results identify a QTL on LG13 that controls differences in tolerance to low Ca:Mg in the McLaughlin population pair which co-localizes with the field survival QTL. This co-localization suggests that differences in the ability to grow in low Ca:Mg

ratios contributes to the survival differences observed in the field. The preliminary analysis of Ca levels in leaf tissue presented in Chapter 1 shows that the Red Hills serpentine population has significantly lower Ca levels from the other serpentine populations examined and these differences suggest that the Red Hills population may have a different underlying physiological basis of tolerance to serpentine soils.

Unfortunately, the McLaughlin serpentine population was not analyzed as a part of that study so a direct comparison of the two populations is not possible but the other serpentine tested are from nearby the McLaughlin population in the Northern Coast Range. The effects of LG13 QTL genotype on leaf Ca levels in both the McLaughlin and Red Hills populations needs to be tested to determine whether this QTL is linked to leaf Ca levels and whether there are differences between the two populations.

### **2.4.3 Candidate genes involved in serpentine adaptation in *M. guttatus***

I have localized the LG13 QTL to a 190kb region which contains 41 genes. The hydroponic tolerance testing and QTL mapping shows that this QTL controls differences in tolerance to low Ca:Mg ratio observed between serpentine and non-serpentine populations of *M. guttatus*. None of the genes within the fine mapped interval have known functions in Ca or Mg uptake or transport. However, there are several genes that have transporter activity as well as a couple genes involved in metal binding or transport (Table 16). Furthermore, this region contains a homolog of one of the putative serpentine adaptation genes in *A. lyrata* (Turner et al. 2010) that is in the RING/U-Box

superfamily and involved in zinc ion binding. A glutathione S-transferase (GST) gene known to be involved in stress response and heavy metal tolerance is also found in this interval. A major consequence of heavy metal accumulation is the production of reactive oxygen species (ROS) which can cause widespread cellular damage.

Glutathione and its metabolizing enzymes such as GST have been found to protect plants from oxidative damage and aid in detoxification, complexation, chelation and compartmentalization of heavy metals. Work in the Ni hyperaccumulating species *Noccea goesingense* suggests that elevated levels of reduced glutathione (GSH) play an important role in Ni tolerance in this species (Freeman et al. 2004). Further fine-mapping will be necessary in order to identify the causal locus within the LG13 QTL region and to determine whether the gene is contained on the fragment of LG13 in this interval which is unassembled.

## **2. 5 Conclusion**

A major goal of current evolutionary genetics is to identify the genes that contribute to adaptive traits. However, this aim of characterizing the genetic basis of adaptive traits by using forward genetic approaches has been questioned because it is argued that the types of variants that QTL mapping approaches are capable of detecting do not represent the vast majority of segregating adaptive variants (Rockman 2012). However, we have very few empirical examples where we understand the fitness effects of naturally segregating variants. Many of the empirical examples do find major gene

control of adaptive traits (e.g. Kohn et al. 2000, Colosimo et al. 2004, Hoekstra et al. 2006, van't Hof et al. 2011) and this study provides another example of this.

While such major genes may not be representative of the vast majority of segregating adaptive variants, they do provide excellent systems for investigating the evolutionary processes that have created and maintain adaptive variation as well as identifying the specific genes involved in order to gain a mechanistic perspective of adaptation. We need more empirical studies that integrate investigations of fitness in the field with genetic and molecular dissection of ecologically relevant traits. By combining field and lab-based QTL mapping studies, the experiments presented here identify a major effect QTL that controls the ability to survive on serpentine soils in *M. guttatus* by altering plants' ability to cope with the low Ca and high Mg levels that characterize these habitats. The strength of selection against non-tolerant alleles is extremely strong in these serpentine habitats and adaptation to serpentine soils has likely occurred in the face of gene flow. Recent theoretical models show that when there is selection for local adaptation with gene flow fewer loci with larger effect sizes are expected to contribute to adaptive differences (Yeaman and Otto 2011, Yeaman and Whitlock 2011). The major gene control of serpentine tolerance in *M. guttatus* is perhaps then not unexpected and provides an example where understanding the evolutionary dynamics of the system can help to predict the genetic architecture of an adaptive trait. Future work aimed at cloning the major LG13 QTL will not only provide insight into the physiological

mechanisms of adaptation to serpentine soils but will also address questions of parallel adaptation to serpentine soils in *M. guttatus* such as whether widespread serpentine populations have adapted to serpentine soils via the same mutations at this shared QTL. These questions of parallel mutation and the reuse of standing variation are central to the study of the genetics of adaptation.

Table 11: Summary of next-gen sequencing data used for bulk segregant analysis. For the Field BSA, pool names refer to F2 survivors from serpentine and non-serpentine field sites. Depth of coverage given is the mean number of reads covering each SNP in the filtered dataset. SNPs numbers given in millions.

Experiment	Bulk	Pool size	# raw reads	Read type	Depth of coverage	# SNPs pre-filtering	# SNPs post-filtering
Field BSA	Serpentine	44	~60	75bp	10x	3.57	1.34
	Non-serpentine	211	~55	SE	8.5x		
Hydroponic BSA	Tolerant	220	~160	100bp	18x	5.21	3.4
	Non-tolerant	140	~160	SE	18x		

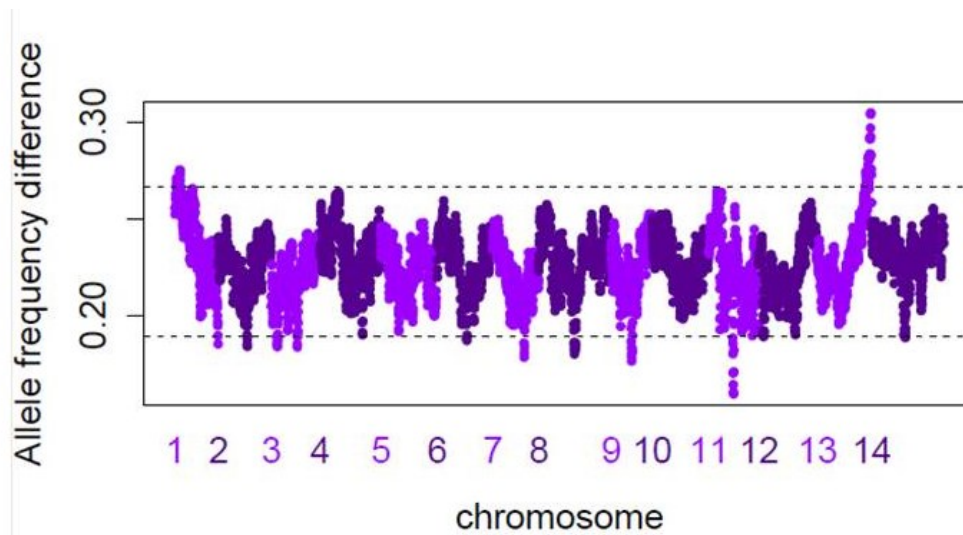


Figure 10: Results from bulk segregant analysis on survival differences at serpentine field sites. The absolute value of allele frequency difference between survivors from the serpentine field sites versus survivors from the non-serpentine site is plotted by genomic position. Each point represents the mean difference between the two pools averaged over 2000 SNPs. Lines represent the 99th percentile.

**Table 12: Markers used in this experiment and their genomic locations.**

<b>Marker</b>	<b>Linkage group</b>	<b>Position (bp)</b>
MgSTS778	13	19547193
MgSTS326	13	20344421
MgSTS117	13	20109853
MgSTS310	13	20445262
MgSTS68	13	20833802
MgSTS419	13	20858778
MgSTS601	13	21049625
MgSTS557	13	21184993
MgSTS508	6	84225
MgSTS25	6	1395489
MgSTS374	4	18859595
MgSTS436	1	2048958

**Table 13: Genotypic ratios for markers within and outside of putative QTLs in the F2 and F3 survivors from serpentine and non-serpentine habitats. Goodness of fit was tested for a 1:2:1 expected ratio of genotypes. SS=homozygous serpentine allele; SN=heterozygous; NN=homozygous non-serpentine allele. Markers used: McL Field 2010 and 2012: LG13=MgSTS419; LG6=MgSTS25; LG1=MgSTS436. RH Field 2010: LG13= MgSTS310. RH Plates: LG13=68; LG6=MgSTS508. \*P<0.05; \*\*, P<0.01, \*\*\*, P<0.0001**

<b>Linkage Group</b>	<b>Experiment</b>	<b>Field site</b>	<b>Genotype ratio (SS:SN:NN)</b>	<b>Goodness of fit</b>
<b>13</b>	McL Field 2010	Serp	17:26:0	$\chi^2=15.33^{***}$
		Off	50:125:45	$\chi^2=4.32$
	McL Field 2012	Serp	40:65:11	$\chi^2=16.19^{***}$
		Off	61:135:64	$\chi^2=0.45$
	RH Field 2010	Serp	9:15:1	$\chi^2=6.12^*$
		Off	20:47:17	$\chi^2=1.40$
	RH Plates	Serp	50:64:5	$\chi^2=34.71^{***}$
		Off	--	--
<b>4</b>	McL Field 2010	Serp	10:20:9	$\chi^2=0.077$
		Off	14:28:9	$\chi^2=2.47$
<b>1</b>	McL Field 2010	Serp	6:9:9	$\chi^2=2.39$
<b>6</b>	McL Field 2010	Serp	9:25:9	$\chi^2=1.14$
	RH Plates	Serp	37:67:18	$\chi^2=7.1^*$

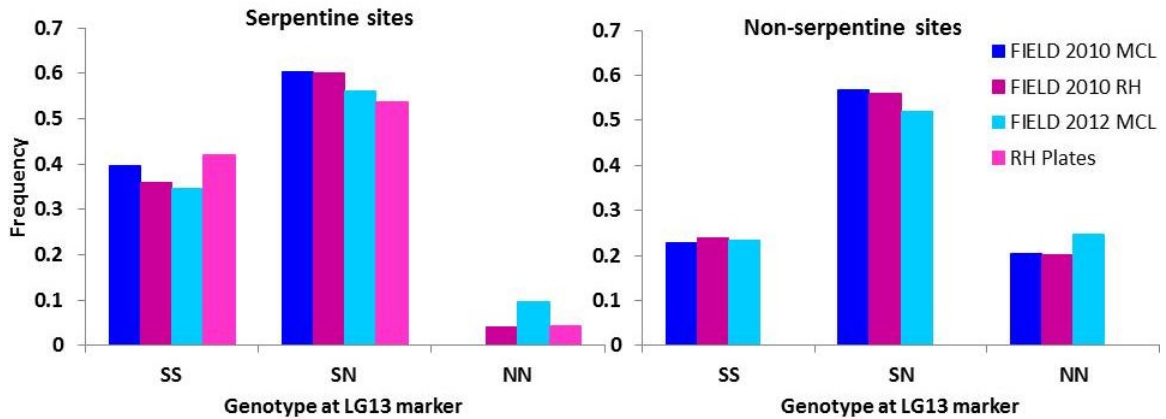


Figure 11: Genotype frequencies at the LG13 QTL region in survivors from serpentine versus non-serpentine field sites from reciprocal transplant experiments. Markers screened in each experiment: McL Field 2010 and 2012 - MgSTS 419; RH Field 2010 - MgSTS310; RH Plates - MgSTS68.

Table 14: Relative fitness and selection coefficients for each genotype at QTL on chromosome 13 on serpentine soil in four different experiments.

Experiment	Genotype	Survival rate	Relative Fitness	Selection coefficient (s)
McL Field 2010	SS	23.4%	1	0
	SN	19.1%	0.82	0.18
	NN	0	0	1
McL Field 2012	SS	42.4%	1	0
	SN	34.4%	0.81	0.19
	NN	11.7%	0.29	0.71
RH Field 2010	SS	12.8%	1	0
	SN	10.7%	0.83	0.17
	NN	1.3%	0.11	0.89
RH Plates Lab	SS	73.6%	1	0
	SN	47.1%	0.64	0.36
	NN	7.3%	0.1	0.9

**Table 15: Results of ANOVA testing for differences between LG13 QTL genotypes in plant size traits at non-serpentine field sites in the three reciprocal transplant experiments. F-ratios and numerator degrees of freedom for each model effect. Degrees of freedom for plot are given as Rosette Diameter, other three traits. McL2010 Rosette diameter (n=215), other traits (n=208). McL2012 Rosette diameter (n=215). RH2010 Rosette diameter (n=78), other traits (n=75). \*P<0.05; \*\*\*P<0.0001.**

	d.f.	Rosette Diameter	Flowering Time	Height	Leaf Length
<b>Genotype</b>					
McL2010	2	1.0	1.8	1.3	0.4
McL2012	2	0.3	--	--	--
RH2010	2	1.4	0.3	0.7	0.3
<b>Block</b>					
McL2010	5	2.5*	2.2	8.1***	5.8***
McL2012	--	--	--	--	--
RH2010	7	1.3	1.6	3.3*	2.5*
<b>Plot(Block)</b>					
McL2010	63, 61	3.7***	3.1***	5.1***	4.9***
McL2012	21	5.6***	--	--	--
RH2010	39, 38	1.4	1.2	1.7	2.0*

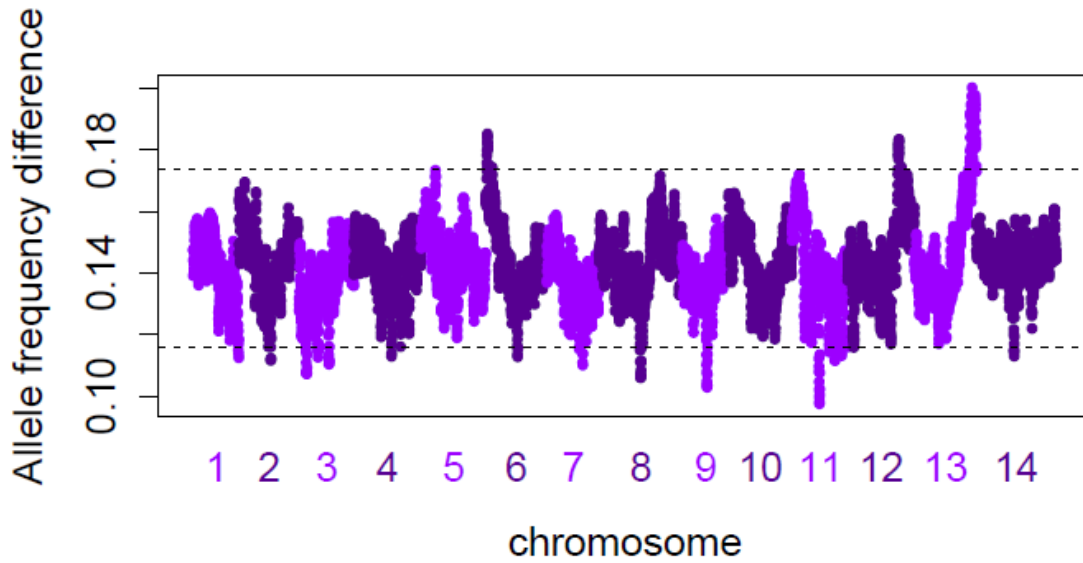


Figure 12: Results from bulk segregant analysis on differences in tolerance to low Ca:Mg ratio in hydroponic feeds. The absolute value of allele frequency difference between the tolerant versus non-tolerant pools is plotted by genomic position. Each point represents the mean difference between the two pools averaged over 2000 SNPs. Lines represent the 99th percentile.

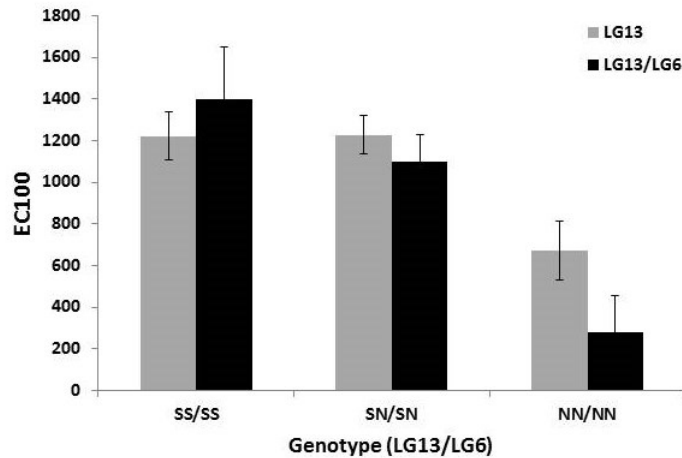


Figure 13: Tolerance to low Ca:Mg ratio for different genotypes at LG13 QTL as well as the two locus genotype including the LG6 QTL.

**Table 16: Counts of two locus genotypes in the Red Hills F2 survivors from lab-based common garden experiment.**

		LG13			
		SS	SN	NN	TOTAL
LG6	SS	17	17	2	36
	SN	25	37	3	65
	NN	7	10	0	17
	TOTAL	49	64	5	118

Line	Marker						% Survival
	778	117	326	419	601	557	
SOD	NN	NN	NN	NN	NN	NN	0
REM	SS	SS	SS	SS	SS	SS	0.92
1-16-A-10	NN	NN	NN	NN	NN	NN	0.026
1-13-B-5	NS	NS	NS	NS	NS	NS	0.458
1-11-A-17	SS	SS	SS	SS	SS	SS	0.792
1-8-A-15	NN	NS	NS	NS	NS	NS	0.521
1-8-B-3	NN	NS	NS	NS	NS	NS	0.232
2-8-D-11	NS	NN	NN	NN	NN	NN	0.021
2-12-A-13	SS	NS	NS	NS	NS	NS	0.274
1-10-D-6	SS	NS	NS	NS	NS	NS	0.821
2-17-A-14	NN	NN	NS	NS	NS	NS	0.463
1-11-C-3	NN	NN	NS	NS	NS	NS	0.552
1-9-C-10	NS	NS	NN	NN	NN	NN	0
2-16-A-7	SS	SS	NS	NS	NS	NS	0.326
2-9-A-3	SS	SS	NS	NS	NS	NS	0.39
1-9-B-11	NN	NN	NN	NS	NS	NS	0.219
2-17-C-12	NN	NN	NN	NS	NS	NS	0.311
1-9-B-12	NS	NS	NS	NN	NN	NN	0.269
2-20-C-11	SS	SS	SS	NS	NS	NS	0.281
2-7-C-6	SS	SS	SS	NS	NS	NS	0.821
1-19-B-12	NN	NN	NN	NN	NS	NS	0.323
2-23-B-15	NN	NN	NN	NN	NS	NS	0.074
2-18-C-13	NS	NS	NS	NS	NN	NN	0.062
1-21-D-2	SS	SS	SS	SS	NS	NS	0.432
2-16-B-16	SS	SS	SS	SS	NS	NS	0.958
1-9-B-9	NS	NS	NS	NS	SS	SS	0.292
1-14-A-5	NS	NS	NS	NS	NS	SS	0.59

Figure 14: Genotypes and percent survival on serpentine soil for backcross progeny used to fine-map LG13 QTL region. Homozygotes for the serpentine allele are designated in purple; homozygotes for the non-serpentine allele are designated in green. Heterozygotes are unshaded. Parental lines and non-recombinant backcross progeny shown at top. Pink box designates fine-mapped interval.

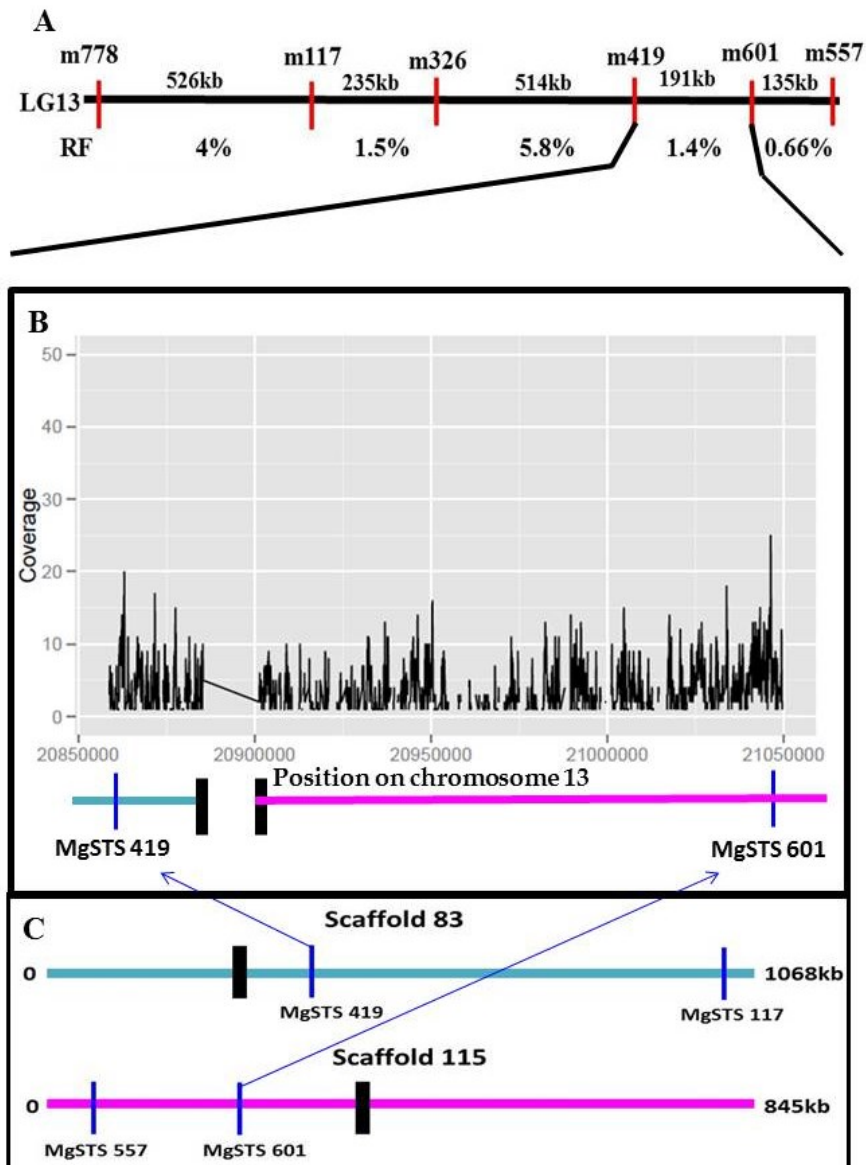


Figure 15: Image of gap in the genome assembly within the fine-mapped interval with positions of MgSTS markers in new versus old genome assemblies. A) Relative positions of the 6 MgSTS markers screened in the hydroponic F2s to identify recombinants for use in the fine-mapping experiment. Positions are based on v2.0 genome assembly. RF=recombination frequency. B) Plot of read depth by position within the LG13 finemapped region between markers 419 and 601 from field survival BSA. C) Old scaffolds (v1.0 genome assembly) and their orientations in the new assembly on LG13 with markers indicated. Black bars represent where the old scaffolds were split in the new assembly.

**Table 17: Candidate genes within the finemapped interval on LG13. Prefix for all *M. guttatus* genes – Migutv20. *A. Thaliana* homologs represent top the BLAST hits based using *M. guttatus* coding sequence as a query. Gene start gives position in basepairs on LG13. Domain/protein name and gene ontology biological process based on annotation for *M. guttatus* on [www.phytozome.net](http://www.phytozome.net). \*\* indicates *A. thaliana* homolog that was identified by Turner et al 2010 as a candidate gene for serpentine tolerance in *A. lyrata*.**

<i>M. guttatus</i> gene	<i>A. thaliana</i> homolog	Gene start	Domain/protein name	GO Biological Process
0425m	AT2G38970	20865350	RING-finger domain, Ca-activated CL channel	Protein binding, transporter activity.
0426m	AT1G22860	20868859	CNH domain, vacuolar sorting protein 39 domain 2	Vesicle-mediated transport, small GTPase regulating protein activity.
0428m	AT1G72700	20876975	ATPase-Plipid	Metabolism, catalytic activity, metal ion binding, nucleotide binding
0431m	AT5G64620	20906912	CELL WALL / VACUOLAR INHIBITOR OF FRUCTOSIDASE 2	Carbohydrate metabolism, stress responses and sugar signaling.
0444m	AT4G19670 **	20952740	IBR domain/Ariadne zinc RING-finger	Zinc ion binding;
0446m	AT2G04032, AT1G31260	20956883	Zinc/Iron transporter	Transmembrane transport, metal ion transporter activity.
0450m	AT1G04750, AT2G33120	20972223	Synaptobrevin/SNARE protein	Integral to membrane, vesicle-mediated transport.
0466m	AT4G19880	21011822	Predicted glutathione S-transferase	Oxio-reductase activity, toxin catabolic process.

## Works Cited

- Abràmoff, M. D., Magalhães, P. J. and Ram, S. J. (2004). "Image processing with ImageJ." Biophotonics international **11**(7): 36-43.
- Ågren, J., Oakley, C. G., McKay, J. K., Lovell, J. T. and Schemske, D. W. (2013). "Genetic mapping of adaptation reveals fitness tradeoffs in *Arabidopsis thaliana*." Proceedings of the National Academy of Sciences.
- Al-Hiyaly, S. A. K., McNeilly, T., Bradshaw, A. D. and Mortimer, A. M. (1993). "The effect of zinc contamination from electricity pylons. Genetic constraints on selection for zinc tolerance." Heredity **70**(1): 22-32.
- Alexander, E. B., Coleman, R. G., Keeler-Wolf, T. and Harrison, S. P. (2007). Serpentine Geocology of Western North America: Geology, Soils, and Vegetation. New York, NY, Oxford University Press.
- Anderson, J. T., Lee, C.-R., Rushworth, C. A., Colautti, R. I. and Mitchell-Olds, T. (2013). "Genetic trade-offs and conditional neutrality contribute to local adaptation." Molecular Ecology **22**(3): 699-708.
- Andrews, S. (2010). "FastQC: A quality control tool for high throughput sequence data." Reference Source.
- Antonovics, J. (1975). Metal tolerance in plants: perfecting an evolutionary paradigm. Proceedings of the International Conference on Heavy Metals in the Environment.
- Antonovics, J. and Bradshaw, A. D. (1970). "Evolution in closely adjacent plant populations VIII. Clinal patterns at a mine boundary." Heredity **25**(3): 349-362.
- Antonovics, J., Bradshaw, A. D. and Turner, R. G. (1971). Heavy Metal Tolerance in Plants. Advances in Ecological Research. J. B. Cragg, Academic Press. **Volume 7**: 1-85.
- Baker, A. J. M. (1987). "METAL TOLERANCE." New Phytologist **106**: 93-111.
- Baxter, I., Hosmani, P. S., Rus, A., Lahner, B., Borevitz, J. O., Muthukumar, B., Mickelbart, M. V., Schreiber, L., Franke, R. B. and Salt, D. E. (2009). "Root Suberin Forms an Extracellular Barrier That Affects Water Relations and Mineral Nutrition in *Arabidopsis*." PLoS Genet **5**(5): e1000492.

- Baxter, I., Ouzzani, M., Orcun, S., Kennedy, B., Jandhyala, S.S., Salt, D.E. (2007). "Purdue Ionomics Information Management System. An Integrated Functional Genomics Platform." Plant Physiol. **143**(2): 600-611.
- Bradshaw, H. D. (2005). "Mutations in CAX1 produce phenotypes characteristic of plants tolerant to serpentine soils." New Phytologist **167**(1): 81-88.
- Brady, K. U., Kruckeberg, A. R. and Bradshaw Jr, H. D. (2005). "Evolutionary ecology of plant adaptation to serpentine soils." Annual Review of Ecology, Evolution, and Systematics **36**(1): 243-266.
- Bratteler, M., Lexer, C. and Widmer, A. (2006). "Genetic architecture of traits associated with serpentine adaptation of *Silene vulgaris*." Journal of Evolutionary Biology **19**: 1149-1156.
- Brooks, R. R. (1987). Serpentine and its vegetation: a multidisciplinary approach, Dioscorides Press.
- Burrell, A. M., Hawkins, A. K. and Pepper, A. E. (2012). "Genetic analyses of nickel tolerance in a North American serpentine endemic plant, *Caulanthus amplexicaulis* var. *barbarae* (Brassicaceae)." American Journal of Botany **99**(11): 1875-1883.
- Callahan, D. L., Kolev, S. D., O'Hair, R. A. J., Salt, D. E. and Baker, A. J. M. (2007). "Relationships of nicotianamine and other amino acids with nickel, zinc and iron in *Thlaspi* hyperaccumulators." New Phytologist **176**(4): 836-848.
- Clausen, J., Keck D.D. and Hiesey, W. M. (1948). Experimental studies on the nature of species. III. Environmental responses of climatic races of *Achillea*. Washington, DC, Carnegie Institute of Washington Publication.
- Clausen, J., Keck, D. D. and Hiesey, H. M. (1940). Experimental studies on the nature of species. I. Effect of varied environments on western North American plants. . Washington, DC, Carnegie Institute of Washington.
- Clemens, S. (2006). "Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants." Biochimie **88**(11): 1707-1719.
- Colosimo, P. F., Peichel, C. L., Nereng, K., Blackman, B. K., Shapiro, M. D., Schluter, D. and Kingsley, D. M. (2004). "The genetic architecture of parallel armor plate reduction in threespine sticklebacks." PLoS biology **2**(5): e109.

- Deinlein, U., Weber, M., Schmidt, H., Rensch, S., Trampczynska, A., Hansen, T. H., Husted, S., Schjoerring, J. K., Talke, I. N., Krämer, U. and Clemens, S. (2012). "Elevated Nicotianamine Levels in *Arabidopsis halleri* Roots Play a Key Role in Zinc Hyperaccumulation." *The Plant Cell Online* **24**(2): 708-723.
- Epstein, E. (1972). *Mineral nutrition of plants: principles and perspectives*.
- Fishman, L., Aagaard, J. and Tuthill, J. C. (2008). "Toward the evolutionary genomics of gametophytic divergence: Patterns of transmission ratio distortion in monkeyflower (*Mimulus*) hybrids reveal a complex genetic basis for conspecific pollen precedence." *Evolution* **62**(12): 2958-2970.
- Freeman, J. L., Persans, M. W., Nieman, K., Albrecht, C., Peer, W., Pickering, I. J. and Salt, D. E. (2004). "Increased Glutathione Biosynthesis Plays a Role in Nickel Tolerance in *Thlaspi* Nickel Hyperaccumulators." *The Plant Cell Online* **16**(8): 2176-2191.
- Gabbrielli, P., Pandolfini, T., Vergnano, O. and Palandri, M. R. (1990). "Comparison of two serpentine species with different nickel tolerance strategies." *Plant and Soil* **122**(2): 271-277.
- Gabbrielli, R. and Pandolfini, T. (1984). "Effect of Mg<sup>2+</sup> and Ca<sup>2+</sup> on the response to nickel toxicity in a serpentine endemic and nickel-accumulating species." *Physiologia Plantarum* **62**(4): 540-544.
- Gardner, M. a. M. M. (2000). "Factors affecting the co-existence of the serpentine endemic *Mimulus nudatus* Curran and its presumed progenitor, *Mimulus guttatus* Fischer ex DC." *Biological Journal of the Linnean Society* **69**: 443-459.
- Gillespie, J. H. and Turelli, M. (1989). "Genotype-environment interactions and the maintenance of polygenic variation." *Genetics* **121**(1): 129-138.
- Hedrick, P. W. (1986). "Genetic Polymorphism in Heterogeneous Environments: A Decade Later." *Annual Review of Ecology and Systematics* **17**: 535-566.
- Hereford, J. (2009). "A Quantitative Survey of Local Adaptation and Fitness Trade-Offs." *The American Naturalist* **173**(5): 579-588.
- Hirschi, K. D., Zhen, R.-G., Cunningham, K. W., Rea, P. A. and Fink, G. R. (1996). "CAX1, an H<sup>+</sup>/Ca<sup>2+</sup> antiporter from *Arabidopsis*." *Proceedings of the National Academy of Sciences* **93**(16): 8782-8786.

- Hoekstra, H. E., Hirschmann, R. J., Bunday, R. A., Insel, P. A. and Crossland, J. P. (2006). "A Single Amino Acid Mutation Contributes to Adaptive Beach Mouse Color Pattern." Science **313**(5783): 101-104.
- Hufford, K. M., Mazer, S. J. and Camara, M. D. (2008). "Local adaptation and effects of grazing among seedlings of two native California bunchgrass species: Implications for restoration." Restoration Ecology **16**(1): 59-69.
- Jurjavcic, N., Harrison, S. and Wolf, A. (2002). "Abiotic stress, competition, and the distribution of the native annual grass *Vulpia microstachys* in a mosaic environment." Oecologia **130**(4): 555-562.
- Kawecki, T. J. and Ebert, D. (2004). "Conceptual issues in local adaptation." Ecology Letters **7**(12): 1225-1241.
- Kay, K. M., Ward, K. L., Watt, L. R. and Schemske, D. W. (2011). "Plant speciation." Serpentine: the evolution and ecology of a model system. University of California Press, Berkeley: 71-96.
- Kazakou, E., Dimitrakopoulos, P. G., Baker, A. J. M., Reeves, R. D. and Troumbis, A. Y. (2008). "Hypotheses, mechanisms and trade-offs of tolerance and adaptation to serpentine soils: from species to ecosystem level." Biological Reviews **83**(4): 495-508.
- Kelly, A. J. and Willis, J. H. (1998). "Polymorphic microsatellite loci in *Mimulus guttatus* and related species." Molecular Ecology **7**(6): 769-774.
- Kohn, M. H., Pelz, H.-J. and Wayne, R. K. (2000). "Natural selection mapping of the warfarin-resistance gene." Proceedings of the National Academy of Sciences **97**(14): 7911-7915.
- Kruckeberg, A. R. (1950). An Experimental Inquiry into the Nature of Endemism on Serpentine Soils. Ph.D., University of California, Berkeley.
- Kruckeberg, A. R. (1954). "The ecology of serpentine soils: A symposium. III. Plant species in relation to serpentine soils." Ecology **35**: 267-274.
- Kruckeberg, A. R. (1967). "Ecotypic Response to Ultramafic Soils by Some Plant Species of Northwestern United States." Brittonia **19**(2): 133-151.
- Kruckeberg, A. R. (1984). California serpentines: flora, vegetation, geology, soils and management problems, University of California Pr.

- Leimu, R. and Fischer, M. (2008). "A Meta-Analysis of Local Adaptation in Plants." PLoS ONE **3**(12): e4010.
- Levene, H. (1953). "Genetic Equilibrium When More Than One Ecological Niche is Available." The American Naturalist **87**(836): 331-333.
- Lexer, C., Welch, M. E., Durphy, J. L. and Rieseberg, L. H. (2003). "Natural selection for salt tolerance quantitative trait loci (QTLs) in wild sunflower hybrids: Implications for the origin of *Helianthus paradoxus*, a diploid hybrid species." Molecular Ecology **12**(5): 1225-1235.
- Li, H. and Durbin, R. (2010). "Fast and accurate long-read alignment with Burrows-Wheeler transform." Bioinformatics **26**(5): 589-595.
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R. and Subgroup, G. P. D. P. (2009). "The Sequence Alignment/Map format and SAMtools." Bioinformatics **25**(16): 2078-2079.
- Linhart, Y. B. and Grant, M. C. (1996). "Evolutionary Significance of Local Genetic Differentiation in Plants." Annual Review of Ecology and Systematics **27**: 237-277.
- Loew O., D. M. (1901). "The relation of lime and magnesia to plant growth." U.S. Dep. Agric. Bur. Plant Ind. Bull **1**: 1-53.
- Lowry, D. B. and Willis, J. H. (2010). "A Widespread Chromosomal Inversion Polymorphism Contributes to a Major Life-History Transition, Local Adaptation, and Reproductive Isolation." PLoS Biol **8**(9): e1000500.
- Macnair, M. R. (1987). "Heavy metal tolerance in plants: A model evolutionary system." Trends in Ecology & Evolution **2**(12): 354-359.
- Macnair, M. R., Smith, S.E., Cumbes, Q. J. (1993). "Heritability and distribution of variation in degree of copper tolerance in *Mimulus guttatus* at Copperopolis, California." Heredity **71**: 445-455.
- Madhok, O. P. and Walker, R. B. (1969). "Magnesium Nutrition of Two Species of Sunflower." Plant Physiol. **44**(7): 1016-1022.
- Magwene, P. M., Willis, J. H. and Kelly, J. K. (2011). "The Statistics of Bulk Segregant Analysis Using Next Generation Sequencing." PLoS Comput Biol **7**(11): e1002255.

- Mari, S., Gendre, D., Pianelli, K., Ouerdane, L., Lobinski, R., Briat, J. F., Lebrun, M. and Czernic, P. (2006). "Root-to-shoot long-distance circulation of nicotianamine and nicotianamine-nickel chelates in the metal hyperaccumulator *Thlaspi caerulescens*." Journal of Experimental Botany **57**(15): 4111-4122.
- McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M. and DePristo, M. A. (2010). "The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data." Genome Research **20**(9): 1297-1303.
- McMillan, C. (1956). "The Edaphic Restriction of *Cupressus* and *Pinus* in the Coast Ranges of Central California." Ecological Monographs **26**(3): 178-212.
- Michelmore, R. W., Paran, I. and Kesseli, R. V. (1991). "Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations." Proceedings of the National Academy of Sciences **88**(21): 9828-9832.
- Morrison, J. M., Goldhaber, M. B., Lee, L., Holloway, J. M., Wanty, R. B., Wolf, R. E. and Ranville, J. F. (2009). "A regional-scale study of chromium and nickel in soils of northern California, USA." Applied Geochemistry **24**(8): 1500-1511.
- Murren, C. J., Douglass, L., Gibson, A. and Dudash, M. R. (2006). "INDIVIDUAL AND COMBINED EFFECTS OF Ca/Mg RATIO AND WATER ON TRAIT EXPRESSION IN *MIMULUS GUTTATUS*." Ecology **87**(10): 2591-2602.
- Nagy, L. and Proctor, J. (1997). "Soil Mg and Ni as causal factors of plant occurrence and distribution at the Meikle Kilrannoch ultramafic site in Scotland." New Phytologist **135**(3): 561-566.
- O'Dell, R. E., and Claassen, V.P. (2008). Parallel evolution and convergent physiological tolerance mechanisms of serpentine-tolerant *Achillea millefolium* (Asteraceae) edaphic ecotypes. Sixth International Conference on Serpentine Ecology. Bar Harbor, ME.
- O'Dell, R. E. and Rajakaruna, N. (2011). Intraspecific variation, adaptation, and evolution. Serpentine: The evolution and ecology of a model system. S. a. R. Harrison, N. Berkeley, CA, University of California Press: 97-137.
- O'Dell, R. and Claassen, V. (2006). "Serpentine and Nonserpentine *Achillea millefolium* Accessions Differ in Serpentine Substrate Tolerance and Response to Organic and Inorganic Amendments." Plant and Soil **279**(1): 253-269.

- Oze, C., Fendorf, S., Bird, D. K. and Coleman, R. G. (2004). "Chromium geochemistry in serpentinized ultramafic rocks and serpentine soils from the Franciscan complex of California." American Journal of Science **304**(1): 67-101.
- Palm, E., Brady, K. and Van Volkenburgh, E. (2012). "Serpentine tolerance in *Mimulus guttatus* does not rely on exclusion of magnesium." Functional Plant Biology **39**(8): 679-688.
- Pollard, A. J., Powell, K. D., Harper, F. A. and Smith, J. A. C. (2002). "The Genetic Basis of Metal Hyperaccumulation in Plants." Critical Reviews in Plant Sciences **21**(6): 539-566.
- Proctor, J. (1970). "Magnesium as a toxic element." Nature **227**: 742-743.
- Proctor, J., Woodell, S. R. J. and MacFadyen, A. (1975). The Ecology of Serpentine Soils. Advances in Ecological Research, Academic Press. **Volume 9**: 255-366.
- Rockman, M. V. (2012). "THE QTN PROGRAM AND THE ALLELES THAT MATTER FOR EVOLUTION: ALL THAT'S GOLD DOES NOT GLITTER." Evolution **66**(1): 1-17.
- Sambatti, J. B. M. and Rice, K. J. (2006). "Local adaptation, patterns of selection, and gene flow in the Californian serpentine sunflower (*Helianthus exilis*)." Evolution **60**(4): 696-710.
- Savolainen, O., Lascoux, M. and Merila, J. (2013). "Ecological genomics of local adaptation." Nat Rev Genet **14**(11): 807-820.
- Schluter, D. and Conte, G. L. (2009). "Genetics and ecological speciation." Proceedings of the National Academy of Sciences **106**(Supplement 1): 9955-9962.
- Sen Tran, T. and Simard, R. R. (1993). Mehlich III-extractable elements. Soil Sampling and Methods of Analysis. M. R. Carter. Boca Raton, FL, USA, Lewis Publishers: 43-49.
- Shat, H. and ten Bookum, W. M. (1992). "Genetic control of copper tolerance in *Silene vulgaris*." Heredity **68**: 219-229.
- Shure, M., Wessler, S. and Fedoroff, N. (1983). "Molecular identification and isolation of the Waxy locus in maize." Cell **35**(1): 225-233.

- Turner, T. L., Bourne, E. C., Von Wettberg, E. J., Hu, T. T. and Nuzhdin, S. V. (2010). "Population resequencing reveals local adaptation of *Arabidopsis lyrata* to serpentine soils." Nature Genetics **42**(2): 260-264.
- Turner, T. L., von Wettberg, E. J. and Nuzhdin, S. V. (2008). "Genomic analysis of differentiation between soil types reveals candidate genes for local adaptation in *Arabidopsis lyrata*." PLoS One **3**(e3183).
- Vacchina, V., Mari, S., Czernic, P., Marques, L., Pianelli, K., Schaumlöffel, D., Lebrun, M. and Lobinski, R. (2003). "Speciation of nickel in a hyperaccumulating plant by high-performance liquid chromatography-inductively coupled plasma mass spectrometry and electrospray MS/MS assisted by cloning using yeast complementation." Analytical Chemistry **75**(11): 2740-2745.
- van't Hof, A. E., Edmonds, N., Dalíková, M., Marec, F. and Saccheri, I. J. (2011). "Industrial Melanism in British Peppered Moths Has a Singular and Recent Mutational Origin." Science **332**(6032): 958-960.
- Verhoeven, H. P., E, N. and A, B. (2008). "Habitat-specific natural selection at a flowering-time QTL is a main driver of local adaptation in two wild barley populations." Molecular Ecology **17**(14): 3416-3424.
- Vickery, R. (1978). "Case studies in the evolution of species complexes in *Mimulus*." Evol Biol **11**: 405-507.
- Vlams, J. (1949). "Growth of Lettuce and Barley As Influenced By Degree of Calcium-Saturation of Soil." Soil Science **67**(6): 453-466.
- Walker, R. B., Walker, H. M. and Ashworth, P. R. (1955). "Calcium-Magnesium Nutrition with Special Reference to Serpentine Soils." Plant Physiology **30**(3): 214-221.
- Weinig, C., Dorn, L. A., Kane, N. C., German, Z. M., Halldorsdottir, S. S., Ungerer, M. C., Toyonaga, Y., Mackay, T. F. C., Purugganan, M. D. and Schmitt, J. (2003). "Heterogeneous Selection at Specific Loci in Natural Environments in *Arabidopsis thaliana*." Genetics **165**(1): 321-329.
- White, P. J. and Broadley, M. R. (2009). "Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine." New Phytologist **182**(1): 49-84.
- Whittaker, R. H. (1954). "The Ecology of Serpentine Soils." Ecology **35**(2): 258-288.

- Wright, J. W., Stanton, M. L. and Scherson, R. (2006). "Local adaptation to serpentine and non-serpentine soils in *Collinsia sparsiflora*." Evolutionary Ecology Research **8**(1): 1-21.
- Wu, C. A., Lowry, D. B., Cooley, A. M., Wright, K. M., Lee, Y. W. and Willis, J. H. (2007). "Mimulus is an emerging model system for the integration of ecological and genomic studies." Heredity **100**(2): 220-230.
- Yang, X., Baligar, V. C., Martens, D. C. and Clark, R. B. (1996). "Plant tolerance to nickel toxicity: I. Influx, transport, and accumulation of nickel in four species." Journal of Plant Nutrition **19**(1): 73-85.
- Yeaman, S. and Otto, S. P. (2011). "Establishment and maintenance of adaptive genetic divergence under migration, selection, and drift." Evolution **65**(7): 2123-2129.
- Yeaman, S. and Whitlock, M. C. (2011). "The genetic architecture of adaptation under migration–selection balance." Evolution **65**(7): 1897-1911.

## Biography

I was born in Madison, Wisconsin on January 12, 1983 to John H. Selby and Denise K. Dipert. I attended Harvard University, where I worked with Professor Kathleen Donohue on the effects of temperature-dependent phytochrome functioning on germination success in *Arabidopsis thaliana*. I received my A.B. in Environmental Science and Public Policy in 2005. From 2006 – 2007, I worked at the Rainforest Alliance in New York, NY as a development assistant. I began my graduate work at Duke University in 2007, became a PhD candidate in 2010, and completed my PhD in the Spring of 2014. I authored one paper from my undergraduate research entitled "A new role for phytochromes in temperature-dependent germination." The first publication from my PhD will be a book chapter, "The ecological genomics of adaptation to harsh environments in *Mimulus guttatus*" to be published in 2014 in *Plant Ecology and Evolution in Harsh Environments*.

While a graduate student, I received an NSF Doctoral Dissertation Improvement Grant in 2011. I also received a Duke University Department of Biology Semester Fellowship and Grant-In-Aid.