Lineage specific inferences about QTL evolution among three *Mimulus* species of contrasting relationship and inbreeding

by

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ABSTRACT

Complex traits including those involved with natural adaptation are determined by the contributions of numerous genes, the environment, and their interactions. Although quantitative trait locus (QTL) mapping approaches have been successful in dissecting complex traits, few studies have adopted a comparative approach of contrasting species pairs that differ in relationship, for the purpose of dissecting evolutionary changes of QTL. Furthermore, no QTL mapping approaches have explicitly inferred QTLs along lineages in a species network. This thesis brings such a comparative approach into QTL mapping.

The evolution of inbreeding in the *Mimulus guttatus* species complex provides an excellent system where lineage-specific QTL changes can be inferred. Three intercrossable species were chosen: *M. guttatus*, *M. platyc*alyx and *M. micranthus*, the latter two taxa being independent derived inbreeders from the first one. Five floral characters were selected as representative traits for the evolution of inbreeding in these species. A three-species crossing design was implemented, upon which QTL analyses were conducted.

As expected in QTL mapping studies, the estimated number of genetic factors varies among crosses. An important role of dominance in the evolution of selfing from outcrossing taxa is supported by the data, owing to the consistency of directional dominance towards selfing taxa. The extensiveness of epistasis identified in this study suggests that in *Mimulus*, genes related to floral characters are co-adapted gene complexes, where genetic interdependency evolves as species diverge. Moreover, such genetic interdependency may be a key element in the evolution of stable mixed mating systems.

A model for the inference of lineage specific QTL in a three-taxon network is described, and used to infer lineage-specific changes for floral traits among the three

Mimulus taxa. After mapping QTL onto lineages, one can determine if QTL at the same map position are homologous (arising in an ancestral lineage leading to two taxa) or non-homologous (arising independently in derived lineages or via convergent evolution). In *Mimulus*, shared negative QTLs of dominant effect that arise from convergent evolution seem to play a prominent role in the early evolution of inbreeding; then derived, independent changes fine-tune further evolutionary changes of inbreeding.

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CO-AUTHORSHIP STATEMENT

For this thesis, Dr. Carol Ritland supervised all the steps from optimizing the protocol for AFLP (amplified fragment length polymorphism) primer testing, genotyping and genotype scoring. I, Charles Chen, conducted all the intercrosses and backcrosses, phenotyping and AFLP genotyping and data analyses. Charles Chen also prepared the manuscripts as his PhD dissertation. Dr. Kermit Ritland supervised the research, the crossing design as well as data analyses. Dr. Kermit Ritland also edited the manuscripts. In Chapter 3 Dr. Kermit Ritland produced the computer programs for the inferred parental genotype and lineage specific QTL inference.

CHAPTER 1. GENERAL INTRODUCTION

The beauty of biological diversity has been more than impressive to all of us. Underlying the morphological differences among species are fundamental genetic changes. One of the ultimate questions in biology, then, is what is the nature of these genetic changes responsible for the evolution of morphological divergence. The questions surrounding numbers of genes, sizes of gene effects, and dominance and epistatic effects is one of the oldest problems in evolutionary biology: the complexity of the genetic changes underlying phenotypic evolution (Orr 2001).

THE BIRTH OF QUANTITATIVE GENETICS

The study of inheritance and evolution began at the end of nineteenth century (Barton and Keightley 2002). The early research of evolutionary genetics started from a motivation to understand the genetic basis of complex traits, particularly for those relating to humans, such as intelligence, temper and artistic faculty. Without an explicit understanding of inheritance, Francis Galton and his student Karl Pearson first established the approaches to statistically describe continuously varying characters (Provine 1971). The multivariate statistical tools of correlation and regression that they developed laid the foundation of the Biometrical school for much of modern statistics (Mauricio 2001).

On 8 February and 8 March of 1865, Gregor Mendel described the results of his research at meetings of the Brunn Natural Science Society (Orel 1984). The following year, in a paper entitled “Experiments in plants hybridization”, Mendel (1866) published

his now famous work in the society's journal, Proceedings of the Brunn Society for the Study of Natural Science (Mendel 1866; Sterb and Sherwood 1966). That paper reported research done by Mendel from 1854 to 1863, involving almost 28,000 plants, in which he claimed he "carefully examined" 12,835 plants. This famous yet under-appreciated experiment involved crosses of two pure-breeding varieties of garden pea (*Pisum sativum*) that differed in many phenotypic character traits. However, the world did not regain its appreciation of Mendel's contribution until 1900, 16 years after Mendel's death. Although the words "heredity" or "inheritance" were not even used in his 1866 report, the results of his work have been credited in the discovery of the first two laws of inheritance, which form the basis of "Mendelian genetics" (Gliboff 1999).

Despite the elucidation and extension of Mendel's laws by the Morgan school with *Drosophila melanogaster*, and ultimately the discovery of the structure of DNA by Watson and Crick, many historians set the year of 1900 as the birth of genetics because the rediscovery of Mendelian inheritance (Zwick et al. 2000). The most successful applications of Mendelian genetics involve traits in which genotypic changes result in large, discrete phenotypic differences ("Mendelian traits"). These include trait differences such as white vs. pink flower color, or smooth vs. wrinkled seed coat, and these differences are primarily governed by the segregation of single genes.

During the late 19th and early 20th centuries, a conflict arose between Mendel's principles of inheritance for discrete variation and the Biometrical principles for continuously varying characteristics. The debate became fierce in the early 20th century, over whether discrete and continuous traits shared the same hereditary and evolutionary

properties. By 1910, it had been shown that continuous variation could result from the action of the environment on the segregation of many Mendelian loci (East 1910, Provine 1971). Nearly a decade after, in 1918, the publication of Ronald Fisher provided a comprehensive framework to unite particular inheritance with continuous variation in evolutionary content, in which Fisher demonstrated that many Mendelian factors of small effect, together with natural environmental variation, could explain continuous trait variability in natural populations (Fisher 1918). This 1918 publication convincingly reconciled discrete Mendelian inheritance with the inheritance of continuous traits. Even now, modern quantitative genetics is mostly based on the statistical foundations laid in 1918 by Ronald A. Fisher (Roff 2007).

After the 1920's, quantitative genetics as we know it today was developed by Fisher and Wright as a synthesis of statistics, Mendelian principles, and evolutionary biology. Quantitative genetics was also embraced by plant and animal breeders after this time for several decades. In 1975, Russell Lande wrote the first in a series of papers that brought quantitative genetics into evolutionary biology (Lande 1975, 1981; Roff 2007). Throughout the 1980s, while quantitative genetics was still increasingly applied in agriculture, Lande developed a comprehensive theory of evolutionary quantitative genetics, including that inferences we might expect about evolution at specific gene loci underlying quantitative traits (Barton and Turelli 1989). With the advent of high-throughput genomic approaches, the genetic basis and evolutionary forces underlying quantitative variation and evolution are now receiving renewed attention, and the wealth of genetic information obtained is having impact upon evolutionary genetics, human health, agriculture and molecular phenotyping (Gibson and Mackay 2002).

EVOLUTIONARY QUANTITATIVE GENETICS

If R.A. Fisher's 1918 publication is taken as the beginning of quantitative genetics, then the centenary of quantitative genetics is only a few years away (Roff 2007). In the 21st century, modern quantitative genetics is considered as the fusion of Mendelian inheritance, biometry and mathematics encompassing this science of heredity (Mauricio 2001). Up until the 1980s, quantitative genetics assumed that phenotypic variation was static, and that the response to natural selection was based upon the standing genetic variance distribution. The "new" evolutionary quantitative genetics seeks to model or infer the underlying genetic architecture that underlies the divergence between individuals, populations and species (Lynch et al. 1999).

"Genetic architecture" refers to the pattern or the collection of genetic effects. That includes allele numbers and effects, genomic distribution, allelic frequency, patterns of pleiotropy, dominance and epistatic interactions of genes. All these build and control a given phenotypic character and its variation (Remington and Purugganan 2003). The genetic architecture of phenotypic variation among individuals within population is typically complex, often with multiple interacting genetic factors that are also sensitive to the external environment (Lyman et al. 2002).

Molecular studies of genetic architecture have become very feasible over the last two decades, largely because of the revolution in DNA marker technologies. In genetic mapping, association of phenotypic trait variation with DNA marker alleles has been successfully used to identify chromosomal regions harbouring individual genes responsible for quantitative variation. These genetic factors, genes, or loci, are referred

to as "quantitative trait loci", or QTL (Geldermann 1975), and constitute the genetic diversity that directly and indirectly contribute to the vast majority of phenotypic variation in natural populations, and perhaps represent the main sites at which selection acts upon phenotypic variation (Price 2006). Most human diseases (diabetes, asthma, arteriosclerosis), agricultural production and yield characters, as well as adaptive traits in wild species, are quantitative traits (Cordell 2002; Mackay 2001a).

MAPPING QUANTITATIVE TRAIT LOCI (QTL) AND TRAIT EVOLUTION IN PLANTS

The basic principle behind finding QTL in experimental organisms was first proposed in 1923, in plant research (Sax 1923). Since then, QTL mapping has been applied to agriculture (Roff 2007), plant domestication (Paterson 2002), and studies of adaptation (Alonso-Blanco et al. 1999), hybridization (Rieseberg et al. 1996) and speciation (Bradshaw et al. 1995).

Genetic basis of adaptation

The genetic basis of adaptation has been one of the most intriguing questions in evolutionary biology (Orr and Coyne 1992). Integrated with modern genetics, the new evolutionary synthesis of neo-Darwinism states that evolution is a gradual continuous process of natural selection acting on small phenotypic variations, and that adaptation results from the fixation of alleles with individually small effects at many loci. This micromutationist viewpoint was first advocated by Fisher (Fisher 1930), suggesting that

adaptation is a process where organisms are fitted to the environment simultaneously for a large number of characters. And, Fisher (1930) also famously demonstrated that, while small mutations are favourable, mutation with large effects are possible but improbable (Orr 1999). Although Fisher's infinitesimal model of genetic basis of adaptation has been challenged almost since its inception (Kimura 1983; Orr and Coyne 1992; Wright 1952), the model still provides a fundamental testable hypothesis. By dissecting the genetic architecture underlying variation of functional traits at the molecular level, QTL mapping can test this hypothesis about infinitesimal genetic changes in adaptation.

Molecular markers have made it possible to map and characterize genetic changes underlying domestication (Tanksley et al. 1982; Paterson et al. 1988; Paterson et al. 1991). QTL mapping studies on crop plants have found that domestication often involves major alleles at genes with pleiotropic effects and epistatic interactions (Doebley et al. 1990; Doebley et al. 1995; Tanksley 1993). Comparative mapping also has further indicated that the same genetic loci are involved in adaptation to domestication (e.g. cereals, Paterson 1995). Domestication has correlated with dramatic increases in fruit size. For example, modern tomatoes (*Lycopersicon esculentum*) can weigh as much as 1,000 grams and exceed 15 cm in diameter, compared to their progenitor (*L. pimpinellifolium*), which had fruits less than 1 cm and only a few grams in weight (Smartt and Simmonds 1995).

In fact, these two species have served as a model system for the study of the genetic basis of domestication. QTL analysis involving a cross between these two species suggested a polygenic system responsible for the domestication process of modern tomatoes (Grandillo et al. 1999). They identified at least 28 QTL responsible for

tomato fruit size, and some of these QTL contribute to over 20% of the phenotypic variance; one of the QTL, *fw2.2*, affecting the size change on tomatoes, accounts for 30% of the variation (Grandillo et al. 1999). Using a transgene complementation as a proof, Frary et al. (2000) utilized a chromosome dissection to identify a 150 kb region that contains the QTL of *fw2.2*, transformed into a large fruited cultivar and one of the cosmids derived from the *fw2.2* region of a small fruited wild species reduced the fruit size by the predicted amount. The cause of the effect of QTL *fw2.2* was determined by a single gene, ORFX, which expresses early in floral development (Frary et al. 2000).

The adaptive importance of flowering phenology has long been recognized, and climatic factors, pollinator adaptations, or deleterious effects of interspecific gene flow may all function as selective mechanisms (Rathcke and Lacey 1985). More importantly, the difference of flowering time can also result in prezygotic isolation, even if they are not selectively advantageous (Remington and Purugganan 2003). A QTL mapping study, using different ecotypes of *Arabidopsis thaliana*, revealed four major QTL responsible for the variation of flowering time with a number of minor QTL (Alonso-Blanco et al. 1998). More interestingly, the four major QTL found in flowering time variation also contribute the variation in *Arabidopsis* shoot architecture, indicating a polygenic system with possible pleiotropic actions and the complexity of flowering phenology in plants (Ungerer et al. 2002).

In a recent review, Ratcliffe and Riechmann (2002) list 38 flowering time genes that have been isolated from *Arabidopsis*, primarily by mutant analysis. These loci include *CONSTANS*, a zinc-finger transcription factor gene (Putterill et al. 1995), the MADS-box transcription factor gene *FLOWERING LOCUS C* or *FLC* (Michaels and

Amasino 1999). Results from molecular studies have shown that flowering time in *Arabidopsis* plants is under complex genetic control (Simpson and Dean 2002). Among those large number of known candidate genes, positional cloning based on QTL mapping research later identified the *FRIGIDA* locus as a major locus affecting flowering time variation among *A. thaliana* ecotypes (Johanson et al. 2000), and *EDI* locus corresponding to a blue-light receptor protein (Alonso-Blanco et al. 1998). These results illustrate the potential power of QTL mapping to address fundamental evolutionary questions (Price 2006).

Genetic basis of species differentiation

A species, as the basic unit of biodiversity, is defined as a discrete interbreeding group of individuals, reproductively isolated from other such groups (Dobzhansky 1935; Mayr 1942). The first genetic survey of species differences appeared in 1938, with J. B. S. Haldane's paper, "The nature of interspecific differences". Current questions about how species differ morphologically largely remain the same as in Haldane's day, involving questions about the genetic architecture of species differentiation (Orr 2001).

The continuing genetic work with sunflower species (Kim and Rieseberg 1999; Rieseberg et al. 1996; Rieseberg et al. 1999b) and Louisiana irises (Arnold 2000; Cruzan and Arnold 1993; Hodges et al. 1996; Hodges et al. 1996; Martin et al. 2005) have demonstrated aspects of the genetic basis of speciation. In both study systems, natural hybrid populations can be found. These hybrids can serve as a genetic bridge between species. In sunflower, studies have shown the evolutionary dynamics of colonizing ability in *Helianthus annuus* via the acquisition of advantageous alleles from the locally

adapted *H. debillis*. Also, "transgressive segregation" in hybrids occurs when new combinations of parental alleles are formed, which enable the survival in novel ecological niches unavailable to either parent (Rieseberg et al. 1999a). This process has also been suggested to arise from non-additive gene action of adaptively important alleles inherited from each parent (Monforte et al. 1997). In addition, by mapping QTL in parents that are responsible for ecological traits such as flood-tolerant versus dry-adapted genotypes, Arnold (2000) and Martin et al. (2005) showed that the survivorship of *Iris* hybrids was strongly influenced by the presence of a number of introgressed alleles with significant epistatic genetic effect throughout the genome. Their results explicitly suggest that introgressive hybridization is an important evolutionary mechanism in *Iris*.

Furthermore, the evolutionary inferences obtained by QTL mapping can be extended beyond a pair of species, yielding more insight into the evolutionary dynamics of species divergence. Diverse taxa in common taxonomic groups often share gene order over large chromosomal segments, and aligned QTL maps of different taxa can reveal important patterns of evolution. For example, the grasses sorghum, rice and maize were each independently domesticated about 10,000 year ago (Mauricio 2001). Each species has been selected to have large seeds, daylength-insensitive flowering and reduced fruit shattering. QTL have been mapped for these traits (Paterson et al. 1995b). Interestingly, the approximate locations of these QTL for each trait were resided in similar map locations in all three species, despite their having 65 million years of reproductive isolation. This conservation of chromosomal regions containing QTL indirectly indicates evolutionarily important genes, upon which selection can act independently across species (Paterson et al. 1995b).

Finally, the most well studied plant species for the genetic basis of reproductive isolation is, perhaps, *Mimulus*. Toby Bradshaw, Douglas Schemske and their colleagues pioneered the QTL approach with the study of speciation (Bradshaw et al. 1998). *M. lewsii* is a bumble-bee pollinated species with pink petals, contrasting yellow nectar guides, wide corolla opening and inserted anthers and stigma. *M. cardinalis* is pollinated by hummingbirds and has red petals, narrow tubular corolla, copious nectar and exerted anthers and stigma (Bradshaw et al. 1998). Although these two *Mimulus* species grow together and flower at the same time, hybrids are not commonly observed in nature. B y crossing these two *Mimulus* species, the study of underlying genetic architecture revealed QTL accounting for more than 25% of the phenotypic variance in floral morphology, and suggested that the evolution of reproductive isolation might involve genes with large effects, representing "speciation genes" (Bradshaw et al. 1998).

In contrast, from the study of floral characters affecting the differences of selfing rate between *M. guttatus* and *M. platycalyx*, Lin and Ritland (1997) found a different result: genes of small effect are responsible to the evolution of mating system in the *M. guttatus* species complex (Lin and Ritland 1997). Table 1.1 lists the studies of genetic architecture of *Mimulus* species differences: 4 out of 24 floral traits were estimated to be one single QTL underlying the divergence; more than 50% of trait difference in these genetic studies had at least 5 QTL loci involved.

The unanswered questions in the study of evolutionary quantitative genetics

Although it is clear that, in theory, gradual micro-evolutionary processes can explain abrupt macro-evolutionary patterns (Charlesworth et al. 1982; Lande 1983) and

genetic dissection techniques like QTL mapping can be used to understand the genetic architecture that underlies trait variation among plant species, the empirical problem remains greatly unsolved. We know little about the actual evolutionary processes and pattern of genetic materials that underlies phenotypic divergence between populations and species. The quantitative genetic scheme of using just a single cross only allows estimates of differences that arise along a single lineage – that separating the two species. In QTL mapping, it does provide fundamental information about the size, location and effects of individual QTL underlying a given species pair difference. However, no information is provided about the timing of QTL evolution; whether the measured QTL arose quite distantly in the past, or are recent.

In this thesis, at the simplest, by bringing just one more species into QTL mapping scheme, and using this third species as an outgroup, a phylogenetic approach to QTL mapping can be developed, in which one can infer the QTL changes along each of the two lineages descending from the outgroup, and also infer QTL changes to the outgroup. The advantage of adding one more species as reference is that with such an approach to QTL mapping, we can go from a directionless comparison to directed comparison, in lineage-specific QTL can be identified. A three-species approach was recently used in genomics to detect non-neutral evolution. From a three-species phylogeny of human, chimpanzee and mouse, several genes related to physiological function like olfaction and nuclear transport were identified as undergoing positive selection along the human-chimp lineage, using the chimpanzee and mouse lineages as outgroups (Clark et al. 2003).

STUDY SPECIES

Owing to the diversity of life history, mating system, and adaptation to novel environments, the genus *Mimulus* has been a model system in plant evolution since 1940 (Clausen et al. 1940). *Mimulus* species occupy habitats from desert to aquatic to alpine, and contain a great degree of genetic diversity (Vickery 1978). More importantly, all species in *Mimulus* are self-compatible and interspecific crossing barrier range from nothing to complete (Vickery 1978). As a result, the *Mimulus* genus is also emerging as a model system for ecological functional genomics (Wu et al. 2007). Studies using *Mimulus* species a model system include those involving the genetics of speciation (Brunet and Eckert 1998; Hiesey et al. 1971; Sweigart et al. 2006), inbreeding depression (Darwin 1876; Dudash and Carr 1998), mating system evolution (Leclerc-Potvin and Ritland 1994; Fishman et al. 2002; Sweigart and Willis 2003), ecological adaptation (Macnair et al. 1993; Angert and Schemske 2005) and cytology (Beardsley et al. 2004; Vickery 1978).

The *Mimulus* genus contains about 160 to 200 species, belonging to two large radiations centered in western North America and Australia (Grant 1924; Beardsley and Olmstead 2002; Beardsley et al. 2004; Vickery 1978). Systematic study has shown that the rapid radiate adaptation in *Mimulus* genus in west North America created approximately 75% of the species of this genus (Whittall et al. 2006), and among those, species in Section *Simiolus* display a high degree of morphological complexity and environmental plasticity (Beardsley et al. 2004). The *Mimulus guttatus* species complex lies within Section *Simiolus* and has about 8 to 12 intercrossable species members

(Campbell 1950; Grant 1924). Natural hybrids are sometimes found co-occupying at wild populations. In this species complex, all of the taxa have a haploid chromosome number of n = 14 (Campbell 1950; Dole and Ritland 1993; Vickery 1964; Vickery 1978).

Yellow monkeyflowers show wide evolutionary changes of mating system, from predominantly selfing to predominantly outcrossing (Ritland and Ritland 1989), but are also intercrossable and of high fecundity, making them valuable material for genetic analysis (Vickery 1978). In a study of 8 different species from *M. guttatus* species complex, Ritland and Ritland (1989) documented a wide range of shift of mating system, and morphological variation related to species reproduction system. Allozyme and chloroplast DNA (cpDNA) RFLP analyses have suggested that among these closely related taxa, inbreeding has multiple, independent origins (Ritland and Ritland 1989; Fenster and Ritland 1992).

The centerpiece of the yellow monkeyflower species complex is *M. guttatus* Fischer ex DC (commonly known as yellow monkeyflower). It is an herbaceous annual and perennial plant that has an extensive distribution throughout western North America in wet, semi-dry meadows and along small streams. *M. guttatus* is often considered the most polytypic species in this species complex, and has been thought as the center of this actively evolving species complex. The diversity within this species complex reflects a rapid adaptation radiation, which has also caused taxonomic confusion, with up to 21 species identified in the group by Pennell (1951), but only four species identified by Campbell (1950). Regardless of this confusion about taxonomy, *M. guttatus* has at least three, independently derived selfing relatives: *M. micranthus*, *M. nasutus* and *M.*

laciniatus (Leclerc-Potvin and Ritland 1994; Ritland and Ritland 1989; Fishman et al. 2002), which can form the basis of replicated studies of the evolution of inbreeding.

The large-flowered *M. guttatus* is herkogamous with high levels of outcrossing (Wright's inbreeding coefficient, $F = 0.38$); and as expected by its small-flower size, the predominantly selfing *M. micranthus* shows high inbreeding (Wright's inbreeding coefficient, $F = 0.73$) (Ritland and Ritland 1989; Fenster and Ritland 1994b; Dudash and Carr 1998). *M. micranthus* Heller is ecologically monotypic and also endemically restricted to the Coastal Range of northern California. As a primarily selfer, *M. micranthus* shows reduced allocation to a number of floral traits that contribute to male function including corolla size and pollen number (Ritland and Ritland 1989). The magnitude of inbreeding depression in outcrossing *M. guttatus* is much greater than in selfing *M. micranthus* in several respects, such as above-ground biomass, pollen production and ovule production (Dudash and Carr 1998). *M. platycalyx*, typically annual, is a mixed-mating derivative of *M. guttatus* with an inbreeding coefficient of $F = 0.54$ (Ritland and Ritland 1989). *M. platycalyx* and *M. guttatus* are sometimes sympatric. Natural hybrids have been identified along Sausal Creek in Marin County, California (Dole and Ritland 1993). Grown in uniform conditions, *M. platycalyx* has floral characters intermediate between *M. guttatus* and *M. micranthus* (Ritland and Ritland 1989). In this study, we used capsule samples collected in Ritland and Ritland 1989) as parental materials. All details about species collection locations, morphological variation, mating system coefficients, phylogenetic genetic distance are in Ritland and Ritland 1989).

OBJECTIVES

The principle objective of this thesis was to develop and implement methodology that can use the information from multiple species comparisons to infer the evolutionary pattern of QTLs. This involves two major components: (1) development of a statistical approach where, within a three-species phylogenetic network, QTL genetic effects residing on specific lineage are inferred and homologous vs. non-homologous QTL identified; and (2), a empirical study with *Mimulus* to demonstrate this approach and address hypotheses about the nature of QTL evolution along lineages that change in inbreeding.

More specifically, the distribution of QTL effects in each of the three lineages of a three-species network allows tests of hypotheses about the pattern of evolution among species. The "null" hypothesis would be that position and effects are randomly distributed among the three branches of an unrooted three species network, and essentially the outcome of mutation and genetic drift. QTLs that occur on a phylogenetic lineage in a non-random way serve as footprint for natural selection. Various hypotheses about the evolution of mating systems and adaptive evolution will be addressed in the following thesis chapters. These include: are phenotypic differences among taxa for the mating system governed by many or few genes? Are these genes recessive in the selfing taxa? Do changes in position of stigmas and anthers result in pleiotropic changes in other floral characters? Are the same processes evident along independent phylogenetic lineages? Also, because the genetic distance between QTL can be inferred from their

phylogenetic map, one can examine the relationship between gene-gene interaction (epistasis) and evolutionary separation.

The specific objectives of each thesis chapter

Chapter 2: Effective number of genetic factors separating inbreeding vs outbreeding species in *Mimulus guttatus* complex

Prior to the development of QTL mapping with molecular markers, Castle and Wright (Castle 1921; Wright 1952, 1968) provided a biometrical method to estimate the number of effective genetic factors underlying quantitative genetic variation. The estimated numbers of genes underlying trait difference are based on the segregational variance of the F2 and the means of the two parental taxa. It provides an estimate of the minimum number of genetic factors, all fixed in the same evolutionary direction that differentiate two morphologically distinct taxa. However, gene number is a result of species evolutionary history, where many different evolutionary forces can alter species morphology. Also, the longer the evolutionary lineages of species are, the greater the opportunity for mutation accumulation at QTL. I therefore expect, as species are separated by longer phylogenetic distances, to detect more genetic factors. In this chapter, evolutionary distance will be estimated for all pairs of *Mimulus* taxa from neutral genetic markers (AFLPs). The number of genetic factors that govern phenotypic changes in pairwise crosses will be obtained by using formula of Fenster and Ritland (1994b), based upon the phenotypic measurements made upon the segregating crosses.

Chapter 3: Lineage specific inferences about QTL evolution among an outcrossing and two derived inbreeding taxa of yellow monkeyflowers

The quantitative genetic scheme of using just a single cross only allows estimates of differences that arise along a single lineage – that separating the two species. In QTL mapping, it does provide fundamental information about the size, location and effects of individual QTL underlying a given species pair difference. However, no information is provided about the timing of QTL evolution: whether the measured QTL arose quite distantly in the past, or are recent. At the simplest, by bringing just one more species into QTL mapping scheme, and using this third species as an outgroup, one can infer the QTL changes along each of the two lineages descending from the outgroup, and also infer QTL changes to the outgroup. Taking the advantage of adding one extra species as reference in classic QTL mapping experiment, one now can go from directionless comparison to having reasonable ability to infer the lineage-specific genetic changes. In this chapter, I first describe an analysis to identify the independent origin of QTL in a species network. An empirical study using *Mimulus* species will then carried out to demonstrate this novel method.

Chapter 4: Epistatic interaction on QTLs involved in the evolution of floral traits in the *Mimulus guttatus* species complex

From QTL-based evidence, epistasis appears to be fairly common in segregating crosses within/between species, suggesting its significance in studying quantitative variation (see review in Mackay 2001a and Malmberg and Mauricio 2005). However,

the importance of epistasis varies among studies (Cockerham and Zeng 1996; Li et al. 1997; Xiao et al. 1995). In theory, epistasis is a property of genetic complexity (Sanjuan and Elena 2006): it is more likely to detect epistasis when there are more genetic loci involved. I expect epistasis to be detected in the comparison of taxa with different mating systems, given the genetic architecture that underlies the difference of selfing rates of *Mimulus* species is polygenic in nature (Fishman et al. 2002; Lin and Ritland 1997). In this chapter, the extent of epistasis for mating system traits between *Mimulus* species will be examined. The major expectation is that species that are more highly diverged will show greater epistasis in the progeny of crosses between them. This is because of the introduction of genes into foreign genetic background, or equilivalently, the disruption of co-adapted genes within species.

Table 1-1. Recent *Mimulus* genetic analyses of species differences

Species	Traits	Number of genes	References
M. lewisi- M. cardinalis	Anthocyanin concentration	> 1	Bradshaw et al. (1998)
	Carotenoid concentration	> 3	
	Lateral petal width	> 8	
	Corolla width	> 8	
	Corolla projected area	> 7	
	Upper petal reflexing	> 5	
	Lateral petal reflexing	> 4	
	Nectar volume	> 3	
	Stamen length	> 7	
	Pistil length	> 7	
	Corolla aperture width	> 8	
	Corolla aperture height	> 4	
M. guttatus- M. platycalyx	Flower length	> 1	Lin and Ritland (1997)
	Pistil length	> 1	
	Long stamen length	> 3	
	Short stamen length	> 3	
	Anther-stigma separation	> 2	
M. guttatus- M. nasutus	Throat width	> 14	Fishman et al. (2002)
	Corolla width	> 14	
	Tube length	> 13	
	Corolla length	> 11	
	Styla length	> 12	
	Stamen length	> 13	
	Stigma-anther separation	> 15	
M. guttatus- M. micranthus	Bud growth rate	> 8	Fenster et al. (1995)
	Flowering time	> 1	Fenster and Ritland (1994b)
	Corolla width	> 9	
	Corolla length	> 10	
	Stamen length	> 8	
	Pistil length	> 13	
	Stigma-anther separation	> 5	

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CHAPTER 2. EFFECTIVE NUMBER OF GENETIC FACTORS SEPARATING INBREEDING VS OUTBREEDING SPECIES IN THE MIMULUS GUTTATUS COMPLEX [1]

INTRODUCTION

Over evolutionary time, the quantitative genetic bases of morphological evolution may involve the accumulation of quantitative trait locus (QTL) differences of either small effect or larger effect. Fisher (1930) argued that adaptation through microevolution should involve the accumulation of many favourable mutations of all small effect, since mutations of large effect less likely to accurately match the optimum. Kimura (1983) reconsidered Fisher's infinitesimal model and derived an expected distribution mutational effect. He noted that while mutations with minor effect may often be favourable, mutations of favourable larger effect are more likely to escape stochastic loss, and hence that mutations with intermediate effect are most likely to be involved in adaptation (Kimura 1983; Griswold and Whitlock 2003). Later, Orr (1999) studied the effects of changes in the distribution of mutational effects and predicted that the genetic basis of phenotypic changes often involves a modest number of factors of large effect and a greater number of factors of small effect.

There have been many empirical studies of the genetic basis underlying species phenotypic differentiation. The genetic basis of phenotypic evolution can be simply controlled by one or a few major genes, or be complex involving many genes with interactions. Orr (2001) reviewed the results studying genetic basis of morphological divergence between species,

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comparing 22 studies of 54 traits involving both plants (mostly *Mimulus* spp.) and animals (mostly *Drosophila* spp.). The most striking feature was the range in the numbers of genetic factors (loci) that distinguish species. For example, Zeng et al. (2000) showed that the difference between *D. simulans* and *D. mauritinan* in the size/shape of the posterior lobe of the male genital arch involved at least 19 loci, where Sucena and Stern (2000) reported the difference in larval morphology between *D. simulans* and *D. sechellia* to be due to as few as one gene. Similarly, wide differences in the numbers of detected genetic factors are also found in plant studies. Studies using genetic analysis of segregation in crosses have shown that many genes are responsible for mating system differences within and among species in *Turnera* (Shore and Barrett 1990), *Clarkia* (Holtsford and Ellstrand 1992) and *Mimulus* (Macnair and Cumbes 1989; Fenster and Ritland 1994b; Fishman et al. 2001). However, studies using the same type of genetic analysis found only a few major genes in *Senecio* (Marshall and Abbott 1982), *Ipomea* (Clegg and Epperson 1988) and *Mimulus* (Bradshaw et al. 1998). Although further studies are needed to gauge the number of genes typically involved with species divergence, different types of inferences, not just on gene number, can also be made.

On such inference is the extent of dominance. Dominance of alleles that confer selfing, over alleles that confer outcrossing, is known to facilitate the evolution of selfing (Haldane 1927). Overdominance was also proposed to facilitate the evolution of a stable, intermediate outcrossing rate (Charlesworth 2006). The extent of dominance for mating system traits has been explored in *Mimulus*, using both QTL mapping techniques (Lin and Ritland 1997; Fishman et al. 2002) and biometric approaches (Macnair and Cumbes 1989; Fenster and Ritland 1994a). However, the extent of dominance has not been systematically examined in a multiple species comparison involving different degrees of evolutionary divergence.

In this study, we first employed the traditional biometric analysis to examine the quantitative genetics basis of the floral character variation associated with the evolution of mating system. The number of genetic factors and the dominance effect were examined on the comparison of one outcrossing *M. guttatus* species and two independent derived inbreeding relatives. With the number of the crosses involving in this *Mimulus* study, we then furthermore addressed the question about the number of genetic factors in relation to species evolutionary divergence. We hypothesized that, if species adaptation is a process of accumulating mutations with different effects, with the drift-mutation balance, the numbers of genetic factors should be greater with greater evolutionary distance. Alternatively, the number of effective genetic factors may only be proportional to the morphological divergence between taxa. The extent of dominance is also examined in this multiple species comparison involving different degrees of evolutionary divergence.

MATERIALS AND METHODS

Study species

Section *Simiolus* in the *Mimulus* genus consists of both predominantly selfing and predominantly outcrossing species (Ritland and Ritland 1989). Many species in Section *Simiolus* are inter-crossable and produce F1 and F2 progeny that are easy to raise and maintain in controlled environments. Those advantages have enabled *Mimulus* species to be the subject of systematic and genetic studies for decades (Vickery 1978).

The *M. guttatus* species complex lies within the Section *Simiolus* and has about 8 to 12 inter-crossable species members (Campbell 1950; Grant 1924). Natural hybrids are sometimes found in the field. All taxa have a haploid chromosome number of n = 14 (Campbell 1950; Dole and Ritland 1993; Vickery 1964; Vickery 1978). Species in this complex have a wide range of natural self-fertilization rates. In a study of 8 different species from *M. guttatus* species complex, Ritland and Ritland (1989) documented such variation, as well as morphological variation related to shifts in allocation to male vs. female reproductive effort. Allozyme and chloroplast DNA (cpDNA) RFLP analyses have also indicated that among these closely related taxa, inbreeding has multiple, independent origins (Ritland and Ritland 1989; Fenster and Ritland 1992).

M. guttatus Fischer ex DC, also known as yellow monkeyflower, is an herbaceous annual and perennial plant that has an extensive distribution throughout western North America in wet, semi-dry meadows and along small streams. *M. guttatus* is the most polytypic species in this species complex, and has been thought as the center of this actively evolving species

complex. *M. guttatus* has at least three, independently derived selfing relatives: *M. micranthus*, *M. nasutus* and *M. laciniatus* (Ritland and Ritland 1989; Fishman et al. 2002). The large-flowered *M. guttatus* are herkogamous with a relatively higher degree of outcrossing rate (Wright's inbreeding coefficient, $F = 0.38$), compared with other smaller-flowered *Mimulus* and predominantly selfing *M. micranthus* (Wright's inbreeding coefficient, $F = 0.73$) (Ritland and Ritland 1989; Fenster and Ritland 1994a; Dudash and Carr 1998). *M. micranthus* Heller is ecologically monotypic and also endemically restricted to the Coastal Range of northern California. As a predominant selfer, *M. micranthus* shows reduced allocation to a number of floral traits that contribute to male function including corolla size and pollen number (Ritland and Ritland 1989). The magnitude of inbreeding depression in outcrossing *M. guttatus* is much greater than in selfing *M. micranthus* for several fitness components, including above-ground biomass, pollen production and ovule production (Dudash and Carr 1998). *M. platycalyx*, an annual like the other two species, is a mixed-mating derivative of *M. guttatus* with an inbreeding coefficient of $F = 0.54$ (Ritland and Ritland 1989). *M. platycalyx* and *M. guttatus* are sometimes sympatric. Natural hybrids have been identified along Sausal Creek in Marin County, California (Dole 1992). Grown in uniform conditions, *M. platycalyx* has floral characters intermediate between *M. guttatus* and *M. micranthus* (Ritland and Ritland 1989). In this study, we used seed collected in Spring 2001 from the same locations as given in Ritland and Ritland (1989).

Pairwise crosses between Mimulus species

All nine pairwise reciprocal backcrosses and F2 intercrosses were conducted between *M. guttatus*, *M. platycalyx* and *M. micranthus* (sample sizes are given in Table 2-1). Parents were

simultaneously grown in different growth chambers. F1 crosses were performed by inter-crossing parents, and maintained in other chamber. F2 progeny were produced by selfing F1 individuals. Backcrosses were performed in both directions to both parents (BC1 and BC2; BC1 is the backcross back to larger flower parent and BC2 is the backcross to smaller flower parent). All plants were grown at 18C/14C day/night temperature, with 18-hour daylight in growth chambers, in the same batch of Pro-Mix soil. To avoid pollen contamination, flowers were bagged after manual pollination. Crosses between parents were performed in 2000; the F2 and two backcrosses from *M. guttatus* x *M. platycalyx* were performed in 2001 under the same growth chamber conditions. Crosses from *M. guttatus* x *M. micranthus* and *M. platycalyx* x *M. micranthus* were performed in the same growth chamber in continuous years.

During the BC and F2 generations, and for parents, the following characters were measured on individuals: (1) corolla length, (2) corolla length, (3) pistil length, (4) stamen length (averaged over the low and high anthers), (5) stigma-anther separation, (pistil length minus the average stamen height). A digital calliper was used to take dimensional measurements.

Gene number estimation

The most widely used method for estimating the effective number of factors (N_e) was developed by Castle (1921) and his graduate student Sewall Wright (Castle 1921; Wright 1968). It utilizes information on the phenotypic means and variance of two parental lines, and their line-cross derivatives. It is known as the Castle-Wright estimator (Eq. 1),

$$N_E = \left(U_{P1} - U_{P2}\right)^2 \Big/ 8\left(s_{F2}^2 - s_E^2\right) \qquad \text{(Eq. 1)}$$

where the *Us* are estimated means in the two parental taxa, and the s_{F2}^2 and s_E^2 are the segregational variance in F2 progeny and the environmental variance, respectively. The estimator has a number of assumptions, including additivity of gene effects, equality of allelic effect and unidirectional gene effect (Lynch and Walsh 1998).

In actuality, there is often dominance of gene effect, which can also vary among loci. If the F1 is not exactly intermediate between the parent means, there is some dominance present (Wright 1968). Here, we also incorporated dominance in our gene number estimation, following Fenster and Ritland (1994b). However, we still assume a uniform dominance coefficient for all loci (as in Fenster and Ritland 1994b). To incorporate dominance, the ratio of dominance to additive effect (D/A) is first estimated as:

$$D/A = \frac{2(U_{F2} - E_{F2})}{U_{P1} - U_{P2}}, \qquad \text{(Eq. 2)}$$

and the gene number estimate, corrected for dominance effects, is then obtained as:

$$N_E(D) = \frac{\left(2 + (D/A)^2\right)(U_{P1} - U_{P2})^2 - C}{16S} \qquad \text{(Eq. 3)}$$

In these expressions, C is a correction factor that equals to the statistical variance of $(1 + (D/A))(U_{P1} - U_{P2})$, and $E_{F2} = (U_{P1} + U_{P2})/2$ and $E_{B1} = \left(3U_{P1} + U_{P2}/4\right)$ are the averages of the two parental means or that expected in the absence of dominance (Fenster and Ritland 1994b). The ratio of dominance to additive effect (D/A) estimators for backcross progeny are:

$$D/A = \frac{4(U_{B1} - E_{B1})}{U_{P1} - U_{P2}} \qquad \text{(Eq. 4)}$$

$$N_{E(D)} = \frac{\left(1 + \left(D/A\right)^2\right)\left(U_{P1} - U_{P2}\right)^2 - C}{16S} \quad . \qquad \text{(Eq. 5)}$$

The estimators for the backcross to second parent are the same except that B1 is replaced by B2 and P1 and P2 are interchanged, resulting in a degree of the sign of degree of dominance (D/A).

RESULTS

Table 2-1 lists the means and the sample sizes for each floral character calculated from each of the parental generation, F1 generations, F2 generations and backcross generations. *M. guttatus* is the most outbreeding in this study and it also shows the largest floral characters. For corolla width and length, *M. guttatus* is about twice as large as the intermediate inbreeder *M. platycalyx*, and almost three times larger than the highly inbreeding *M. micranthus*. Correspondingly, *M. guttatus* has the longest pistil and stamens. *M. guttatus* also shows the greatest separation between the height of stigma and anther (character stigma-anther separation mean = 2.02). The intermediate inbreeder, *M. platycalyx*, shows very little stigma-anther separation (mean = 0.26), while for inbreeding *M. micranthus*, stigma-anther separation was negative (mean = -1.35).

Figure 2-1 shows distribution of corolla length across generations for the cross of *M. guttatus* with *M. micranthus*. The F1 distribution is slightly skewed toward *M. micranthus* parents, but the distribution of F2 falls, as expected, in between the two *Mimulus* parents. Figure 2-2 plots the mean vs. the variance for corolla length for the same cross. With respect to the mean, the F1 and the backcrosses were approximately intermediate, while the variances were inflated for the backcross to *M. guttatus* and especially the F2, suggesting transgressive segregation against the *M. guttatus* genetic background.

Degree of dominance

Estimates of dominance based on Eq. 2 (for F2s) and Eq. 4 (for backcrosses) are given in Table 2-2. The differentiation of floral characters among species within *M. guttatus* species complex was governed by a great degree of dominance. Among all characters, except the character of stigma-anther separation, estimates of dominance are mostly negative, suggesting that the direction of dominance is that inbreeding alleles are dominant over outbreeding alleles (the more outbred species was parent #1, the more inbred parent #2, so that this direction of dominance would give a negative *D/A* ratio). For example for corolla length, dominance estimates range from almost zero (-0.06; F1 of *M. platycalyx* - *M. micranthus*) to strongly negative (–1.40; backcross *M. platycalyx* - *M. micranthus*). There are some exceptions: in the cross of *M. platycalyx* and *M. micranthus* dominance shows changes of direction between the F1 and F2 generations for corolla width and pistil length (Table 2-2). More importantly, not only did stigma-anther separation show some extreme variation for dominance, but all estimates were positive (Table 2-2).

Gene number estimates with the correction of uniform dominance

Estimates of the number of genetic factors underlying the differentiation of taxa are listed in Table 2-3. The numbers of factors ranged from 13.96 (pistil length in the backcross of *M. guttatus* and *M. micranthus* to *M. micranthus*) to 0.45 (pistil length, *M. guttatus* x *M. platycalyx* backcross to *M. platycalyx*). A low number of genetic factors was also found between *M. guttatus* and *M. platycalyx*, ranging from 4.73 (in the backcross to *M. guttatus* for corolla length) to as little as 0.82 (in the backcross to *M. platycalyx* for corolla length). Larger estimates were found between *M. guttatus* and *M. micranthus*. The lowest estimate in this cross

was 2.14 genetic factors (in the backcross to *M. guttatus* for stigma-anther separation) and the greatest one was 13.96 (in the backcross to *M. micranthus* for pistil length).

Number of genetic factors in relation to species evolutionary divergence versus morphological divergence

Figure 2-3 shows the results for the number of effective genetic factors with the species genetic divergence. The genetic divergence between *Mimulus* species was estimated from AFLP (amplified fragment length polymorphism) variation (see Chapter 3). The estimated genetic distance between *M. guttatus* and *M. micranthus* was 0.08 (S.E.=0.01), between *M. guttatus* and *M. platycalyx* was 0.19 (S.E.=0.02), and between *M. platycalyx* and *M. micranthus* was 0.20 (S.E.=0.02) (Table 2-4). This three species phylogeny is displayed in Figure 3-6 (Chapter 3). Euclidean distances between *Mimulus* species for each trait were given as morphological divergence. Standard errors of morphological divergence were estimated from taking squared root of variance among the 1,000 bootstraps. To properly compare crosses with different degree of genetic divergence, we only select the data from F2 crosses in this analysis. In the cross of *M. guttatus* x *M. micranthus*, the species with greatest difference in floral morphology but least degree of genetic differentiation, shows the largest number of estimate number of effective genetic factors (Table 2-4). The number of genetic factors is smallest in the cross between *M. guttatus* and *M. platycalyx*, which paradoxically had the greatest genetic differentiation (Figure 2-3). Summarized in Table 2-4, the hypothesis of increasing number of genetic factors with genetic distance is not supported; however, the species with greatest morphological divergence does still show the largest number of effective genetic factors (the cross of *M. guttatus* x *M. micranthus* in Table 2-4).

DISCUSSION

In this study, we followed the traditional biometric approach to estimate the number of effective genetic factors differentiating floral characters among *Mimulus* taxa. The individuals used as parents in this study were all collected directly from field populations. F1 and later generation crosses were manipulated and grown under consistent growth chamber conditions. We then applied Fenster and Ritland's (1994b) formula to correctly estimate the number of genetic factors by incorporating dominance into the estimation procedure. We found the numbers of genes underlying floral morphological difference among *Mimulus* species to range from 1.11 to 6.53 (for corolla width) and 0.7 to 7.04 (for corolla length) (Table 2-3). Although the numbers estimated in our study assumed a uniform dominance, our results here still reveal a small to intermediate number of effective genetic factors underlying *Mimulus* species phenotypic differentiation. A similar result of few genetic factors was also found for floral traits that cause autogamous selfing in *M. cupriphilus*, as well as for traits associated with the adaptation to heavy-metal sites (Macnair and Cumbes 1989).

Violations of the Wright-Castle estimator

A variety of statistical approaches have been proposed for estimating the effective (or minimum) number of genetic loci contributing to a quantitative trait (Serebrovsky 1928; Tan and Chang 1972; Wright 1952, 1968). Among those, the original method of Wright (in Castle 1921; Wright 1952, 1968) is most commonly used. While the requirement of inbred lines in Wright method is sometimes violated in practice, and may produce unwanted complications of

inbreeding depression on the mean and developmental stability of the lines. However, Wright's method can be applied to crosses between genetically heterogeneous populations (Lande 1981); this minimizes the extent of inbreeding depression and reduces the total time necessary to perform the experimental crosses.

In addition, even though the effect of dominance was discussed in his later review (Wright 1968), the original Wright-Castle estimator assumed additivity among loci underlying the given morphological divergence. Non-additive genetic effects may either increase or decrease the estimate of gene number, but is perhaps small compared with the effect of linkage, which could possibly lead to a serious underestimation of the number of gene controlling a trait difference (Zeng et al. 1990). To ignore the effect of dominance would certainly underestimate the gene number separating taxa (Wright 1968). In the current work, we have dealt with the issue of dominance via using the estimators of Fenster and Ritland (1994b).

Comparisons to previous QTL studies in Mimulus

The *M. guttatus* species complex is a well-known system to study mating system divergence. The most common species in this complex, *M. guttatus*, is a predominantly outcrossing species (Latta and Ritland 1994b), and self-fertilization appears to have evolved several times within this species complex (Vickery 1978; Fenster and Ritland 1994b). The autogamous self-fertilization species, *M. micranthus* and *M. nasutus*, have striking reductions in corolla size and stigma-anther separation, as well as with changes in the production of male and female gametes (Fishman et al. 2002). The molecular evidence for the independent origin of selfing in this group suggests that evolution of selfing in this group may involve different genes or different genetic mechanisms (Ritland and Ritland 1989; Fenster and Ritland 1992, 1994a).

Because of the intercrossibility of species in this species complex, the genetic architecture of their difference has been characterized with both traditional and advanced molecular quantitative genetic approaches (Fenster and Ritland 1994a; Fishman et al. 2002; Macnair and Cumbes 1989). However, the estimated number of effective genetic factors underlying the mating system difference was not consistent across studies. Fenster and Ritland (1994a) analyzed the effective number of genetic factors differentiating 6 floral traits among 4 *Mimulus* taxa, and reported that the mean number of genes separating selfer and outcrosser *Mimulus* in all F2 generations and backcrosses was ranged from 5.3 (for stigma-anther separation) to 12.8 (for pistil length). The effective number of genes estimated by the biometric approach cannot exceed the number of chromosomal segments segregating independently in one generation, which equals to, in most cases, the haploid number of chromosomes (Darlington 1937; Lande 1981). The estimated numbers of genetic factors using biometric approach are close to the upper limit (n = 14 for *Mimulus*, Vickery 1978).

A polygenic evolutionary system has also been shown in studies using molecular quantitative genetic approaches by Fishman et al. (2002) and Fishman and Willis (2001), who used 255 AFLP and microsatellite markers and constructed a framework genetic linkage map of hybrid genome of *M. guttatus* and *M. nasutus*. They then analyzed the genetic basis of 16 floral characters in a large segregating F2 population and identified 24 QTL underlying interspecific differences in seven floral traits (Fishman et al. 2002). In the absence of epistasis, at least 18 additional QTL could be added on to the effect responsible to corolla width differentiation. It is seemingly conceivable that, based on both biometric and molecular quantitative genetic models, divergence between outcrosser *M. guttatus* and selfer *M. nasutus* is a polygenic system.

However, in the comparison of the studies targeting the same interest, studies have identified relatively small numbers of effective genetic factors responsible for the divergence of *Mimulus* mating system. *Mimulus* floral size features, such as floral width and floral length, are known to influence mating system through pollinator attraction during reproduction (Karron et al. 1997; Chang and Rausher 1998). An early QTL study of floral divergence between *M. guttatus* and *M. platycalyx*, a selfer with relatively large flowers but no degree of stigma-anther separation, found a relatively low number of QTLs (one to three) affecting five mating system characters, and each QTL explained 7.6% to 28.6% of the phenotypic variation (Lin and Ritland 1997). The genetic control of 12 morphological differences on floral characters between the bumblebee-pollinated *M. lewisii* and hummingbird-pollinated *M. cardinalis* was carried out in a large linkage mapping population of F2 plants by Bradshaw et al. (1998), who identified one to six QTLs for each trait with most traits appearing to have at least one major QTL explaining larger than 25% of phenotypic variation. This research implied an oligo-genetic model, where single genes of individually large effect or clusters of tightly linked genes with large cumulative effect play important role in the evolution of floral characters in *Mimulus*.

The incongruent results of gene numbers vs. morphological divergences raises questions about the strength of selection vs. the amount of standing variation (Orr 2001). In this thesis, a wide range of effective number of genetic factors was identified (Table 2-3). Given the assumptions of additivity and uniformity of gene action in the traditional biometric method, our findings can only suggest the minimal mutational steps that allow populations to reach another fitness peak. The actual number of genetic changes is undoubtedly larger than what we have estimated in this thesis. Also, the divergence of traits driven by natural selection, i.e. mutations toward selfing favoured on inbreeding lineage, could generate a covariance of alleles at

underlying QTL/effective genetic factors. This would decline the expectation in gene number estimates with greater species evolutionary divergence under the drift-mutation hypothesis.

Dominance and the evolution of inbreeding

We observed a consistent dominance of alleles for inbreeding over alleles for outbreeding. In our study, significant dominance was found for almost all crosses and floral traits (Table 2-2). As illustrated in Figure 2-4, a general pattern of directional dominance was identified. In Figure 2-4, three F2 crosses between *Mimulus* were chosen for the comparison, (1) the cross between highly outbreeder *M. guttatus* and highly selfer *M. micranthus*, (2) the cross between *M. guttatus* and intermediate outbreeder *M. platycalyx*, and (3) the cross between intermediate outbreeder *M. platycalyx* and highly selfing *M. micranthus*. A directional dominance towards selfing taxa of *Mimulus* is clearly evident, with the exception being stigma-anther separation where dominance was found in the direction of outcrossing over selfing (large separation over small separation).

In a large outcrossing population, the probability of fixation of completely recessive advantageous new mutations is much less than that for favorable mutation with some expression in heterozygotes, making dominance important in the evolution of selfing at early stage (Haldane 1927). Dominance therefore increases the probability of the evolution for selfing. Our results support this hypothesis. Our study further suggests the initial stage of evolving selfing is likely caused by a small number of major genetic factors with dominance (Chapter 3).

Such findings of partial dominance towards inbreeding characters was also found in Fenster and Ritland (1994b). Further, as depicted in Figure 2-4, dominance is greater when comparing species with high outcrossing rate with species of intermediate outcrossing (e.g. *M. guttatus* vs. *M. platycalyx*), compared to the intermediate outcrossers vs. high selfers (e.g. *M.*

platycalyx vs. *M. micranthus*). Our result also supports the theoretical work of Latta and Ritland (1994a), who demonstrated a stable intermediate outcrossing is more likely to occur if selfing alleles were dominant and when multiple genes are involved in controlling outcrossing rate.

In theory, characters that closely associated with fitness are expected to be controlled by genes with non-additive genetic effects (Falconer 1981); and nevertheless, dominance has also been recognized its importance role in shaping mating system characters in *Mimulus* study systems. For example, using QTL mapping between *Mimulus* species pair, *M. guttatus* and *M. nasutus*, Fisherman et al (2002) identified a polygenic system with many QTL; partial dominance was observed and the directionality of dominance was found nearly equal between the two parental species. Lin and Ritland (1997) also found mixed results, in that dominance was found toward both parents; however a greater number of QTL had dominance towards the selfing taxa.

In the *M. guttatus* species complex, the reduced stigma-anther separation is directly indicative of increased selfing (Carr and Fenster 1994; Dole 1992; Ritland and Ritland 1989). Stigma-anther separation in our study interestingly shows dominance of outcrossing alleles over selfing alleles (Figure 2-4). This type of dominance would initially maintain stigma-anther separation at the early stages of the evolution of inbreeding, which may be advantageous, since it helps to maintain the standing genetic variation. However, as flowers evolve towards smaller size, the stigma-anther separation would likewise become smaller, leading to a higher self-fertilization rate. Also, when species become more inbreeding, genes and the associated genetic dominance towards selfing taxa that reduce the size of flowers make self-fertilization more efficient, due to changes of sex allocation (Ritland and Ritland 1989). Thus the dominance for stigma-anther separation may be a transient phenomenon.

Table 2-1. Generation means and sample sizes for each character

		Characters				
Cross	**Sample size**	**Corolla length**	**Corolla width**	**Pistil length**	**Stamen length**	**Stigma-anther separation**
M. guttatus* X *M. platycalyx						
P- *M. guttatus*	10	34.20 (1.07)	30.25 (0.79)	18.96 (0.53)	16.95 (0.25)	2.02 (0.39)
P- *M. platycalyx*	9	23.23 (0.57)	17.77 (0.40)	13.15 (0.31)	12.89 (0.29)	0.26 (0.26)
F1	20	24.08 (0.60)	19.61 (0.54)	14.72 (0.20)	12.52 (0.22)	2.20 (0.14)
F2	100	24.70 (0.3)	19.88 (0.3)	14.80 (0.2)	12.98 (0.2)	1.81 (0.07)
BC to *M. guttatus*	216	29.83 (0.19)	24.16 (0.18)	18.09 (0.11)	14.64 (0.10)	3.4 (0.07)
BC to *M. platycalyx*	173	23.71 (0.32)	18.58 (0.28)	14.35 (0.18)	12.03 (0.16)	2.24 (0.10)
M. guttatus* X *M. micranthus						
P – *M. guttatus*	10	34.20 (1.07)	30.25 (0.79)	18.96 (0.53)	16.95 (0.25)	2.02 (0.39)
P – *M. micranthus*	11	11.39 (0.36)	8.10 (0.30)	6.38 (0.22)	7.21 (0.23)	-1.35 (0.15)
F1	35	14.88 (0.84)	12.26 (0.82)	9.17 (0.52)	8.82 (0.33)	0.34 (0.23)
F2	184	16.97 (0.23)	13.71 (0.22)	10.41 (0.25)	9.19 (0.09)	1.22 (0.05)
BC to *M. guttatus*	149	26.71 (0.33)	22.34 (0.33)	16.09 (0.15)	13.86 (0.14)	2.21 (0.07)
BC to *M. micranthus*	122	13.86 (0.22)	10.67 (0.22)	8.51 (0.11)	7.89 (0.10)	0.62 (0.05)
M. platycalyx* X *M. micranthus						
P – *M. platycalyx*	9	23.23 (0.56)	17.77 (0.40)	13.15 (0.31)	12.89 (0.29)	0.26 (0.26)
P – *M. micranthus*	11	11.39 (0.36)	8.10 (0.30)	6.38 (0.22)	7.21 (0.23)	-1.35 (0.15)
F1	36	16.58 (0.23)	16.98 (0.23)	10.39 (0.09)	9.76 (0.11)	0.63 (0.06)
F2	221	11.74 (0.22)	9.95 (0.14)	8.43 (0.08)	7.76 (0.12)	-0.31 (0.04)
BC to *M. platycalyx*	72	16.13 (0.28)	11.66 (0.22)	9.82 (0.14)	10.03 (0.17)	-0.2 (0.09)
BC to *M. micranthus*	166	11.74 (0.25)	8.13 (0.20)	7.09 (0.14)	7.76 (0.14)	-0.67 (0.06)

Values in parentheses are standard errors to the mean

Table 2-2. Estimates of degree of dominance (D/A ratio) in F1, F2 and backcross generations

	Characters				
Cross	**Corolla length**	**Corolla width**	**Pistil length**	**Stamen length**	**Stigma-anther separation**
M. guttatus* X *M. platycalyx					
F1	-0.42 (0.07)	-0.30 (0.05)	-0.23 (0.06)	-0.59 (0.09)	0.60 (0.27)
F2	-0.73 (0.11)	-0.66 (0.08)	-0.43 (0.10)	-0.96 (0.15)	0.89 (0.41)
BC to *M. guttatus*	-0.59 (0.26)	-0.95 (0.14)	0.40 (0.30)	-1.28 (0.21)	4.52 (1.80)
BC to *M. platycalyx*	-0.82 (0.22)	-0.74 (0.14)	-0.17 (0.21)	-1.85 (0.37)	3.74 (1.11)
M. guttatus* X *M. micranthus					
F1	-0.35 (0.04)	-0.31 (0.04)	-0.28 (0.05)	-0.34 (0.04)	0.01 (0.09)
F2	-0.51 (0.04)	-0.49 (0.03)	-0.36 (0.05)	-0.68 (0.05)	0.39 (0.15)
BC to *M. guttatus*	-0.31 (0.14)	-0.43 (0.11)	-0.09 (0.13)	-0.32 (0.10)	1.23 (0.45)
BC to *M. micranthus*	-0.57 (0.07)	-0.54 (0.66)	-0.32 (0.07)	-0.93 (0.10)	1.34 (0.27)
M. platycalyx* X *M. micranthus					
F1	-0.06 (0.03)	0.42 (0.04)	0.11 (0.03)	-0.05 (0.04)	0.74 (0.21)
F2	-0.61 (0.02)	-0.62 (0.06)	-0.39 (0.06)	-0.58 (0.08)	0.32 (0.22)
BC to *M. platycalyx*	-1.40 (0.04)	-1.53 (0.14)	-0.97 (0.13)	-1.22 (0.16)	-0.10 (0.53)
BC to *M. micranthus*	-0.88 (0.15)	-0.98 (0.13)	-0.58 (0.14)	-0.98 (0.20)	0.72 (0.37)

Values in parentheses are standard errors to the mean

Table 2-3. Estimates of effective number of genetic factors underlying *Mimulus* floral character divergence, assuming an uniform correlation of uniform dominance coefficient among loci.

	Characters				
Cross	**Corolla length**	**Corolla width**	**Pistil length**	**Stamen length**	**Sigma-anther separation**
M. guttatus* X *M. platycalyx					
F2	2.08(1.03-3.37)	2.90 (1.51-4.19)	2.66 (1.27-5.81)	1.54 (0.89-2.87)	2.13 (0.64-4.9)
BC to *M. guttatus*	2.79(1.05-3.3)	4.73 (2.88-7.84)	4.44 (1.09-8.67)	1.78 (1.17-2.74)	4.26 (1.27-11.07)
BC to *M. platycalyx*	0.82(0.48-1.19)	1.59 (0.92-2.03)	0.45 (0.28-0.71)	1.37 (0.89-2.21)	2.10 (0.58-18.0)
M. guttatus* X *M. micranthus					
F2	5.17 (3.42-6.38)	5.12 (3.47-6.19)	10.2 (8.69-15.14)	8.42 (6.82-10.55)	2.85 (2.64-2.91)
BC to *M. guttatus*	2.89 (1.8-3.78)	3.08 (1.98-3.99)	7.58 (3.07-13.56)	2.14 (1.53-3.05)	2.57 (1.54-2.64)
BC to *M. micranthus*	9.87 (6.06-12.08)	9.07 (5.92-10.69)	13.96 (8.76-32.35)	8.27 (6.01-11.86)	6.93 (4.13-7.17)
M. platycalyx* X *M. micranthus					
F2	5.23 (3.32-6.51)	4.58 (3.27-6.3)	5.8 (4.07-8.5)	3.47 (2.15-6.65)	2.35 (1.01-5.97)
BC to *M. platycalyx*	7.08 (3.79-9.72)	8.90 (4.66-10.93)	5.24 (3.24-10.31)	4.27 (2.64-8.06)	0.46 (0.2-1.11)
BC to *M. micranthus*	1.7 (1.2-2.29)	2.04 (1.52-2.91)	1.23 (0.92-1.74)	1.18 (0.83-1.74)	0.67 (0.29-1.38)

Values in parentheses are 95% confidence intervals.

Table 2-4. Estimates of effective number of genetic factors, morphological divergence and genetic distance in *Mimulus* F2 crosses

Cross	Estimate number of effective genetic factors in F2 cross[1]	Morphological divergence[2]	Genetic distance[2]
Corolla width			
M. guttatus x *M. micranthus*	2.90 (1.5-4.2)	22.15 (0.08)	0.08 (0.01)
M. guttatus x *M. platycalyx*	5.12 (3.5-6.2)	12.48 (0.08)	0.19 (0.02)
M. platycalyx x *M. micranthus*	4.58 (3.3-6.3)	9.67 (0.05)	0.20 (0.02)
Corolla length			
M. guttatus x *M. micranthus*	5.17 (3.2-6.4)	22.81 (0.11)	
M. guttatus x *M. platycalyx*	2.08 (1.0-3.4)	10.98 (0.11)	
M. platycalyx x *M. micranthus*	5.23 (3.3-6.5)	11.83 (0.06)	
Pistil length			
M. guttatus x *M. micranthus*	10.2 (8.7-15.1)	12.59 (0.05)	
M. guttatus x *M. platycalyx*	2.66 (1.3-5.8)	5.81 (0.05)	
M. platycalyx x *M. micranthus*	5.80 (4.1-8.5)	6.78 (0.03)	
Stamen length			
M. guttatus x *M. micranthus*	8.42 (6.8-10.6)	9.20 (0.04)	
M. guttatus x *M. platycalyx*	1.54 (0.9-2.9)	4.02 (0.04)	
M. platycalyx x *M. micranthus*	3.47 (2.2-6.7)	5.17 (0.03)	
Stigma-anther separation			
M. guttatus x *M. micranthus*	2.85 (2.6-2.9)	3.39 (0.04)	
M. guttatus x *M. platycalyx*	2.13 (0.6-4.9)	1.78 (0.04)	
M. platycalyx x *M. micranthus*	2.35 (1.0-5.0)	1.61 (0.03)	

1. Values in parentheses are 95% confidence intervals.
2. Values in parentheses are standard errors to the mean.

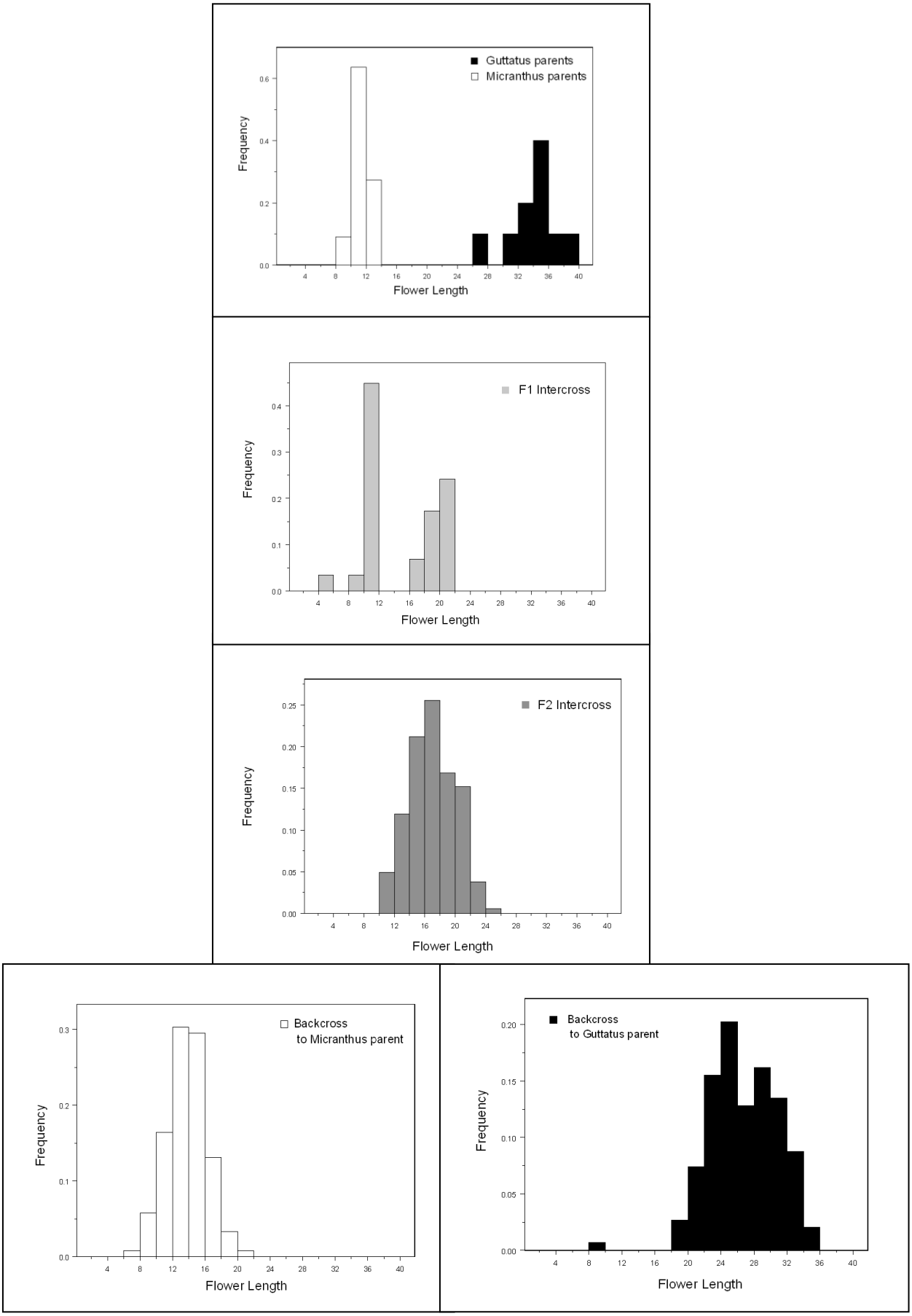

Figure 2-1. Distribution of corolla lengths for parental, F1, F2 and backcrosses in the cross of *M. guttatus* and *M. micranthus*.

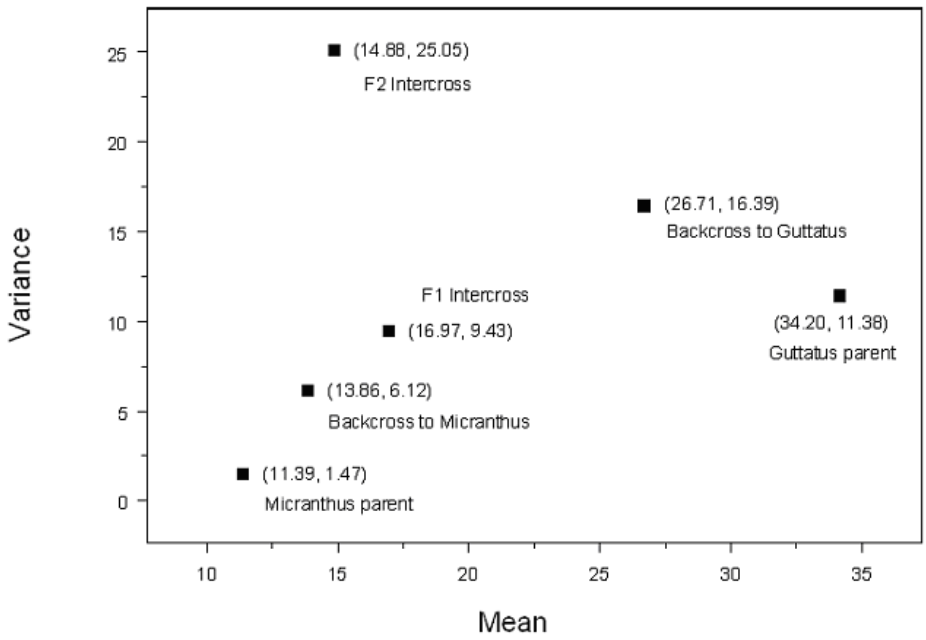

Figure 2-2. The distribution of means and variances of corolla length trait variation among parental, F1, F2 and backcrosses in the cross of *M. guttatus* and *M. micranthus*.

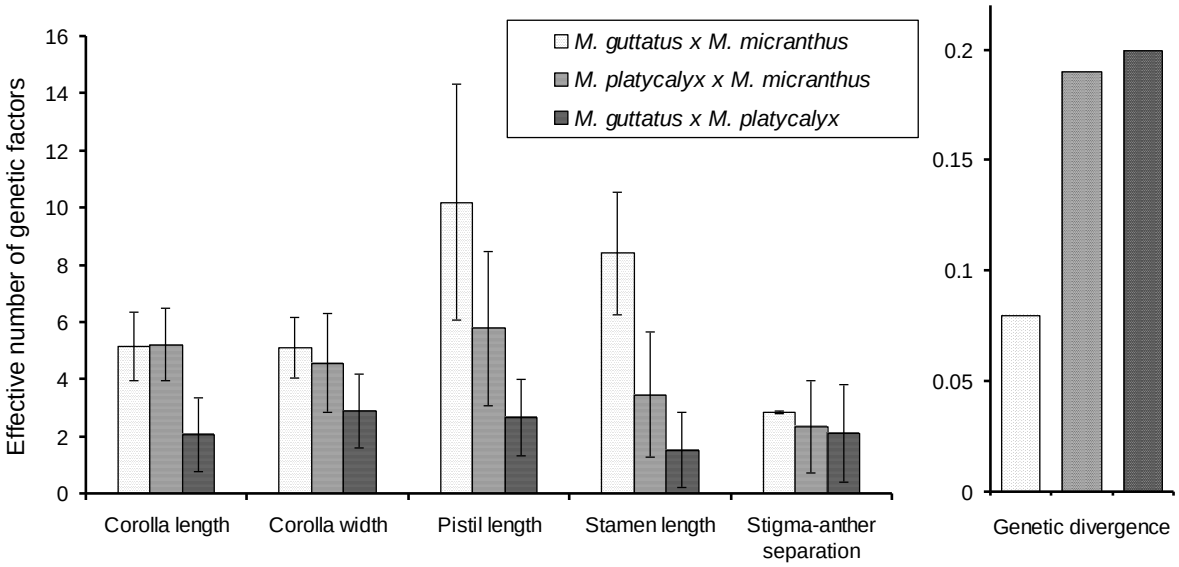

Figure 2-3. The pattern of estimated number of genetic factors upon species evolutionary relationship

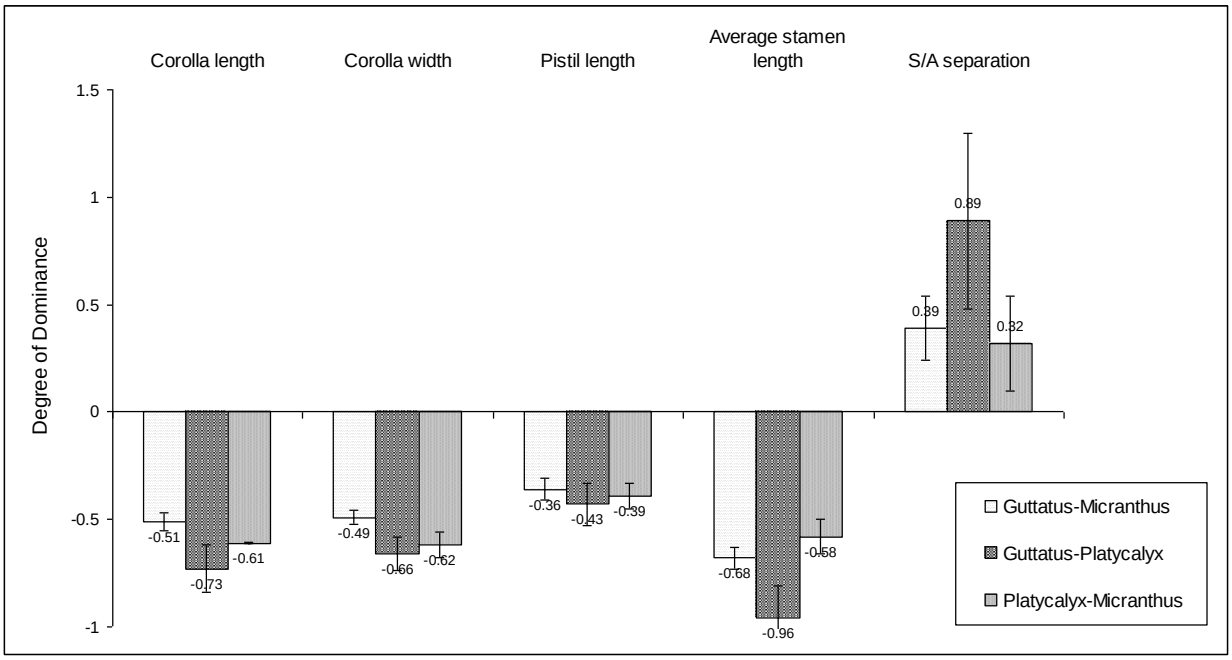

Figure 2-4. The distribution of degree of dominance among *Mimulus* F2 crosses

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CHAPTER 3. LINEAGE SPECIFIC INFERENCES ABOUT QTL EVOLUTION AMONG AN OUTCROSSING AND TWO DERIVED INBREEDING TAXA OF YELLOW MONKEYFLOWERS [1]

INTRODUCTION

A major challenge of evolutionary biology is to understand the molecular genetic basis of complex traits that differentiate taxa. A crude characterization of the genetic architecture of species differences can be obtained with "classical" biometric approaches, where the number of genetic factors that distinguish two taxa is estimated using the segregation variance in artificial crosses and the difference of parental means (Wright 1968; Chapter 2). With developments in genotyping technologies and statistical genetics, quantitative trait locus (QTL) mapping has become a powerful means of ascertaining the genetic architecture of species differences (Tanksley 1993; Westerbergh and Doebley 2004). Regardless, the identification of genes affecting complex traits, including those of evolutionary significance, is considered to be one of the most challenging tasks of genetics (Risch 2000).

Although problems exist with the accuracy of QTL mapping, QTL analysis still provides fundamental information about the size, location and effects of individual QTL that differ between the two parents of the cross (Broman 2001; Price 2006). QTL mapping techniques have been used for a large variety of traits, including those involved with human diseases (Cardon and Bell 2001), adaptation in natural populations (Slate

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2005) and breeding of animals (Mott et al. 2000). QTL mapping is also used to dissect the genetic architecture of complex traits in model organisms such as *Arabidopsis* (Ungerer et al. 2002), *Drosophila* (Mackay 2001b), maize (Westerbergh and Doebley 2002) and mouse (Cheverud et al. 1996).

In plants, a classic example of QTL mapping for adaptive traits has involved the comparison between bumblebee pollinated *M. lewsii* and hummingbird pollinated *M. cardinalis* (Bradshaw et al. 1995; Schemske and Bradshaw 1999). These studies found that 9 of 12 traits related to the shift of pollination syndrome have at least one QTL that explained more than 25% of variation between species. At the *yup* locus, a single QTL accounted for 83% of phenotypic variation for carotenoid concentration between species. This is probably the best known case of a major QTL for morphological differentiation between species (Orr 2001). However, studies such as this mainly use pairwise comparisons, which allow estimates of those differences only along a single lineage. Within this lineage, no information is available about the evolutionary pattern and process of genetic changes, such as time of appearance of new QTL along the lineage.

In this paper, we develop a phylogenetic approach for QTL mapping, in which the genetic effect of QTL along phylogenetic lineages is inferred. At the simplest, by bringing in a third taxa, one can infer the QTL changes that have occurred along the two lineages that lead to the two most closely related taxa. The third lineage traces from the common ancestor of these two taxa, back to the common ancestor of all three taxa, and forwards again to the third taxa, making lineage specific inferences more complicated. In the case of a star phylogeny, QTLs can be unambiguously identified in all three lineages. After mapping QTL onto lineages, we can determine if QTL at the same map position are

homologous (arising in an ancestral lineage leading to derived taxa) or non-homologous (arising independently in derived lineages). The distribution of homologous QTL on a species network can also be used to examine hypotheses such as drift-mutation balance versus directional selection model in the evolution of quantitative trait variation.

A related approach of using a three- taxa phylogeny was recently applied to mammals. From a three-species phylogeny involving human, chimpanzee and mouse, several genes related to physiological function like olfaction and nuclear transport were identified as undergoing positive selection along the human-chimp lineage, using the mouse lineage as an outgroup (Clark et al. 2003).

In the yellow monkeyflower (*Mimulus guttatus*) species complex, self-fertilization has presumably arisen several times from on outcrossing ancestor. The evolution of selfing is accompanied by changes of an entire syndrome of floral traits, including male allocation, reduced size of floral characters, reduced attraction to pollinators and reduction of the spatial and temporal separation of male and female reproductive organs within the flower (Jain 1976; Ritland and Ritland 1989). We selected five quantitative floral characters as representative traits for the evolution of inbreeding in *Mimulus* species and focused on three intercrossable taxa: *M. guttatus*, *M. platycalyx* and *M. micranthus*, the latter two being presumed inbreeding derivatives of the first taxa. Specifically we expect that non-homologous QTLs, e.g., those shared between *M. platycalyx* and *M. micranthus* via convergent evolution, will be of larger effect as compared to those that occur later in derived lineages, as initial evolution towards selfing is more likely to occur with few loci as major genes allow associations to easily develop

between loci affecting inbreeding depression and loci controlling selfing (Holsinger 1991; Uyenoyama and Waller 1991).

MATERIALS AND METHODS

The genus *Mimulus* (Scrophulariaceae) consists of about 160 species in 10-12 taxonomic sections (Grant 1924; Pennell 1951; Beardsley and Olmstead 2002; Vickery 1995; Beardsley and Olmstead 2002). In the section *Simiolus*, the *M. guttatus* species complex consists of 8-12 inter-crossable species mainly occurring in California (Vickery 1964, 1978). Its populations mostly occur in stream edges and wet meadows and grow at a variety of elevations (Fenster and Ritland 1994b). A previous study of reproductive traits and isozymes in 8 taxa of this species complex found that inbreeding, and a suite of traits associated with inbreeding, has evolved at least twice in this group (Ritland and Ritland 1989); evolutionary increases of selfing also correlated with decreases of maleness (flower size, pollen number) (also see Fenster and Ritland 1992).

For this study, we selected three inter-crossable, herbaceous annual *Mimulus* species from the *M. guttatus* species complex, *M. guttatus*, *M. platycalyx* and *M. micranthus*, based on their morphological and mating system differences. *M. guttatus* is extensively distributed in western North America, with a relatively low inbreeding coefficient of 0.38 (Ritland and Ritland 1989). *M. platycalyx* occurs in the coast ranges north of San Francisco (Dole 1992) and is a moderately inbred species with an inbreeding coefficient of 0.54 (Ritland and Ritland 1989). *M. micranthus* is endemic to the Coast Range foothills of California, and is a highly selfing species with an inbreeding coefficient of 0.73 (Ritland and Ritland 1989). The shape and size of the flowers of these species is illustrated by Figure 3-1.

Hypotheses about the homology of QTLs

Homology is the common ancestry of a trait or gene, as opposed to functional similarity. It is mainly applied to gene sequences and gene products (Fitch 1970), but in the case of quantitative trait loci, homology has been thought as the sharing of QTL in a genome interval between related taxa. In Ritland and Ritland (1989), *M. tilingii* appears to be the outmost lineage in the dendrogram of this *M. guttatus* complex. Owing its large sized floral characters, outcrossing mating system and closer relationship to the abovementioned three *Mimulus* species, we considered the positive QTL genetic effect that increases the size of traits to be the ancestral. Together with the common assumption that inbreeders are "dead end" species and hence recent derivatives from outbreeders, and also because of the observation that *M. platycalyx* has a very restricted distribution (Marin Co., CA) while *M. guttatus* is distributed throughout western North America, we consider that *M. platycalyx* (as well as *M. micranthus*) are taxa derived from a *M. guttatus* type ancestor. In this study, we infer QTL homology using a well supported *Mimulus* phylogeny and map QTLs on species lineages.

In Figure 3-2 (using results from Figure 3-6, which shows the ancestor lies in the *M. platycalyx* lineage), we depict the scenarios that QTLs can evolve at a single homologous chromosomal interval. In Figure 3-2A, a positive (larger flowered) QTL (bestowing larger flowers) appears in the common ancestor and the two larger flower species. This represents a true orthology of shared positive QTL between the two derived species, *M. guttatus* and *M. platycalyx*. The negative QTL found on *M. micranthus* is then an independent arrival QTL change. Distinguishing ortholog and paralogy can be difficult (Petsko 2001) and the current confusion about the meaning of these terms has

not gone unnoticed (Fitch 2000). With only QTL data, a clear distinction of paralogy from orthology would be difficult to draw, owing to the lack evidence of QTL duplication event. However, ancestral type of QTLs occurs when a positive QTL arises by the shared common ancestry between the two derived lineages, and this is represented by Figure 3-2A.

Assuming ancestral status being positive QTL, Figure 3-2B and Figure 3-2C depict the contrasting case of QTL homology of shared negative QTL between derived relatives. As in our *Mimulus* study, in Figure 3-2B a negative QTL genetic change (promote the reduced size of flower) found on derived selfer lineages, *M. platycalyx* and *M. micranthus*, but they are unrelated QTL. A case of homoplasy is thus defined in Figure 3-2B. In contrast, Figure 3-2C is the parsimonious scenario when negative QTL found on selfer lineages (*M. platycalyx* and *M. micranthus*) could possibly be orthologous; a negative QTL change has to happen onto ancestral lineage and the possibility of positive QTL, by a reverse mutation, on the larger flower lineage of *M. guttatus*. A phylogenetic reference, such as ancestral status and the evidence of +QTL versus –QTL on species specific lineage, is required in distinguishing scenarios in Figure 3-2B from Figure 3-2C.

More complicated scenarios are possible. As those cases are not the most parsimonious scenarios, we regard such patterns as much rarer than the single changes in Figures 3-2A to 3-2C.

Three-taxa crossing design and quantitative trait measurement

All three inter-taxon crosses were performed (*M. guttatus* x *M. platycalyx*, *M. guttatus* x *M. micranthus* and *M. platycalyx* x *M. micranthus*). F1's were maintained in the same growth chamber, with the parents. Backcross progeny were produced by crossing F1 individuals back with both parent species, using parent individuals as pollen resource. Overall, 6 reciprocal backcrosses were generated in this experiment. To avoid the pollen contamination, flowers were bagged immediately after crossing. All plants, including parental, F1, and backcrosses, were grown on the same batch of Pro-Mix soil in the growth chambers in the Forest Sciences Centre, University of British Columbia, with growing conditions of 14C/8C day/night and 18 hours of daylight. To avoid a possible block effect, the seedlings from all 6 reciprocal backcrosses were labelled and sowed on growing trays, each of the growing trays contained the same number of seedlings from every backcross and we also periodically rotated the growing trays among growth chambers.

Five floral traits were measured, as diagrammed in Figure 3-3: corolla width, corolla length, pistil length, stamen length (there are two sets of anther that differ in length), and stigma-anther separation (the difference between the previous two traits). In a combined-cross analysis, crosses with greater variability in the phenotype will have a greater influence (Li et al. 2005). Hence, prior to QTL analysis, we standardized the corolla width measurements by its standard deviation to stabilize the variance.

AFLP (amplified fragments length polymorphism) genotyping

In total, 675 individuals from all of six reciprocal backcrosses were used. Specifically, the number genotyped for each backcross was: (GxP)xG=135, (GxP)xP=112, (PxM)xP=68, (PxM)xM=135, (GxM)xG=124 and (GxM)xM=121. Fresh leaf tissue from every offspring was collected while the floral sizes were measured. Leaf tissue for DNA isolation were immediately stored at - 80°C. Genomic DNA was isolated from frozen leaf tissue of all backcross families via the CTAB method (Doyle and Doyle 1990). AFLP was performed as described in Vos et al. (1995) and Remington et al. (1999) as modified for the LiCor 4200 DNA sequencer.

Templates for AFLP reactions were prepared using 500 ng of genomic DNA for restriction enzyme digests with *Eco*RI and *Mse*I, and ligation adapters Remington et al. (1999). The restriction-ligation mixture was diluted 1:100 in deionized water prior to preamplification. Preamplification was carried out using standard AFLP *Eco*RI and *Mse*I primers containing the selective nucleotides *Eco* + C and *Mse* + CC. Selective final amplifications were conducted by combinations of *Eco* primers with three nucleotides and *Mse* primers with three nucleotides as listed in Table 3-1.

Reaction products were resolved on denaturing gel containing 3.5% of Long Ranger polyacrylamide, 7.5 M urea and 1X TBE. Loading buffer (10 µl) consisting of 95% deionised formamide, 20 mM EDTA pH8.0, and 1 mg/ml bromophenol blue (USB) was added to each amplification product. Prior to loading the gel, the mixture of amplified product and loading buffer was heated at 94C for 3 minutes and then quickly cooled down on ice. Electrophoresis was carried out on the Li-Cor 4200 sequencer using 1X TBE running buffer. IRD-labelled molecular weight markers were loaded in the first

and last lane as standards. Polymorphic fragments were first scored by eye in TIFF image files for primer selection and repeatability test of primers. RFLPscan Version 3.0 (Scanalytics) program scored segregating loci. AFLP markers were tested for a departure from the 1:1 (backcross) and 3:1 (intercross) ratios for presence:absence of bands. Markers showing significant segregation distortion (p <0.05) were excluded. Out of 614 polymorphic loci scored, a total of 368 polymorphic loci were obtained in later analyses.

Inferred parent genotype and linkage map construction via joint analysis

Owing to the fact that heterozygous parents cannot be identified by direct genotyping of AFLPs, but are essential for QTL mapping, we inferred parent genotype using the progeny of all backcrosses. For a single locus with two alleles (one recessive), Table 3-2 lists the segregation probabilities for bandless vs. banded progeny, conditioned on parent genotype (AA, Aa or aa). Let these probabilities be defined as $p_u(i, j)$ for the bandless (unbanded) phenotype and $p_b(i, j)$ for the banded phenotype. Any given parent has three possible genotypes and across all six parents, there $3^6 = 729$ possible configurations of parent genotypes. For any particular genotypic configuration, depicted as $g(k)$, for k=1,6, the likelihood of the observed data across the six crosses is

$$\prod_{k=1}^{6} p_u(g(k_1), g(k_2))^{N_{u,k}} \; p_b(g(k_1), g(k_2))^{N_{b,k}} \qquad \text{Eq. 1}$$

where $N_{u,k}$ is the number of bandless progeny and $N_{b,k}$ is the number of banded progeny in cross k; and $k_1$and k_2 are the male and female parents of cross k. A computer program was written that enumerated all 729 possible parental genotype configurations, and chose the most likely configuration of the six parents for each AFLP locus. The likelihood of

the second most likely parent genotype configuration for each locus was also examined, which allows reassuring the uncertainty of the inferred parent genotypes.

To account for genotyping error, we first found parental genotypes using the above procedure. Then at each cross, the less frequent phenotype is truncated to zero and the likelihood of the data again estimated. If the increase in likelihood is greater than expected by a 5% genotyping error, the data was modified to assume the less frequent category were genotype errors (this normally occurs with rather extreme ratios). This procedure was repeated six times at each locus to ensure convergence (as the crosses are interdependent for parental inference).

A joint likelihood function, which combines information across the six crosses, was used to estimate pair-wise recombination fractions between dominant markers. This approach not only efficiently combines genotype information across crosses, but also infers linkage phase of parents, and is particularly more informative with dominant markers (Hu et al. 2004). Estimated recombination fractions were then converted to map distances using Kosambi mapping function, and the linkage groups constructed with JoinMap (Stam 1995; Stam and van Ooijen 1995).

The analysis of lineage specific QTL genetic effect

Figure 3-4 illustrates the crossing design employed in this study. In the figure, the expected QTL effect along each branch emanating from the ancestral taxa is denoted as U_G, U_P and U_M, for *M. guttatus*, *M. platycalyx* and *M. micranthus*, respectively. Note that U_G includes any effect on the ancestral lineage leading to *M. platycalyx* and *M. micranthus*. Now, arrange the expected means into the matrix

$$\mathbf{u} = \begin{bmatrix} U_P & U_M & U_M & U_G & U_G & U_P \\ U_M & U_P & U_G & U_M & U_P & U_G \end{bmatrix}$$

where columns index the six crosses, and for the backcross denoted as AAxAB, the first row is the mean for the species "A" and the second row is the mean for species "B". These means within this matrix are indexed as U_{jk} (j=1..2, k=1..6). At a marker locus, for cross k and progeny i in cross k with quantitative trait Q_{ik}, and with the total number of progeny in cross k being n_k, the joint likelihood is

$$L(\mathbf{u}) = \prod_{k=1}^{6} \prod_{i=1}^{n_k} \exp(-(Q_{ik} - E_{ik})^2 / 2)$$

where $E_{ij} = U_{1k}$ if the progeny ik has marker genotype aa, or $E_{ij} = (U_{1k} + U_{2k})/2$ if the progeny ik has marker genotype A_ (dominant phenotype). This assumes a normal distribution of quantitative traits with unit variance; as discussed earlier we did such a transformation.

Percentage variance explained was calculated as the differences between the two variances $\sum_{k=1`}^{6} \sum_{i=1}^{n_i} \exp(-(Q_{ik} - E_{ik})^2 / 2)$ for E_{ik} = 0 (no model) vs. E_{ik} = estimated model parameters. Explained variance is (no model – estimated model)/(no model) variances.

Note that for this crossing design to be informative for all U_G, U_P and U_M, at least two crosses involving expected means must be segregating. For example, the first and the third crosses, just by themselves, are informative about all three means, while the first two crosses are not (Figure 3-4). Simpler designs involving fewer backcrosses are possible, but to maximize the chance of having an informative cross, we assayed all six backcrosses.

The above estimation formula assumes that the magnitude of QTL effect between any two species is the sum of the two lineage specific QTLs which lie between each of the two species, i.e., that QTLs evolve in an additive manner. More elaborate designs involving dominance and epistasis would be worthwhile to research and implement, but are outside the scope of our current experimental design involving the three *Mimulus* taxa. The formula also assumes that QTLs are fixed between taxa, and none are segregating within taxa. This is justified because we are examining QTL differences that distinguish phylogenetic lineages, and also these QTLs are likely of much greater effect than QTLs that are segregating within contemporary populations.

Furthermore, since we are using dominant genetic markers, in the particular cross design given in Figure 3-4, we can only estimate QTLs when the F1 is heterozygous and the backcross parent is homozygous recessive (if both parents of the backcross are heterozygous, the cross is non-informative for QTLs, and if the parent taxa is heterozygous and the F1 homozygous, the cross is also non-informative for QTLs that differentiate taxa). Unfortunately, this limits the numbers of loci which are informative for mapping QTL. More informative markers such as single nucleotide polymorphisms (SNPs) or particularly microsatellites, would of course be desired. Finally, because of the relative complexity of the analysis and the lack of any previous computer programs developed for this type of work, we use single-marker QTL detection (as opposed to interval mapping or other multi-marker analyses for QTL). In effect we employ a "genome scan", examining markers one-by-one down a genetic map. To avoid problems with numerical estimation of the maximum likelihood, we used a "brute force" evaluation of the likelihood surface across all possible values of U_G, U_P and U_M, each ranging from -

1 to +1 in increments of 0.05. The joint estimate was chosen as that combination of the three values that gave the highest likelihood.

Statistical significance was ascertained in two ways. First, we permuted quantitative traits and markers 1000 times (in this procedure, traits are randomized among genotypes, and estimates redone). The likelihood of these permutated data were compared to the original unpermuted data; in general, if 50 or less of the permuted data were more likely than the original data, the estimates are deemed significant. In the second way, we use the bootstrap to estimate standard errors of individual branches. Progeny were re-sampled within crosses, estimates recalculated, and the square root of the variance among the 1000 bootstraps was found. Also, if more than one significant QTL was detected within a window of 20 cm, the QTL with the highest percentage variance explained was chosen, and other adjacent markers showing QTL excluded.

We placed no constraints upon the joint space of U_G, U_P and U_M. In the case of a two taxa comparison, say between taxa *G* and *P*, then obviously U_G=1-U_P (no lineage specific estimates can be obtained). We tried the constraint U_G = 1-U_P- U_M, but this model as compared to the full model of jointly estimating U_G, U_P and U_M gave a much worse fit as revealed by variance explained. In the three taxa case, some type of constraint does exist. However, for our purposes, the relative QTL changes among taxa do reveal the changes during the evolution of selfing from outcrossing.

RESULTS

AFLP marker distribution and linkage map

A total of 8 AFLP primer pairs were used to genotype all offspring of 6 backcrosses in the study (Table 3-1). From these, 614 polymorphic AFLP loci were genotyped and scored. After excluding the AFLP markers with unexpected segregation ratios, 368 AFLP loci were used for linkage map construction. We identified 14 linkage groups containing 99 markers covering 482 cM (Figure 3-5), and the average marker spacing was 4.9 cM.

Pairwise genetic distance between Mimulus parents

Over 8 AFLP primer pairs, a total of 368 AFLP loci were selected for further analyses. Using the likelihood method described in this chapter, *Mimulus* parent genotypes for these 368 AFLP loci were inferred from the information given by all 6 backcrosses. It appears that parentage inference was quite reliable, as relative to the most likely set of six parent genotypes, the next most likely set of six-parent genotypes were 1000 times less likely 90% of the time, and 10 times less likely 97% of the time.

All 368 AFLP loci were included in the analysis in estimating Nei's genetic distance (Nei 1972) between *Mimulus* parents. Standard errors were estimated from taking squared root of the variance among the 1000 bootstraps over the 368 AFLP loci. The estimated genetic distances between *M. guttatus* and *M. micranthus* was 0.08 (S.E.=

0.01), between *M. guttatus* and *M. platycalyx* was 0.19 (S.E.=0.02), and between *M. platycalyx* and *M. micranthus* was 0.20 (S.E.=0.02).

Figure 3-6 shows the branch length and the three species network. The branch length for each of the *Mimulus* lineage was calculated from the genetic distance between species pairs. The standard errors were also estimated from the bootstraps (numbers in parentheses). We also estimated the genetic distance with band sharing index (Nei and Li 1979), and the same topology of Figure 3-6 was obtained, with only slightly larger genetic distances between taxa.

The analysis of lineage specific QTL effects

Table 3-3 lists the markers that gave significant lineage specific QTL genetic estimates for all three lineages. Among the 99 markers in the genetic map, 9 markers had significant estimates of lineage specific QTLs. Across the six quantitative traits, 24 QTL were found: 7 for corolla width, 6 for corolla length, 4 for pistil length, 5 for stamen height, and 2 for stigma-anther separation (Table 3-3). The percentage variance explained by each marker ranged from 1.5 to 9.6, and averaged about 5.

QTL of positive effect increases the size of the trait (promoting outcrossing), while negative QTL effect decreases it (promoting selfing). In general, we expect the *M. guttatus* lineage to show positive QTL effects, and the *M. platycalyx* and *M. micranthus* lineages to show negative effects. By and large this was true; for corolla width, 5 of 6 significant QTL were of positive effect in the *M. guttatus* lineage, while 10 of 12 significant QTL were of negative effect in the other two lineages. However, there were exceptions; for example, for corolla width, marker C1_200 showed a significantly

negative QTL genetic effect of -0.22 on the *M. guttatus* lineage, and significantly positive QTL genetic effect of 0.83 on the *M. platycalyx* lineage.

Many of the markers showed QTL genetic effects for several of the floral traits. For example, QTL B2_229 affected all five traits on all three lineages, and QTL C1_378 and QTL C7_210 affected four of five traits (the exception being stigma-anther separation). The sign of lineage specific effect was also consistent among traits. Also, one marker (B4_410) exhibited a significant QTL in just one lineage: the *M. micranthus* lineage.

Based on allozymic variation, Ritland and Ritland (1989) presented a phylogenetic dendrogram of taxa in *M. guttatus* species complex, in which the larger flowered *M. tilingii* was the most outlying species indicated that outbreeding is the ancestral condition of our currently studied species. We therefore expect the sign of lineage specific QTL effect to be positive in the *M. guttatus* lineage (evolution towards larger flowers) and negative in the other two (evolution towards smaller flowers). This was the classic expectation of our study, and this occurs with markers QTL B2_229, B5_292 and B5_535 for many of the traits including corolla width (Table 3-3) and also presents the most common case in our study. Regardless, many of these QTL also appear to be of relatively large effect (absolute values of 0.5 or greater).

However, contrasting cases occur with QTL C1_200 and QTL B5_394, for corolla width and corolla length, respectively. Both cases showed the opposite: negative effects in the *M. guttatus* lineage and positive effects in the *M. platycalyx* lineage. For corolla width, QTL B4_410 was significant only in the *M. micranthus* lineage, conferring smaller size, and QTL C7_210 was of significant opposite sign between the two

inbreeding lineages, with smaller size conferred in *M. platycalyx* and larger size conferred in *M. micranthus*. These results also hold for the other four floral traits.

Because the floral characters analyzed in this study are all dimensional traits, and are likely under same type of selection, a consistent homology pattern for a given QTL locus would be expected among all floral traits, and indeed this was generally observed. An interesting exception was QTL locus B3_166 for stamen length and stigma-anther separation (Table 3-3).

DISCUSSION

The reconstruction of the evolution of inbreeding via the analysis of lineage specific QTL effects

Here we have presented a new approach for QTL mapping, "lineage specific QTL mapping". In addition to inferring number of genes and magnitudes of gene effects, we infer the lineages where QTL changes occur in a network. We considered only the simplest of phylogenies, that of three species, and where the ancestral state is represented by one of the three species. Nevertheless from the results of our QTL analysis of three *Mimulus* taxa, we infer that the evolution of inbreeding in two derived inbreeding *Mimulus* taxa involved major genes causing reduced floral size (increased inbreeding). Independent QTL substitutions of smaller effect can also subsequently occur on species with higher selfing rate (for example, QTL B4_410 for corolla width and corolla length). Not all QTL involving with the evolution of inbreeding were of smaller flower size effect, however.

Our results accord with the expectation of Holsinger (1991) and Uyenoyama and Waller (1991). They worked with models for the evolution of selfing where inbreeding depression must be purged before genes favouring self-fertilization can spread. They found that initial evolution towards selfing is more likely to occur with few loci of major effect because associations easily develop between loci affecting inbreeding depression and loci controlling selfing.

In a study of the genetic architecture of floral differences between *M. guttatus* and *M. micranthus*, Lin and Ritland (1997) suggested that genes with small to intermediate effects were considered responsible to the evolution of mating system. They speculated that the evolution of self-fertilization in *Mimulus* involves the initiation of selfing by a few genes with relatively larger effects and followed by subsequent minor changes of minor modifiers loci (Lin and Ritland 1997). In accordance with this expectation, our lineage specific QTL mapping shows that the QTL appears on both inbreeding lineages, for example of B5_535, explains the largest percentage of variance for corolla width (9.6%, Table 3-3). The largest QTL effect for pistil length was also found significant on all lineages, B2_229 (9.6% variance explained, Table 3-3). For stamen length, B5_535 and B2_229 each explained 6.4% and 6.6% of total variance, respectively, also the two largest QTL. There are only two QTL for stigma-anther separation: QTL B2_229 and QTL B3_166. The percentage of variance explained by shared B2_229 is higher than B3_166 (Table 3-3). Moreover, independent derived QTL that arrives on one lineage, such as B4_410 in *M. micranthus*, shows a smaller genetic effect of 2.1% variance explained in the variation of corolla width (Table 3-3). The hypothesis of Lin and Ritland (1997) is thus supported by the analyses of our lineage specific QTL mapping.

One result that is somewhat paradoxical is the large distance inferred for the *M. platycalyx* lineage (Figure 3-6). It is over twice the length of the other two lineages. Under neutrality and a molecular clock, this topology implies that the common ancestor of the three taxa is on the *M. platycalyx* lineage. If so, then *M. platycalyx* is an independently derived inbreeder from *M. micranthus*, assuming *M. guttatus* is the progenitor. Together with the common assumption that inbreeders are “dead end” species

and hence recent derivatives from outbreeders, and also because of the observation that *M. platycalyx* has a very restricted distribution (Marin Co., CA) while *M. guttatus* is distributed throughout western North America, we consider that *M. platycalyx* (as well as *M. micranthus*) are taxa derived from a *M. guttatus* type ancestor, despite the results of Figure 3-6. We also note that because inbreeding causes increased rates of evolution and loss of homozygosity, and because estimators of genetic distance assume constancy of population size and homozygosity, that these estimates of genetic distance may not reflect evolutionary time among species that vary for levels of inbreeding.

Directional selection and the evolution of selfing

The signs of QTL can be used to indicate whether the trait variation has been under selection, as opposed to the neutrality of antagonistic QTL in a given line (Orr 1998; Rieseberg et al. 2002). Under random genetic drift, there should be roughly equal numbers of "+" and "–" QTL between taxa (Orr 1998). However, we observed an excess of one over the other, indicated a role of natural selection in selective pressure the evolution of inbreeding in *Mimulus*.

Specifically, in the *M. guttatus* lineage five QTL for corolla width show significant lineage specific effect, one negative and four positive. In the *M. micranthus* lineage, six QTL for corolla width were identified, one positive and five negative. The rapid change of directionality of lineage specific QTL effects between *M. guttatus* and *M. micranthus* suggests a role of directional selection in the shift of mating system in *Mimulus*.

Our novel approach for lineage specific QTL mapping allows for a second type of Orr-type non-neutrality test. We note that if the genetic basis on the evolution of mating system was solely based on drift-mutation balance, the lineages with larger branch length (such as *M. platycalyx* lineage in Figure 3-6) should have more QTL. For corolla width, the number of lineage specific QTL identified on each of *Mimulus* lineages are five in *M. guttatus*, six in *M. platycalyx* and six in *M. micranthus*, yet *M. platycalyx* has a significantly longer lineage as estimate from the AFLP data. Results from our lineage specific QTL mapping do not support the pure drift-mutation model in the evolution of mating systems.

The novelty of lineage specific QTL inference

In Chapter 4 of this thesis, we used the classical QTL mapping method, involving crosses between a single pair of taxa, for example, between *M. guttatus* and *M. platycalyx*. Indeed, the same marker in both this and that study, C1_200, exhibited a QTL for corolla width. Here, by adding a third species (*M. micranthus*) and adopting our new analysis which maps QTL onto species lineages, we further found that QTL C1_200 was an independent QTL mutation, one arising after speciation of *M. micranthus* from *M. platycalyx*, as there was no sign of significance of QTL effect in the *M. micranthus* lineage (Table 3-3).

Moreover, one cannot detect QTL of the same sign between two taxa; only by introducing a third taxon (of the opposite QTL sign) can QTL of the same sign between two taxa be identified. For example, QTL were found on linkage group 8, around marker C7_210 for two of the crosses in Chapter 4: *M. guttatus* x *M. platycalyx* and *M.*

platycalyx x *M. micranthus* (QTL8_38, Table 4-3 in Chapter 4). On the same homologous chromosomal position, there was no QTL identified between *M. guttatus* and *M. micranthus* (Chapter 4). However, lineage-specific QTL mapping revealed QTL C7_210 in all lineages (Table 3-3). Lineage specific QTL are both positive in *M. guttatus* and *M. micranthus* lineages, but negative in the *M. platycalyx* lineage (Table 3-3). Thus lineage specific QTL analysis can reveal QTL not seen in two-taxon crosses.

The homology of QTL among lineages

The term homology was introduced by Richard Owen in 1843 as the similarity of characters due to shared ancestry (Panchen 1999; Owen 1848). This concept of "derived from an equivalent characteristic of the common ancestor" has been extensively applied in classical phylogenetic comparisons, where homology is the opposite of analogy and characters can therefore be similar without being homologous or homologous without being identical.

Ancestral QTL effects are those that arise prior to speciation, and share true common ancestry between derived taxa. They are not detected by crosses between these derived taxa, but require a third taxa, representing the ancestral outgroup. Figure 3-2A represents such scenario of a true orthology of positive QTL on homologous chromosomal location of derived lineages. In this study, we however suspect that this true common ancestry of lineage specific QTL genetic effects is in fact not as common as we thought. With our *Mimulus* data, the supporting evidence of such type of QTL is not found (Table 3-3).

In Table 3-3, we found that the *M. guttatus* lineage harbours several positive QTLs, and most of negative QTL were on the selfer lineages, *M. platycalyx* and *M. micranthus*. These negative QTLs that arrive on the same chromosomal location in derived selfer lineages show interesting but conflicting cases in homoplasy versus homology (Figure 3-2B versus Figure 3-2C). In the previous section of this thesis, an important role of directional selection was suggested by the rapid change of directionality of QTL genetic effect found on inbreeding lineages. The scenario described in Figure 3-2B is likely, when selection favours negative QTL changes (that reduce flower size and thus increase inbreeding) on inbreeding lineages. Without the evidence of negative mutation that occurred before spreading out into selfer lineages (Figure 3-2C), those shared negative QTLs on both *M. platycalyx* and *M. micranthus* could have arisen through convergent evolution. Such QTLs are however common. In our study, 4 out of 7 lineage specific QTL genetic effects are under this category (Table 3-3). In addition, these shared negative QTLs on derived selfer lineages are often larger in size, suggesting an important role of convergent evolution of derived genetic changes in the evolution of inbreeding. Our findings in convergent evolution of QTL genetic changes also raise the question about inferences of QTL orthology, where orthologous QTLs are thought to be the common key genetic regulators of morphological development (Pereira and Lee 1995; Hu et al. 2003).

Moreover, those that arise subsequent to speciation, control the same quantitative trait but locate solely on one of the lineages are the cases of independent arrival of QTL. In our case, most of those on the derived lineages are often found with smaller genetic effect, to "fine tune" the trait. This is supported by the QTL B4_410 in *M. micranthus*

lineage (Table 3-3). In the evolution of inbreeding of these two related *Mimulus* taxa, we found that shared QTL were predominant. Our analysis shows as well the strength in quantifying the role of lineage specific genetic changes in the evolution of quantitative variation.

Conclusion

The basic idea in the analysis of lineage specific QTL effect allows integration of QTL with species evolutionary history. Superimposing QTL changes on species evolutionary history helps to re-examine important evolutionary and biological phenomenon such as differential adaptation of speciation (Wu 2001), evolution of selfing (Charlesworth et al. 1993), the origin of disease-producing allelic variation in human population (Kidd et al. 2000) and the evolution of Dobzhansky-Muller hybrid incompatibility (Brideau et al. 2006). This approach will gain more statistical and biological power as additional lineages are added, especially when crosses to more evolutionarily distant species are available, and when more informative markers are used.

Table 3-1. AFLP primers and polymorphism of primer pairs. Primer sequence of *Eco*RI is GACTGCGTACCAATTC. Primer sequence of *Mse*I is GATGAGTCCTGAGTAA

AFLP Primers for pre-amplification: Primer names and sequences	Primer name	AFLP primer pair for final amplification	Number of amplified polymorphic fragments
Primer B set:	B2	Eco + ACA / Mse + CCT	25
*Eco*RI* + AC / *Mse*I** + CC	B3	Eco + ACA / Mse + CGC	54
	B4	Eco + ACA / Mse + CCA	59
	B5	Eco + ACT / Mse + CGG	38
Primer C set:	C1	Eco + CA / Mse + CCG	61
*Eco*RI + C / *Mse*I + CC	C3	Eco + CCG / Mse + CCG	31
	C4	Eco + CCA / Mse + CCT	54
	C7	Eco + CTC / Mse + CCT	46
Total number of polymorphic fragments			368

Table 3-2. Probabilities of bandless (first number) vs. banded progeny (second number), conditioned on the genotypes of the two parents of a cross (bandless is the recessive condition).

		Parent 2	
Parent 1	**AA**	**Aa**	**aa**
AA	0, 1	0, 1	0, 1
Aa	0, 1	¼, ¾	½, ½
aa	0, 1	½, ½	1, 0

Table 3-3. Estimates of lineage specific QTL genetic effects (significant effects, as determined by bootstrapping, are indicated by standard errors, SE, in parentheses and by asterisks, *).

				Lineage specific QTL effect				
Traits	**AFLP marker**	**Linkage group**	**cM**	***M. guttatus* effect (SE)**	***M. platycalyx* effect (SE)**	***M. micranthus* effect (SE)**	**% of variance explained**	**Permutation probability**
Corolla width	C1_200	1	13.95	-0.22 (0.11) *	0.83 (0.25) *	0.19 (0.25)	4.5	0.044
	B2_229	1	29.34	0.55 (0.13) *	-0.94 (0.12) *	-0.74 (0.21) *	9.1	0.009
	C1_378	2	22.30	0.02 (0.10)	-1.00 (0.00) *	-0.15 (0.06) *	1.7	0.003
	B5_292	3	19.40	0.46 (0.12) *	-0.62 (0.24) *	-0.70 (0.22) *	5.3	0.006
	B4_410	3	39.78	-0.09 (0.18)	0.14 (0.20)	-0.60 (0.33) *	2.1	0.029
	B5_535	4	0.00	0.22 (0.08) *	-0.95 (0.10) *	-0.75 (0.24) *	9.6	0.000
	C7_210	8	41.18	0.46 (0.27) *	-0.37 (0.21) *	0.28 (0.18) *	2.0	0.053
Corolla length	B2_229	1	29.34	0.49 (0.13) *	-0.87 (0.16) *	-0.66 (0.21) *	7.1	0.015
	C1_378	2	22.30	0.05 (0.09)	-1.00 (0.00) *	-0.17 (0.07) *	2.3	0.002
	B4_410	3	39.78	-0.06 (0.17)	0.07 (0.19)	-0.51 (0.35) *	1.5	0.077
	B5_535	4	0.00	0.22 (0.08) *	-0.95 (0.10) *	-0.63 (0.25) *	9.1	0.000
	B5_394	5	60.81	-0.55 (0.26) *	0.18 (0.13) *	-0.07 (0.11)	1.6	0.045
	C7_210	8	41.18	0.68 (0.24) *	-0.51 (0.20) *	0.34 (0.17) *	3.4	0.002
Pistil length	B2_229	1	29.34	0.51 (0.13) *	-0.97 (0.07) *	-0.63 (0.25) *	9.6	0.022
	C1_378	2	22.30	0.05 (0.09)	-1.00 (0.00) *	-0.15 (0.06) *	2.1	0.001
	B5_535	4	0.00	0.19 (0.09) *	-0.94 (0.11) *	-0.50 (0.30) *	7.7	0.001
	C7_210	8	41.18	0.60 (0.25) *	-0.51 (0.17) *	0.34 (0.17) *	3.5	0.007
Stamen length	B2_229	1	29.34	0.47 (0.14) *	-0.86 (0.19) *	-0.63 (0.25) *	6.4	0.041
	C1_378	2	22.30	0.04 (0.11)	-1.00 (0.00) *	-0.17 (0.07) *	2.0	0.003
	B5_535	4	0.00	0.20 (0.08) *	-0.87 (0.15) *	-0.62 (0.32) *	6.6	0.003
	B3_166	7	0.00	0.15 (0.14) *	-0.29 (0.16) *	0.40 (0.38) *	2.3	0.058
	C7_210	8	41.18	0.72 (0.24) *	-0.54 (0.21) *	0.38 (0.17) *	4.8	0.000
Stigma-anther separation	B2_229	1	29.34	0.35 (0.14) *	-0.84 (0.18) *	-0.31 (0.27) *	5.3	0.025
	B3_166	7	0.00	-0.06 (0.13)	0.19 (0.13) *	-0.82 (0.24) *	4.3	0.008

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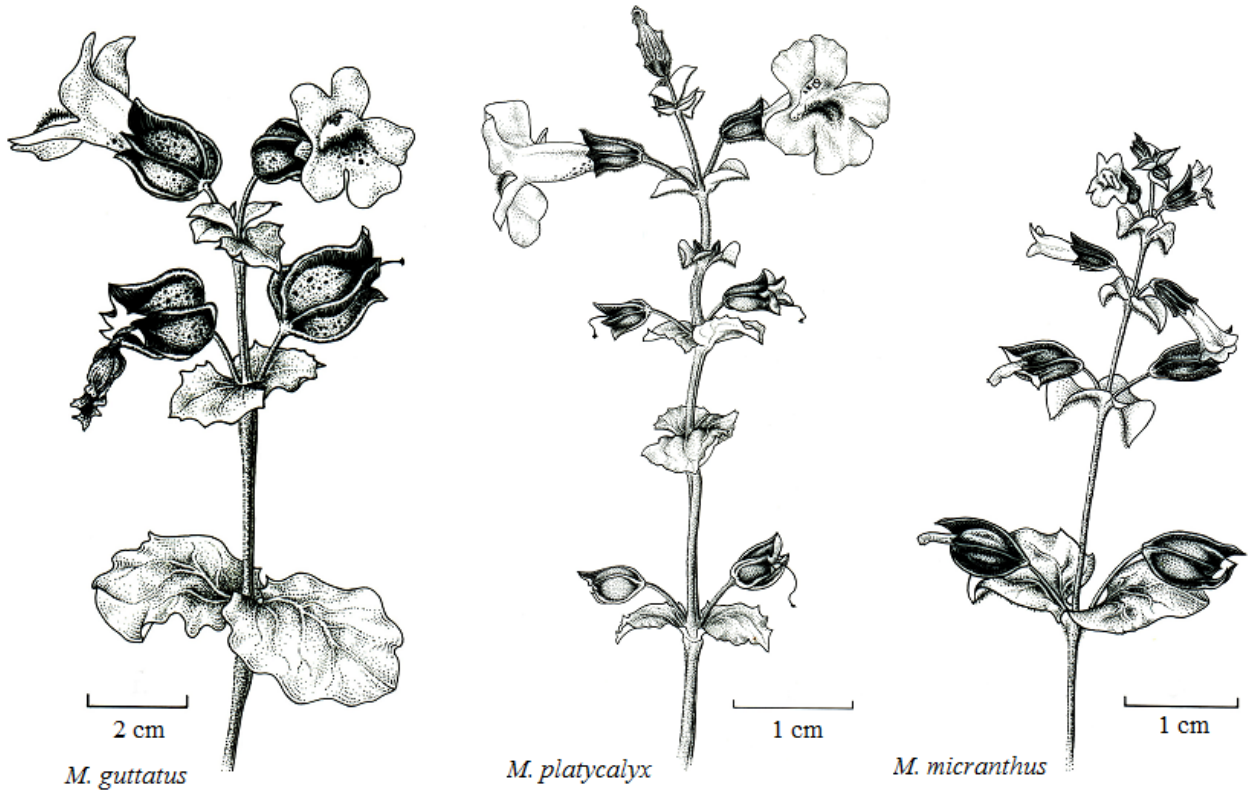

Figure 3-1. The three intercrossable *Mimulus* taxa used for our QTL phylogenetic analysis

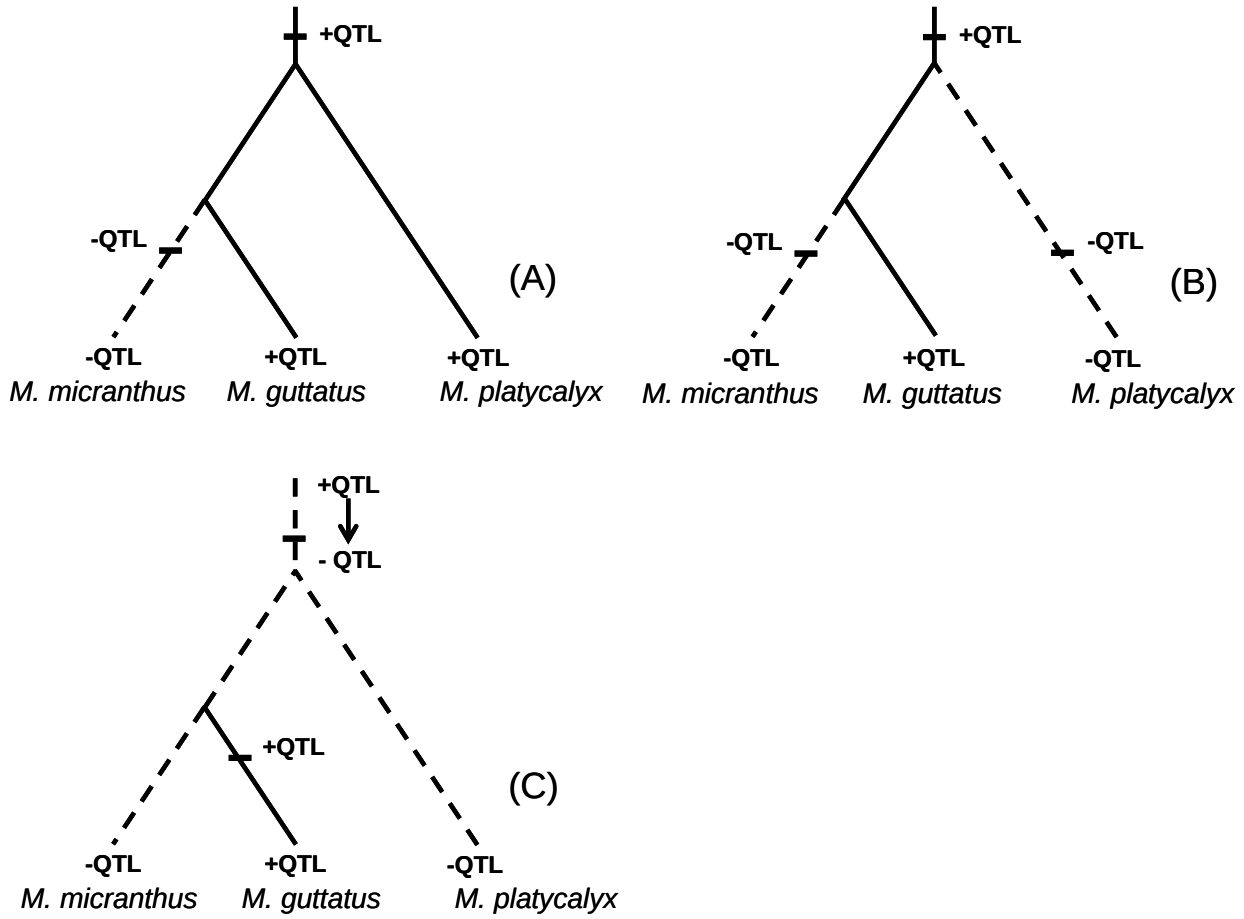

Figure 3-2. Possible patterns of QTL evolution and homology. The dashed lines indicate lineages that have evolved towards QTL of smaller effect (towards inbreeding).

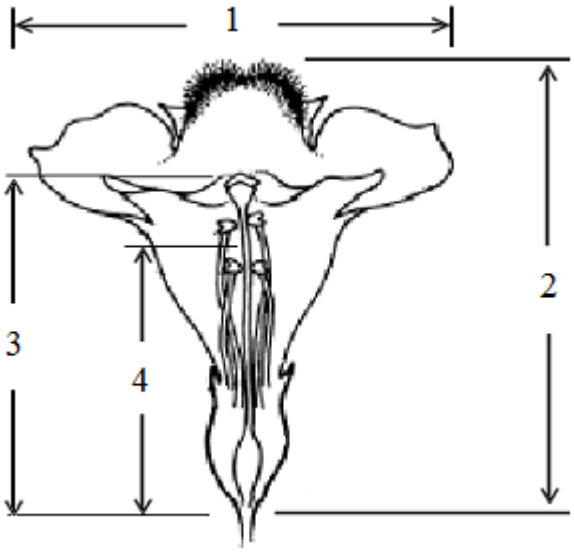

Figure 3-3. A central dissection of a *Mimulus guttatus* flower and the floral traits measured in this study.

Parent		Backcross	Expected mean for a quantitative trait *Conditioned on progeny genotype and parental configuration of the backcross* *Genotype* ***Aa***	***aa***
M. platycalyx (U_P)				
	X →	$BC_{(PxM)\rightarrow P}$	$\frac{1}{2}(U_P + U_M)$	U_P
$F1_{PxM}$				
	X →	$BC_{(PxM)\rightarrow M}$	$\frac{1}{2}(U_P + U_M)$	U_M
M. micranthus (U_M)				
	X →	$BC_{(GxM)\rightarrow M}$	$\frac{1}{2}(U_G + U_M)$	U_M
$F1_{GxM}$				
	X →	$BC_{(GxM)\rightarrow G}$	$\frac{1}{2}(U_G + U_M)$	U_G
M. guttatus (U_G)				
	X →	$BC_{(GxP)\rightarrow G}$	$\frac{1}{2}(U_G + U_P)$	U_G
$F1_{GxP}$				
	X →	$BC_{(GxP)\rightarrow P}$	$\frac{1}{2}(U_G + U_P)$	U_P
M. platycalyx (U_P)				

Figure 3-4. Crossing design and expected means for a given quantitative trait.

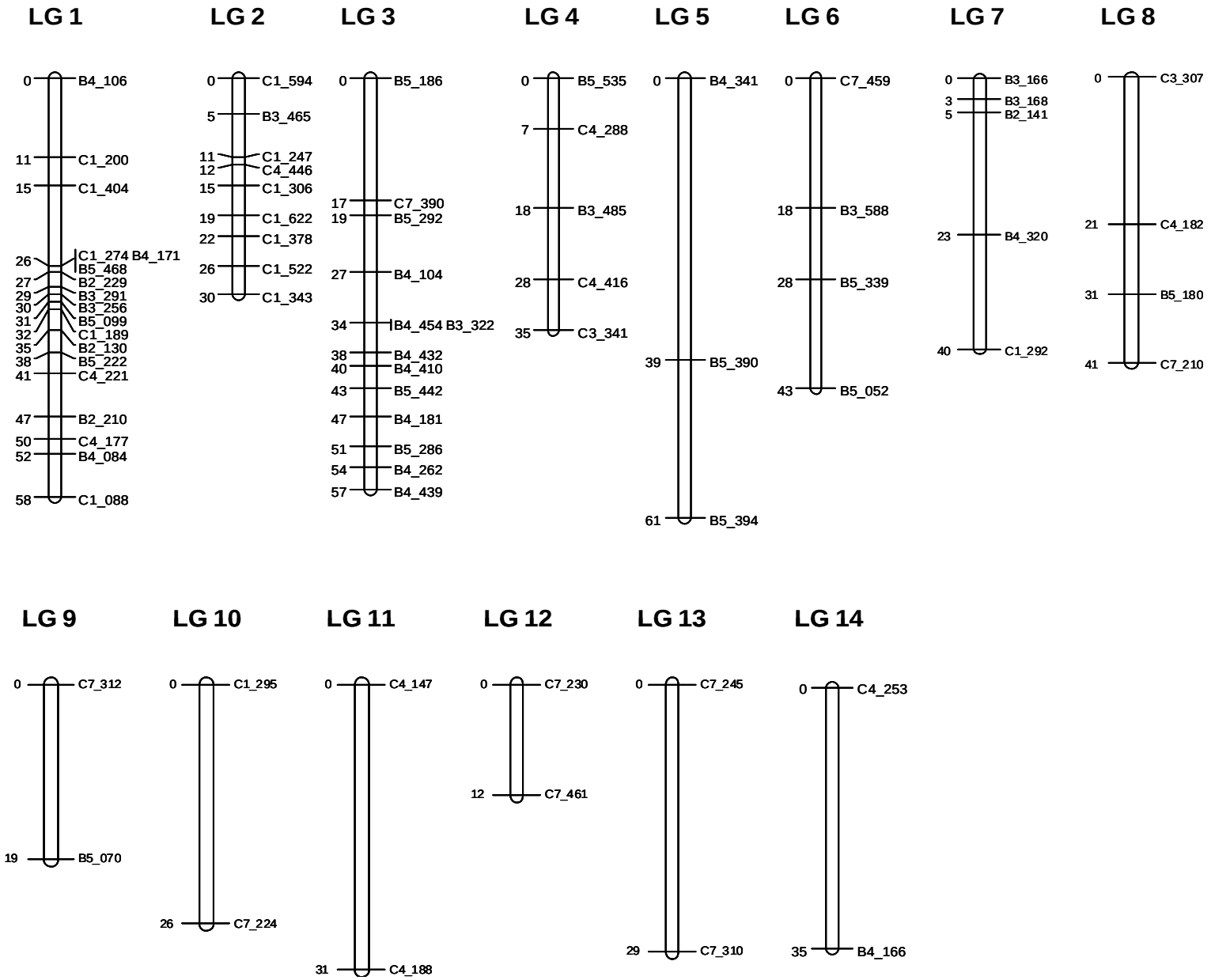

Figure 3-5. AFLP linkage map as inferred from segregating progeny in 6 backcrosses involving 3 *Mimulus* taxa.

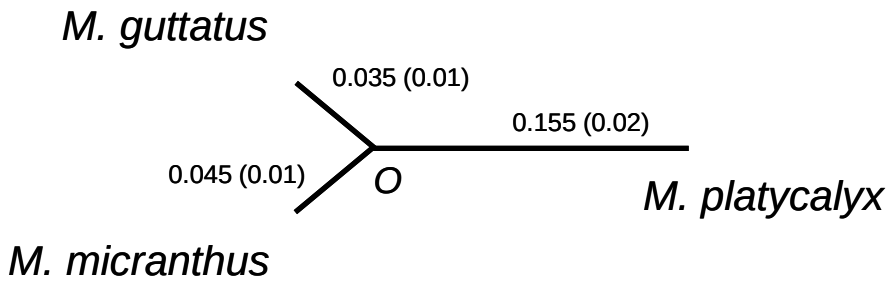

Figure 3-6. Estimated genetic distances between *M. guttatus*, *M. platycalyx* and *M. micranthus*..

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CHAPTER 4. EPISTATIC INTERACTION OF QTLS INVOLVED IN THE EVOLUTION OF FLORAL TRAITS IN THE MIMULUS GUTTATUS SPECIES COMPLEX [1]

INTRODUCTION

Complex traits such as human disease, growth rate, or crop yield are polygenic, or determined by the contributions from numerous genes in a quantitative manner. Although many studies have successfully identified quantitative trait loci (QTL), our knowledge of QTL underlying complex traits is largely constrained to QTL with relatively large effect. In addition, QTL are often inferred without incorporating genetic background, or the effects of a combination of other loci (Carlborg and Haley 2004). Genetic interaction is often ignored, in part due to the difficulty of analysis (Barton and Turelli 2004; Cheverud 2000). In fact, the statistics used to detect single QTLs can mask the significance of interaction terms, as the genetic effects of interacting loci are summed and overall have no influence on the prior (Holland 2001), and can therefore be biased against detecting significant interactions (Templeton 2000).

There have been a number of recent methods proposed to infer the epistatic interaction, including (1) the one dimensional scan that searches for genetic interactions of a given allele with the genetic background (Jannink and Jansen 2001), (2) simultaneous two-way searches at multiple, selected pair of loci (Kao et al. 1999) and more recently, (3) a genome-wide scan that simultaneously considers all locus pairs

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(Broman et al. 2003; Sen and Churchill 2001). With genomic approaches, more comprehensive inferences are possible. Examples can be seen in *Arabidopsis thaliana*, where epistasis has been shown to underlie genetic determination of flowering time (Juenger et al. 2005), juvenile growth rate (Kroymann and Mitchell-Olds 2005), and response to water availability (Hausmann et al. 2005). In *Drosophila*, a major QTL on chromosome 3 and minor QTL on chromosome 2 were initially identified affecting variole number between *D. sechellia* and *D. simulans* (Jones 2005). With a rather fine physical map and a large selection of 1038 additional recombinants in the chromosome regions of interest, the study also discovered that a previously identified major QTL on chromosome 3 was, in fact, a pair of epistatically interacting QTL (Orgogozo et al. 2006).

How much is this epistatic genetic variance involved in local adaptation, population differentiation, and speciation? This question dates back to 75 years ago, to the differing viewpoints about the genetic basis of evolutionary change that Fisher (1958) and Wright (1984) held. Their famous debate on the important of epistasis in adaptation and population differentiation has greatly influenced theoretical studies and our understanding of evolutionary, population and quantitative genetics (Malmberg and Mauricio 2005). When strong selection acts on the additive component of the genetic variance, it will eventually exhaust the overall additive genetic variance and leave segregating loci with primarily dominance and epitasis effects (Wade 1992; Roff and Emerson 2006). Although mutational variation is commonly thought to be the primary source to the long term selection response (Keightley 2004), one long term selection experiment found a surprisingly important role of epistasis, where genetic interactions

among four loci mediated a considerably larger response to selection than predicted by a single locus model (Carlborg et al. 2006).

Lately, the role of epistasis has re-gained attention in theoretical evolutionary biology research, with the analytical tools and experimental approaches having been improved (Wolf et al. 2000). The resurgent interest in the role of epistasis has included how epistatic variance can be converted into additive variance after a population bottleneck (Barton and Turelli 2004), and the role of epistasis in the evolution genetic recombination (Otto and Lenormand 2002). It is important to empirically study the "character" of genetic interaction, specifically the role it plays in the evolution of complex traits.

In this chapter, we study the genetic architecture of epistasis for mating system traits that differ among three monkeyflower species (*Mimulus*), using quantitative trait locus (QTL) mapping. Our study examined the ubiquity of epistasis using all pairwise comparisons (crosses) of three *Mimulus* species that differ for selfing rate. Our three species phylogenetic comparison further allows us to examine the relationship between the degree of genetic divergence among species and the extent of epistasis.

MATERIALS AND METHODS

Study species

Species in the genus *Mimulus* have become model systems for the study of evolutionary processes in nature due to the diversity of life histories and mating systems in the genus, as well as the ease with which they can be grown and manipulated. The *Mimulus* genus currently contains about 160 species, of which approximately 75% occur only in western North America (Beardsley and Olmstead 2002; Grant 1924). *Mimulus* species vary with respect to ploidy level (Vickery 1978), breeding system (including frequent shifts among pollinators and to self-fertilization), and acclimation to extreme environments. Species within *Mimulus* genus species are model systems to study the genetics of speciation (Bradshaw et al. 1998), inbreeding depression (Darwin 1876; Dudash and Carr 1998), mating system evolution (Leclerc-Potvin and Ritland 1994), the evolution of heavy metal tolerance (Macnair et al. 1993) and cytological pattern of evolution (Vickery 1978).

Studies have shown that *Mimulus* has likely gone through an adaptive radiation in western North America (Whittall et al. 2006). Species in Section *Simiolus* display a high degree of morphological complexity and environmental plasticity (Beardsley et al. 2004). With dramatic differences in mating systems, from predominantly selfing to predominantly outcrossing (Ritland and Ritland 1989), the compatibility of crosses among species, and the large numbers of seeds produced by artificial crosses, species

from Section *Simiolus* in *Mimulus* genus are ideal material for genetic analyses of evolutionary changes (Vickery 1978).

The *Mimulus guttatus* species complex lies within the Section *Simiolus* and is comprised of 8 to 12 inter-crossable species (Campbell 1950; Grant 1924). Although each species is morphologically distinct, natural hybrids are sometimes found (Vickery 1978). All taxa in this species complex have a haploid chromosome number of n=14 (Campbell 1950; Dole and Ritland 1993; Vickery 1964;Vickery 1978). Species in this section display a wide range of mating system and floral morphology variation (Ritland and Ritland 1989). *M. guttatus* has at least three, independently derived selfing relatives: *M. micranthus*, *M. nasutus* and *M. laciniatus* (Ritland and Ritland 1989, Fenster and Ritland 1992, Leclerc-Potvin and Ritland 1994; Fishman et al. 2002).

The large-flowered *M. guttatus* is a herkogamous species with a fairly high outcrossing rate (Wright's inbreeding coefficient, $F = 0.38$), while the smaller-flowered *M. micranthus* is predominately selfing (Wright's inbreeding coefficient, $F = 0.73$; Ritland and Ritland 1989). As a predominant selfer, *M. micranthus* shows reduced allocation to the floral traits that contribute to male function, including corolla size and pollen number (Ritland and Ritland 1989). In addition, the magnitude of inbreeding depression in selfing *M. micranthus* is much lower than in outcrossing *M. guttatus*, based upon fitness measures such as biomass, pollen production and ovule production (Dudash and Carr 1998). Lastly, *M. platycalyx*, is intermediate between these two species in terms of outcrossing (Wright's inbreeding coefficient $F = 0.54$) and floral size traits (Ritland and Ritland 1989). *M. platycalyx* and *M. guttatus* are sometimes sympatric. Natural hybrids have been identified along Sausal Creek in Marin County, California

(Dole and Ritland 1993). In this study, we used samples collected in Ritland and Ritland (1989) as parents of the crosses we conducted. Further details about locality, morphological variation, mating system coefficients, phylogenetic genetic distance are given in Ritland and Ritland (1989).

The shift of mating system in *M. guttatus* species complex has been discussed widely and thought to be associated with the change of an entire syndrome, including male allocation, reduced size of floral characters, reduced attraction to pollinators and reduction of the spatial and temporal separation of male and female reproductive organs within the flower (Jain 1976; Ritland and Ritland 1989). Thus, measures of a relatively few floral traits (corolla width, anther length, etc.) capture the majority of evolutionary differences between species.

Backcrosses between Mimulus species

Three interspecific crosses were performed using three *Mimulus* species as parental material, *M. guttatus*, *M. platycalyx* and *M. micranthus*. All parent plants in this study were simultaneously grown in the same growth chamber. Three intercross F1 were produced, *M. guttatus* x *M. platycalyx*, *M. guttatus* x *M. micranthus* and *M. platycalyx* x *M. micranthus*. F1s were maintained in the same growth chamber, while all the parent material were still kept growing. Backcross progeny were produced by crossing F1 individuals back with both parent species, using parent individuals as pollen resource. As a result, a total of 6 reciprocal backcrosses were established. Figure 4-1 shows the crossing scheme and the composition of mapping populations in this study. BC1 refers to a backcross to the larger flower size parent and BC2 for the smaller flower size parent.

To avoid the contamination from inter-pollination, flowers were bagged right after crossing. All plants, including parental, F1, and backcrosses, were grown on the same batch of Pro-Mix soil in the growth chambers in Department of Forest Sciences, University of British Columbia, where the growing conditions was kept as 14C/8C day/night temperature with 18 hours daylight for all the chambers. To avoid the possible block effect, the seedlings from all 6 reciprocal backcrosses were labelled and sowed on the growing trays, each of the growing trays contained the same number of seedlings from every backcross and we also periodically rotated the growing trays among growth chambers. The pairwise crosses between parents were performed in the year 2000; due to the limitation of growth chamber space, the backcrosses from *M. guttatus* x *M. platycalyx* were conducted in 2001 under the same growth chamber conditions. Crosses from *M. guttatus* x *M. micranthus* and *M. platycalyx* x *M. micranthus* were done in the same growth chamber in the continuous years. The *Mimulus* parents used to generate later crosses in this chapter and therefore offspring are the same individuals that are described in Chapter 3.

Measurement of floral traits

The following traits were measured on individuals, with a digital calliper used in taking measurements: (1) widest corolla width, (2) corolla tube length, (3) pistil length, (4) stamen height (averaged over the low and high anthers), (5) stigma-anther separation (pistil length minus the average stamen height) (Figure 4-2). Those 5 floral characters are correlated with the evolution of mating system in the *M. guttatus* species complex (Carr

and Fenster 1994; Dole 1992; Ritland and Ritland 1989). The means and standard errors of these floral characters are given in Table 4-1.

DNA isolation and AFLP (Amplified Fragments Length Polymorphism) genotyping

Fresh leaf tissue from every individual in the crossed progeny was collected while the floral trait data was collected. Leaf tissues for DNA isolation were immediately stored in the minus 80°C fridges in Department of Forest Sciences, University of British Columbia. Genomic DNAs were isolated from frozen leaf tissue via the CTAB method (Doyle and Doyle 1990). Table 4-2 lists the sample size for each backcross family. The cross made in between *M. platycalyx* and *M. micranthus* and then backcross to *M. platycalyx* had reduced progeny number so we collected all available seeds.

AFLP assay was performed as described in Vos et al. (1995), Remington et al. (1999) and modified for the LiCor 4200 automatic DNA-sequencer. Templates for AFLP reactions were prepared using 500 ng of genomic DNA for restriction enzyme digests with *Eco*RI and *Mse*I, and ligation adapters (Remington et al. 1999). The restriction ligation mixture was diluted 1:100 in deionised water prior to preamplification. Preamplification was carried out using standard AFLP *Eco*RI and *Mse*I primers containing selective nucleotide *Eco* + C and *Mse* + CC. Selective final amplifications were conducted using various combinations of *Eco* primers with three nucleotides and *Mse* primers with three nucleotides. The primers in preamplification and selective amplification are given in Table 4-3.

Joining linkage map construction using joint likelihood function

AFLP markers were tested for a departure from the 1:1 and 3:1 Mendelian ratio for the presence:absence of a fragment (according to whether the cross was a backcross of F2 with respect to the markers), using chi-square test with $\alpha = 0.05$. Markers showing segregation distortion ($\alpha < 0.05$) were excluded and not used to construct the linkage map. Out of 614 polymorphic AFLP loci scored, 368 are included in later analyses. A joint likelihood function using combined information across crosses was used to estimate the pair-wise recombination fractions between AFLP markers. This recently developed approach is not only able to use the genotype information from crosses when the knowledge of parental genotype, or linkage phase, is absent, but also improves the estimates with higher precision and accuracy, particularly when dominant markers are used (Hu et al. 2004). Recombination fractions were then converted to map distances using the Kosambi mapping function, then linkage groups were found using the JoinMap program (Stam 1995; Stam and van Ooijen 1995).

Prior to the combined cross analysis, a process of "calc.genoprob" in R using a hidden Markov model to calculate the probabilities of the true underlying genotypes was performed. We then examined the segregation of every marker used on the genetic map in each pair of backcross progeny, re-coded the genotypes in the progeny that represent the parental configuration with the reference from all the 6 crosses together and the function "c.cross" of R/QTL to combine the two backcrosses from the same parents as one intercross (Broman et al. 2003; Sen and Churchill 2001).

Among the three *Mimulus* species, *M. guttatus* is the most outbreeding and indeed shows the largest flowers, averaging 30.25 mm on corolla width (Table 3-1). In general,

M. guttatus corolla size is about twice the size of the intermediate inbreeder *M. platycalyx*, and almost three times larger than the highly inbreeding species of *M. micranthus* (Table 3-1). In a combined-cross analysis, crosses with greater variability in the phenotype would have a greater influence (Li et al. 2005). As a result, we then standardized the corolla traits with their standard deviations, in order to stabilize the variance before jointly analyzing the QTL that contribute to the variation among *Mimulus* species.

QTL mapping using R/QTL genome scan

Interval mapping (IM) was used to find QTL at each map position across the whole genetic map (Lander and Botstein 1989). At each position, the likelihood of a QTL was estimated as the product of the prior probability of the QTL, times the likelihood of the QTL effect given the genotype of the flanking markers. The QTL effect is estimated as that maximizing the likelihood; it is numerically estimated using the expectation-maximization (EM) algorithm. The logarithm of odds (LOD) is estimated as the $\log_{10}$ of the likelihood of the estimated effect, minus the likelihood under zero QTL effect.

The genome-wide scans of QTL were implemented with the "scanone" function in R/QTL software (Broman et al. 2003; Sen and Churchill 2001). Prior to the execution of scanone, the required multipoint genotype probabilities for EM algorithm were first calculated using the "calc.genoprob" function in R/QTL. The probabilities were calculated at 1 cM intervals for the maximum distance between positions.

A proper LOD threshold, which identifies statistically significant QTL, should take into account genome size, progeny number, and the density of the markers genotyped on the map (Churchill and Doerge 1994**)**. In each mapping population, significance thresholds to identify QTL presence were estimated through tests of 10,000 permutations. The permutation thresholds were determined by the "n.perm" command in the scanone, as described in Churchill and Doerge (1994). In this study, cut-off threshold genome wide significance of α =0.05 was used. The upper 5% bound of estimated QTL effects under this null condition of no QTL effect (as simulated by permutation) gives the significance threshold.

Pairwise R/QTL genome scans

To infer interactions between QTL, pairwise genome scans were implemented using the "scantwo" function of R/QTL (http://www.rqtl.org; Broman et al. 2003). As the number of pairwise comparisons between QTL is enormous, to reduce computational load a 5 cM interval scanning interval was used. The scantwo function searched through all pairs of loci with a two-way ANOVA model. The likelihood under the full regression model with interaction terms is

$$Y = \mu + b_1 [Q1] + b_2 [Q2] + b_3 [Q1 \times Q2] + A_g + Z_d [Q1] + Z_d [Q2] + Z_d [Q1 \times Q2] + \varepsilon.$$

This was first compared to the null model with no genetic effects,

$$Y = \mu + A_g + \varepsilon$$

where Q_1 and Q_2 are unknown QTL genotypes at two map locations, A is a matrix of covariates and Z is the matrix of covariates that interact with QTL genotypes. This joint-LOD likelihood is the "full" model of the joint effect of two QTL underlying floral trait

variation. The "joint LOD" score provides an estimate of the entire suite of two-locus effects. R/QTL then considers another linear model that includes only additive effects (no interaction):

$$Y = m + h_1 [Q1] + h_2 [Q2] + A_g + Z_d [Q1] + Z_d [Q2] + e.$$

Comparing the full vs. reduced model results in an "interaction LOD"; this statistic measures the statistical significance of the two locus interaction (epistatic) effect.

Genome-wide significance thresholds for the interaction LOD score were established through permutation tests by n.perm involving 1,000 permutations by scantwo. Following Lander and Kruglyak (1995), the cut-off threshold was set at $p <$ 0.63, to pre-select possible interacting pairs of loci. This procedure in using less stringent significance threshold to identify "suggestive linkage" was also used in other combined cross QT: analysis (Li et al 2005). In this procedure, as there are many suggestive estimates of QTLs, all of the pre-selected QTL loci and chromosome regions then subject to a backward selection procedure with interaction terms, to search the best fitting model involving the fewest parameters, using function "stepwiseqtl" and "makeqtl" in R/QTL. Details of the methods can be seen in http://www.rqtl.org/manual/html/stepwiseqtl.html.

First, the suggestive QTLs as well as interacting pair of QTL were estimated. Then, the suggestive QTL or interaction that is least insignificant in the overall ANOVA result was eliminated. Recalculating the overall ANOVA, insignificant QTL and interacting gene pairs were successively eliminated, until only highly significant main and interaction effects remained. The same procedure of model selection for the best fit QTLs and interacting pairs can be seen in Zou and Zeng (2008) and Tsudzuki et al. (2007).

RESULTS

QTL analysis using single locus R/QTL genome scans

In the single-locus R/QTL genome scan of QTL for the 5 floral traits, 21 QTLs were found between *M. guttatus* and *M. platycalyx*, 12 QTLs between *M. platycalyx* and *M. micranthus* and 19 QTLs between *M. guttatus* and *M. micranthus*. The number of QTLs differentiating *M. platycalyx* and *M. micranthus* is the least of the three pairwise comparisons.

Table 4-3 gives results from both the single locus and locus-pair analysis (two-way interaction ANOVA model). With the resolution offered by R/QTL pairwise genome scans, a few previously undetected QTLs were identified. In total, 34 QTLs were identified between *M. guttatus* and *M. platycalyx*, 31 QTLs between *M. platycalyx* and *M. micranthus* and 27 QTLs between *M. guttatus* and *M. micranthus* (Table 4-3). The largest QTL was found in *M. platycalyx* and *M. micranthus*, for stamen length (*QTL8_38* in Table 4-3).

QTLs were named according to their linkage group and chromosome location (in map units). For example, for corolla width, one QTL was found in linkage group 1, on chromosome location of 19 cM around AFLP marker C1_200, so it is termed *QTL1_19*. QTL for two different floral traits that reside on the same location were considered the same QTL, and thus will be given the same name, to account for probable pleiotropy (Togawa et al. 2006). The QTL locus *QTL1_19*, for example, was found to control both stamen length and corolla length between *M. guttatus* and *M. micranthus* (Table 4-3).

Our analyses identified QTLs in almost all 14 linkage groups, the exception being group 5. Moreover, linkage group 2 and linkage group 8 have considerably more QTLs than other linkage groups. On average, QTLs in linkage group 8 were found to account for 14.4% of PVE across all crosses (Table 4-3). QTLs in linkage group 2 were found associated with almost every floral trait in all crosses, except for the corolla length and stigma-anther between *M. guttatus* and *M. platycalyx* and corolla width and length in *M. guttatus* and *M. micranthus*.

Between *M. guttatus* and *M. platycalyx*, linkage group 8 showed significant QTLs for every floral trait in the analysis. *QTL10_26* was, in contrast, a minor QTL with a marginal but significant effect of 3.7% PVE (pistil length) to 2.8% PVE (corolla length) which was revealed only when epistasis was included (Table 4-3). Between *M. platycalyx* and *M. micranthus*, linkage groups 2, 8 and 11 nevertheless showed important genetic roles not only in terms of number of QTLs, but also by PVE explained by each QTL (Table 4-3). For example, two corolla width QTLs were found in group 8, *QTL8_0* and *QTL8_38*, with 2.7% of PVE and 21.2% of PVE, respectively. Together, they also displayed significant epistatic genetic interaction (Table 4-4). Stigma-anther separation showed fewest QTL, with only 3 (*QTL2_28*, *QTL8_38* and *QTL13_25*).

A less polygenic system with multiple QTL between *M. guttatus* and *M. micranthus*, with number of QTL detected in the range of 3 (stamen length) to 6 (corolla width and stigma-anther separation). Interestingly, more QTL had negative genetic effect of reducing floral size. In comparison, QTL genetic effects also were more minor, with PVE ranging from 1.2% (*QTL13_18* for stigma-anther separation) to 10.1% (*QTL1_1* and *QTL9_19* for corolla width).

Pairwise QTL genome scans

Among the variation of 5 floral traits in three *Mimulus* species, up to 167 pairs of possible gene-gene interaction were suggested by the pairwise genome scans (Figure 4-3 to Figure 4-7). Table 4-4 summarizes significant epistatic locus pairs for each of the three *Mimulus* pairwise comparisons, tested by fitting two way ANOVA models. Overall, 57 pairs were significant across the five floral traits.

In general, results from the two-way ANOVA vary among crosses. Between *M guttatus* and *M. micranthus*, only 4 epistatic pairs were found, while 25 were found between *M. guttatus* and *M. platycalyx* and 28 between *M. platycalyx* and *M. micranthus* (Table 4-4). The percentage of variance explained by epistasis also varied among crosses. For example, for corolla width, the full ANOVA model explained 71.61 percent of overall variance of the corolla width between *M. guttatus* and *M. platycalyx*, and of this, 6.21 percent of overall variance was explained by epistasis. Between *M. platycalyx* and *M. micranthus*, the full ANOVA model explained 76.45 percent of overall variance while the interaction effect was stronger with 16.03% variance explained. For the same trait, the variance explained by epistasis was only 6.18% in the cross between *M. guttatus* and *M. micranthus*.

Pairwise QTL genome scans: corolla width

Between *M. guttatus* and *M. platycalyx*, 4 interacting locus pairs for corolla width were identified. The most significant involved *QTL8_38* (Table 4-3) and a new QTL (undetected in the single-locus analysis) on linkage group 3, with 2.3% of variance

explained by this interaction (Table 4-4). Two mapped QTL genes, *QTL4_32* and *QTL8_38*, both significant in the single-locus analysis, had epistasis that explained 1.37% of the variance. In addition, epistasis was also found between chromosome regions that had no QTL in the R/QTL single-locus analysis. For example, the two-way ANOVA detected epistasis between linkage group 9 and linkage group 12, which had no QTLs detected by the R/QTL analysis (Table 4-3 and Table 4-4).

Seven pairs of interacting loci were found in the cross of *M. platycalyx* and *M. micranthus*. Epistasis appears to be an important genetic character in this cross, giving that there were originally only 3 QTL genes mapped by R/QTL with single locus genome scans, but with the two-way ANOVA analysis, 8 QTL were found. Two of the mapped QTL loci, *QTL2_28* and *QTL8_38*, not only showed additive effects, but jointly demonstrated epistasis that accounted for 4.66% of the segregational variance, the largest degree of epistasis found in this study. Also, a great proportion of epistasis was found to involve *QTL8_38*, with 5 out of 7 epistasis interactions involving this QTL (Table 4-4). Again, R/QTL failed to detect additive QTL that interact epistatically; e.g., epistasis number (2) and number (3) between *M. platycalyx* and *M. micranthus*, although each explained a relatively small amount of trait segregation, 1.48% and 1.85%, respectively (Table 4-4).

In the cross of *M. guttatus* and *M. micranthus*, two epistatic interactions were found, both involving previously mapped QTL (Table 4-4).

Pairwise QTL genome scans: corolla length

Although the R/QTL single locus scans revealed similar QTL for corolla length and corolla width between *M. guttatus* and *M. platycalyx* (Table 4-3), the analysis of epistasis revealed detailed differences for QTL for corolla length. Eight pairs of epistatic QTL were found and epistasis explained up to 10.5% of the segregational variance (Table 4-4). Two significant epistasic pairs were found in linkage group 10 (*QTL10_26*) and linkage group 13 (*QTL13_25*); both were not initially revealed by R/QTL analysis (Table 4-4).

Similar results were also found between *M. platycalyx* and *M. micranthus*. Epistasis was mostly found between mapped QTL and the chromosome regions (Table 4-4). No epistasis was found between *M. guttatus* and *M. micranthus*.

Pairwise QTL genome scans: pistil length

Five cases of epistasis were found between *M. guttatus* and *M. platycalyx*, with PVE ranging from 1.51% to 3.32%. Interestingly, the highest epistasis was found between two chromosome regions that contained no significant QTL in the single-QTL R/QTL analysis (epistasis number (3) between *M. guttatus* and *M. platycalyx*, Table 4-4). Between *M. platycalyx* and *M. micranthus*, we identified six epistatic QTL most of them between mapped QTL and previously unidentified QTL (Table 4-4). Between *M. guttatus* and *M. micranthus*, we found two significant epistatic QTL between previously identified QTL loci, with a combined PVE of 5.24% (Table 4-4).

Pairwise QTL genome scans: stamen length

In total, 13 cases of epistasis were identified for stamen length among all comparisons; 7 between *M. guttatus* and *M. micranthus*, 6 between *M. platycalyx* and *M. micranthus*, and none between *M. guttatus* and *M. micranthus*. Although the analysis of R/QTL single locus genome scans did not suggest significant major QTL on linkage group 10, actually in this group a few epistatic interactions were identified with the two-way ANOVA. For example, between *M. guttatus* and *M. platycalyx*, genetic interaction was found within linkage group 10 between chromosome region 0 cM (*QTL10_0*, nearby marker C1_295) and 26 cM (*QTL10_26*, nearby marker C7_224).

Epistasis from two interacting chromosome regions on the same linkage group can also be seen on linkage group 11: epistasis number (6) between *M. platycalyx* and *M. micranthus*. However, the epistasis found within linkage group 11 was generated between a previously mapped QTL (*QTL11_19*) and the chromosome region identified QTL related gene, *QTL11_13* (around 10 cM and the nearby marker is C4_147), in which R/QTL failed to detect a QTL. Interestingly, this *QTL11_13* was a QTL for other floral traits (Table 4-3). This epistasis on linkage group 11 between *M. platycalyx* and *M. micranthus* could be due to a pair of closely linked genes, or several genes over a larger chromosome region, with pleiotropic effects upon floral traits.

Pairwise QTL genome scans: stigma-anther separation

Compared to the other floral traits, epistasis for stigma-anther separation was much less. Between *M. guttatus* and *M. platycalyx*, we found only one significant epistasic pair of QTL, that involving *QTL8_38* and a location in linkage group 5 (Table

4-4), even though we found several (8) QTL previously via single-locus analyses (Table 4-3). Between *M. platycalyx* and *M. micranthus*, again only one was detected, between *QTL8_38* and *QTL13_25* (Table 4-4). Between *M. guttatus* and *M. micranthus*, no significant epistasis was found.

DISCUSSION

The focus of this study was to determine the extent of epistasis for QTL underlying mating system variation in the *Mimulus guttatus* species complex. A basic understanding of genetic architecture not only involves the number of QTL and their magnitude of effect, but also their genetic interactions. This provides a complete understanding of the evolutionary transition of mating system from outcrossing to self-fertilization.

In our analyses, both "joint" and "interaction" LOD scores were calculated for all pairwise combinations of QTL loci. The joint LOD score provided an estimate of the entire two-locus interaction, and the interaction LOD score specifically indicated epistasis. Genome-wide significant thresholds identified significant interactions via permutation tests. We found 25 pairs of QTL to exceed the threshold of both joint and interaction LOD scores between *M. guttatus* and *M. platycalyx*, 28 between *M. platycalyx* and *M. micranthus* and 4 between *M. guttatus* and *M. micranthus.*

The importance of epistasis in population differentiation has been emphasized by Sewall Wright in a series of papers. It was summarized in 1980 by Wright (1980), represented in his "shifting balance" theory that explained selection as a counterpoint to the selection of single mutations according to classical Darwinian theory. By his interpretation, a selective advantage is attained when a particular combination of alleles is created by population admixture, rather than by single point mutations. His idea was originally founded on experimentation, not theory. Observations of domestic livestock suggested that overall improvement takes place not from within a particular herd, but

when interbreeding takes place between herds, thus generating novel combinations of alleles that foster interacting co-adapted gene complexes. A number of theoretical models have demonstrated that favourable interacting co-adaptive gene complexes can evolve with similar selective pressures.

Breaking the co-adaptive gene complex by intercrossing populations or species can decrease fitness, and this phenomenon of hybrid-breakdown is the basis of the Dobzhansky-Muller speciation model (Dobzhansky 1937). For example, in the genetic architecture of population differentiation of *Chamaecrista fasciculate*, the enhanced performance of F1 to parents suggestively represented that increased fitness (heterosis) was due to dominance that masked deleterious recessive alleles and the expression of positive epistasis (Fenster and Galloway 2000; Lynch 1991; Lynch and Walsh 1998; Lynch et al. 1999). More interestingly, these studies documented a consistent hybrid-breakdown in the F3, suggesting that combining genes from different populations can decrease vigour beyond what was due to the expected loss of heterozygosity (Fenster and Galloway 2000). Such findings of epistasis contributing to population divergence in a local scale can also be seen in a number of other studies (Templeton et al. 1976; Price and Waser 1979; Waser and Price 1989, 1994; Burton 1987, 1990; Deng and Lynch 1996). In *Drosophila*, the incongruence of epistasis estimates, both between studies and traits, can be generally related with the degree of differentiation presented by the populations in each trait (Rego et al. 2007), while considering studies involving populations from the same species (Edmands 1999; Bieri and Kawecki 2003; Teotónio et al. 2004).

Statistical detection of epistasis

An interesting observation from studies that address the importance of epistasis was that while some studies have shown little evidence for significant epistasis (Edwards et al. 1987; Xiao et al. 1995), others have reported major interaction effects (Cockerham and Zeng 1996; Li et al. 1997). It is often emphasized that the most important problem in studying epistasis in QTL experiments is the relatively poor statistical power, owing to the small sample size, limiting numbers of individuals in each of the genotype classes, and questionable level for declaring significance of epistasis (Tanksley 1993; McMullen et al. 2001, Yi and Xu 2002).

In this study of floral trait variation among *Mimulus* species, we noticed that there were only a few cases of epistasis found in between the QTLs as identified via single-locus analyses (Table 4-4). Given the nature of the R/QTL single locus genome scan that we used to identify QTL and the calculation of significance threshold by the permutation method, we could have likely missed QTL due to the stringent cut-off threshold. Such results could have biased us towards detection of too few QTL loci with too large effects (Beavis 1998).

As shown in Figures 4-3 to Figure 4-7, our multi-dimensional genome scans revealed a higher degree of complexity of the genetics in floral character evolution. On those graphical examples of two-dimensional linkage maps, the highlighted (darker) points in the figures are indicative of the potential genetic interactions in *Mimulus*. For the example in the cross of *M. guttatus* and *M. micranthus*, additively, both *QTL1_1* and *QTL12_8* affect the variation of corolla width, with significant amount of genetic effect (Table 4-4). Together, Figure 4-3(C) displays a highlighted LOD score to genetic

interaction in between *QTL1_1* and *QTL 12_8* ((1) in Figure 4-3(C)), in the comparison with other genome regions.

In addition to the epistasis found in between mapped QTL, much epistasis was found between mapped QTL and previously unidentified chromosome regions in these two dimensional analysis (Table 4-4). These chromosome regions with only little marginal genetic effect, the potential QTL, were the chromosome regions that only showed their genetic effect while epistasis is considered. Without the restriction to just the "known" QTL (as inferred by single QTL analysis), the multidimensional genome scan technique we utilized allows a more systematic and powerful search for interacting locus pairs (Malmberg and Mauricio 2005). This is because of the well-known fact that in two-way ANOVA models, main effects may not be present but interactions terms can be.

For example, on linkage group 10, 26 cM nearby AFLP marker C7_224, using R/QTL single locus genome scan with the threshold set up by 10,000 permutations, the linkage group did not succeed in supporting significance of *QTL10_26* in the cross between *M. guttatus* and *M. platycalyx*. Next, by fitting in a two-way ANOVA, this locus later reveals its genetic effect by exhibiting an epistatic effect jointly with another locus locating on 0 cM position of the same linkage group (*QTL10_0*) in the variation of corolla width (1.53% of PVE), corolla length (1.15% of PVE) and average stamen length (1.57% of PVE) (Table 4-4). *QTL10_26* was then reckoned to be likely involved in the two-locus epistatic interaction. In the final ANOVA model together with all the interaction terms, some visible genetic effect was also uncovered associated with this *QTL10_26* locus, contributing to 3.09 % PVE for corolla width, 2.38% PVE for corolla

length and 3.02% PVE for stamen length. Such additively silent but epistatically acting genetic loci are also frequently observed on linkage group 7, 12 and 13 from the comparison of *M. platycalyx* and *M. micranthus* (Table 4-4).

Epistasis found in previous studies

The same QTL mapping technique as we have employed have also been used in studies of genome-wide epistatic interactions that determine the genetic basis of circadian behaviours (Shimomura et al. 2001) as well as the diabetes related phenotypes in mice (Togawa et al. 2006). In plants, Malmberg et al. (2005) studied fruit number, germination and seed size in field-grown *Arabidopsis thaliana*. The number of additive QTL varied from 2 to 4; in each case the number of QTL loci estimated with epistatic interactions was approximately double, varying from 5 to 8.

In a maize domestication study, residing on the long arm of chromosome 3, the effect of QTL-3L was found only weak or non-significant when transferred into a NIL background (Doebley and Stec 1993; Doebley et al. 1995). The significance of QTL-3L can however only be detected when the epistasis with QTL-1L was also considered (Lukens and Doebley 1999). The interesting findings of QTL on maize domestication traits, the silent loci (e.g. *QTL10_26*) involved in *Mimulus* mating system evolution or the additional QTLs found in *Arabidopsis* suggests that the alleles like *QTL10_26* in *Mimulus* case could have resided in natural populations without affecting the fitness of the population, while not segregating. The joint presence of teosinte and maize alleles in the domestication process, or the *QTL10_26* of *Mimulus* on different genetic background, allows detectable genetic effects of QTL-3L and *QTL10_26*, and thus is indicative that

selection in the evolution of mating system shift (or during domestication of maize species) was acting on a gene complex, rather than single locus in strictly additive fashion.

Epistasis in the evolution of selfing in Mimulus

Kelly (2005) found considerable epistasis for flower morphology in *Mimulus.* However, epistasis in *Mimulus* might be variable across genetic loci underlying these traits, as well as among different genetic crosses (Fishman et al. 2002; Kelly 2005). Here, in the cross of *M. guttatus* and *M. micranthus*, not only was the proportion of variance explained by epistasis small, but the two-way ANOVA model also failed to find epistasis in a number of floral traits, including corolla length, stamen length and stigma-anther separation (Table 4-4). Given that inbreeding depression is high in *M. guttatus* population (Dole and Ritland 1993; Latta and Ritland 1994), the partially recessive mating system modifier loci likely drive the evolution of selfing from outcrossing in *Mimulus* (Lin and Ritland 1997). Although we did not explicitly estimate dominance genetic variance in this study, directional dominance was evident in the biometric comparison of *M. guttatus* and *M. micranthus* (Chapter 2). With the little proportion of epistasis variance found in this analysis, we speculate the genetic basis of evolution of selfing from outcrossing, in *M. guttatus* species complex, involves genes with predominantly additive and dominance effect, with few epistatic interactions.

Moreover, in theory, population or species that have longer independent evolution histories should also evolve more closely co-adapted gene complexes owing to the lack gene flow and recombination. Thus one could expect a greater degree of epistasis when

crossing populations or species with greater degree of genetic divergence, because of the novel combinations of alleles from different loci brought by recombination. Estimated by 368 neutral AFLP markers, *M. guttatus* and *M. micranthus* are about twice as closely related to each other compared to the *M. guttatus*-*M. platycalyx* and *M. micranthus*-*M. platycalyx* species pairs (Chapter 3). Our findings of little epistasis in between closely related *M. guttatus*-*M. micranthus* taxa are compatible with Moller et al. (1965), who found that crosses between more distant populations had a favourable epistatic effect upon grain yield (Lynch 1991).

Finally, the differentiation of *M. micranthus* from *M. guttatus* might have been a rapid event with only a few co-adapted gene complex involved. This is supported by the evidence that these interacting QTL pairs, involving epistasis found in the cross of *M. guttatus* and *M. micranthus*, are among those homologous lineage specific QTLs found in these *Mimulus* lineages (e.g., QTL B5_535 in Chapter 3).

Epistasis in stable mixed mating systems

The genotypic association between inbreeding depression loci and modifiers of mating system, as studied by numerous workers (Campbell 1986; Holsinger 1991 and Uyenoyama et al. 1993), offers a scenario that may help explain stable mixed mating systems. As overdominance cannot be purged, and therefore inbreeding depression maintained, the evolution of selfing from outcrossing is prevented and stable partial selfing maintained. The heterozygous modifier loci needed for such a condition should occur in either outcrossing and intermediate selfing species, like *M. guttatus* and *M. platycalyx*, respectively, and not in a fully selfing species like *M. micranthus*. Now, in

the multi-locus model, when there is partial selfing, it is known that different loci do not behave independently, a situation referred as “identity disequilibrium” (Charlesworth and Charlesworth 1990; Ohta and Cockerham 1974; Tachida and Cockerham 1989). When loci are in identity disequilibrium, heterozygosity is correlated among loci. Higher than expected multilocus heterozygosity might lead to elevated epistasis. This genetic scenario is supported by our results: epistasis was greater in crosses with *M. platycalyx*, the intermediate selfer, suggesting a role of co-adapted gene complex and epistasis in maintaining mixed mating systems.

Conclusion

Epistasis was suggested here by the pairwise genome scans for floral character evolution in *Mimulus*. It was further revealed by the two-way ANOVA model, where epistasis was further supported between *M. guttatus* and *M. platycalyx* and between *M. platycalyx* and *M. micranthus*. The comparison between *M. guttatus* and *M. micranthus* however failed to show epistasis, although results from single locus genome scans did identify polygenic genetic basis with averaged 5.8 QTL genes for each floral trait variation. Interesting, these two species have half the genetic distance of the other two pairings of taxa, suggesting that epistasis increases in the progeny of wider crosses (Figure 4-8).

Table 4-1. Generation means and sample sizes for each corolla trait in *Mimulus* pedigrees (P=parental species, F1=first generation cross, BC=backcross to parental species).

		Corolla trait				
Population	**Sample size**	**Corolla width**	**Corolla length**	**Pistil length**	**Stamen length**	**Stigma-anther separation**
Cross: *M. guttatus* X *M. platycalyx*						
P- *M. guttatus*	10	30.25 (0.79)	34.20 (1.07)	18.96 (0.53)	16.95 (0.25)	2.02 (0.39)
P- *M. platycalyx*	9	17.77 (0.40)	23.23 (0.57)	13.15 (0.31)	12.89 (0.29)	0.26 (0.26)
F1	20	19.61 (0.54)	24.08 (0.60)	14.72 (0.20)	12.52 (0.22)	2.20 (0.14)
BC to *M. guttatus*	216	24.16 (0.18)	29.83 (0.19)	18.09 (0.11)	14.64 (0.10)	3.4 (0.07)
BC to *M. platycalyx*	173	18.58 (0.28)	23.71 (0.32)	14.35 (0.18)	12.03 (0.16)	2.24 (0.10)
Cross*: M. guttatus* X *M. micranthus*						
P – *M. guttatus*	10	30.25 (0.79)	34.20 (1.07)	18.96 (0.53)	16.95 (0.25)	2.02 (0.39)
P – *M. micranthus*	11	8.10 (0.30)	11.39 (0.36)	6.38 (0.22)	7.21 (0.23)	-0.35 (0.15)
F1	35	12.26 (0.82)	14.88 (0.84)	9.17 (0.52)	8.82 (0.33)	0.34 (0.23)
BC to *M. guttatus*	149	22.34 (0.33)	26.71 (0.33)	16.09 (0.15)	13.86 (0.14)	2.21 (0.07)
BC to *M. micranthus*	122	10.67 (0.22)	13.86 (0.22)	8.51 (0.11)	7.89 (0.10)	0.62 (0.05)
Cross: *M. platycalyx* X *M. micranthus*						
P – *M. platycalyx*	9	17.77 (0.40)	23.23 (0.56)	13.15 (0.31)	12.89 (0.29)	0.26 (0.26)
P – *M. micranthus*	11	8.10 (0.30)	11.39 (0.36)	6.38 (0.22)	7.21 (0.23)	-0.35 (0.15)
F1	36	16.98 (0.23)	16.58 (0.23)	10.39 (0.09)	9.76 (0.11)	0.63 (0.06)
BC to *M. platycalyx*	72	11.66 (0.22)	16.13 (0.28)	9.82 (0.14)	10.03 (0.17)	-0.2 (0.09)
BC to *M. micranthus*	166	8.13 (0.20)	11.74 (0.25)	7.09 (0.14)	7.76 (0.14)	-0.67 (0.06)

Values in parentheses are standard error to the mean
All measures are in mm

Table 4-2. AFLP primers used in this study and the extent of polymorphism.

AFLP Primers for pre-amplification: Primer names and sequences	Primer name	AFLP primer pair for final amplification	Number of amplified polymorphic fragments
Primer B set:	B2	Eco + ACA / Mse + CCT	25
*Eco*RI* + AC / *Mse*I** + CC	B3	Eco + ACA / Mse + CGC	54
	B4	Eco + ACA / Mse + CCA	59
	B5	Eco + ACT / Mse + CGG	38
Primer C set:	C1	Eco + CA / Mse + CCG	61
*Eco*RI + C / *Mse*I + CC	C3	Eco + CCG / Mse + CCG	31
	C4	Eco + CCA / Mse + CCT	54
	C7	Eco + CTC / Mse + CCT	46
Total number of polymorphic fragments			368

* Primer sequence of *Eco*RI is GACTGCGTACCAATTC.
** Primer sequence of *Mse*I is GATGAGTCCTGAGTAA.

Table 4-3. Significant QTL identified from R/QTL single locus and pair-wise genome scans. The significance was determined by calculating the genome-wide thresholds of α = 0.05 by 10,000 permutations. The genetic effect in percentage of variance explained and significance of selected genetic loci were all tested in a two-way ANOVA model when interaction was taken account. The nearest marker denoted the AFLP marker residing closest to the position where exhibits the maximum LOD score that defines the QTL gene on the linkage group. Name of QTL was given by the linkage group and the chromosome location of the resulting QTL. QTL genes that are identified from the same linkage group and share the nearest AFLP marker are considered to be the same genetic locus, and therefore given the same name.

Cross: *M. guttatus* x *M. platycalyx*

Trait	Linkage group	Name of QTL	QTL map position cM (nearest marker)	LOD score	Genetic effect [1], PVE [2]
Corolla width	1	*QTL1_19*	19.1 (C1_200)	31.1	4.7, 1.7%
	2	*QTL2_12*	12.0 (C4_446)	33.4	-4.5, 1.8%
	4	*QTL4_32*	32.0 (C3_341)	35.6	5.5, 1.7%
	8	*QTL8_38*	38.2 (C7_210)	50.6	4.5, 12.9%
	9[3]	*QTL9_0*	0.0 (C7_312)	3.51	1.6, 1.8%
	10[3]	*QTL10_26*	26.0 (C7_224)	7.95	-1.1, 3.1%
	12[3]	*QTL12_12*	12.0 (C7_461)	5.6	-1.8, 2.6%
Corolla length	1	*QTL1_20*	20.0 (B4_106)	46.1	6.4, 1.7%
	4	*QTL4_32*	32.1 (C3_341)	52.8	7.0, 2.8%
	8[3]	*QTL8_10*	10.0 (C3_307)	32.2	0.2, 3.7%
	8	*QTL8_38*	38.0 (C7_210)	63.3	5.9, 18.4%
	10[3]	*QTL10_0*	10.0 (C1_295)	3.3	2.2, 2.8%
	10[3]	*QTL10_26*	26.0 (C7_224)	7.7	-1.2, 2.4%
	11	*QTL11_25*	25.0 (C4_188)	67.5	6.5, 2.8%
	13[3]	*QTL13_25*	29.0 (C7_310)	2.9	2.0, 2.6%
Pistil length	1	*QTL1_20*	20.2 (B4_106)	44.4	6.3, 2.9%
	2[3]	*QTL2_12*	12.0 (C4_446)	31.9	-5.1, 3.3%
	4	*QTL4_32*	32.1 (C3_341)	49.4	6.0, 1.9%
	8	*QTL8_38*	38.0 (C7_210)	59.5	5.4, 9.3%
	9	*QTL9_0*	8.0 (C7_312)	4.4	2.1, 1.9%
	10[3]	*QTL10_26*	26.0 (C7_224)	7.9	-1.2, 3.7%
	11	*QTL11_25*	25.0 (C4_188)	64.6	6.3, 1.8%
Stamen length	1	*QTL1_19*	20.0 (C1_200)	28.6	4.4, 2.5%
	2	*QTL2_12*	12.0 (C4_446)	30.0	-4.1, 2.3%
	4	*QTL4_32*	30.0 (C3_341)	29.2	5.0, 5.0%
	8	*QTL8_38*	41.0 (C7_210)	46.6	1.4, 11.5%
	9[3]	*QTL9_0*	5.0 (C7_312)	4.5	1.8, 2.3%
	10[3]	*QTL10_0*	0.0 (C1_295)	2.7	1.7, 2.0%
	10[3]	*QTL10_26*	26.0 (C7_224)	7.8	-1.0, 3.0%
	11	*QTL11_25*	25.0 (C4_188)	45.8	5.1, 4.5%
	12[3]	*QTL12_12*	12.0 (C7_461)	6.3	-1.9, 2.4%
Stigma-anther separation	6	*QTL6_43*	43.0 (B5_052)	3.8	-0.6, 3.0%
	8	*QTL8_38*	41.0 (C7_210)	17.5	0.1, 11.5%
	11	*QTL11_25*	29.0 (C4_188)	12.2	1.4, 3.3%

Cross: *M. platycalyx* x *M. micranthus*

Trait	Linkage group	Name of QTL	QTL map position cM (nearest marker)	LOD score	Genetic effect [1], PVE [2]
Corolla width	1[3]	*QTL1_58*	58.0 (C1_088)	1.7	0.2, 1.9%
	2	*QTL2_28*	28.5 (C1_522)	14.1	2.1, 6.9%
	7[3]	*QTL7_0*	0.0 (B3_166)	2.8	-0.9, 3.5%
	8[3]	*QTL8_0*	0.0 (C3_307)	7.5	1.7, 2.7%
	8	*QTL8_38*	41.2 (C7_210)	32.7	0.8, 21.2%
	11	*QTL11_13*	13.0 (C4_147)	21.8	2.7, 2.1%
	12[3]	*QTL12_5*	5.0 (C7_230)	5.8	3.9, 5.8%
	13[3]	*QTL13_25*	25.0 (C7_310)	1.1	-0.3, 4.0%
Corolla length	2	*QTL2_28*	28.2 (C1_522)	16.2	2.7, 5.2%
	6[3]	*QTL6_35*	35.0 (B5_339)	12.4	-3.7, 1.7%
	8	*QTL8_38*	41.2 (C7_210)	45.5	0.9, 25.8%
	9[3]	*QTL9_0*	5.0 (C7_312)	14.7	3.3, 19.0%
	11	*QTL11_13*	15.0 (C4_174)	26.0	3.4, 9.6%
	12[3]	*QTL12_12*	12.0 (C7_461)	10.0	-0.3, 4.4%
	13[3]	*QTL13_5*	5.0 (C7_245)	2.1	1.0, 1.9%
Pistil length	2[3]	*QTL2_28*	30.0 (C1_522)	17.6	3.0, 4.2%
	7[3]	*QTL7_0*	0.0 (B3_166)	2.14	0.9, 4.2%
	8	*QTL8_38*	41.2 (C7_210)	50.0	0.9, 26.6%
	9[3]	*QTL9_0*	5.0 (C7_312)	17.8	4.0, 2.2%
	11	*QTL11_13*	17.0 (C4_188)	29.3	4.0, 5.1%
	12[3]	*QTL12_12*	10.0 (C7_461)	14.3	-0.4, 2.8%
Stamen length	2[3]	*QTL2_28*	28.0 (C1_522)	16.3	3.4, 3.9%
	4[3]	*QTL4_0*	2.0 (B5_535)	18.0	4.7, 3.5%
	8	*QTL8_38*	41.2 (C7_210)	59.6	0.8, 37.7%
	9[3]	*QTL9_0*	3.0 (C7_312)	24.3	1.3, 10.4%
	11[3]	*QTL11_13*	10.0 (C4_147)	16.8	5.0, 2.6%
	11	*QTL11_25*	19.0 (C4_188)	34.8	5.0, 3.2%
	12[3]	*QTL12_12*	12.0 (C7_461)	19.1	0.1, 1.3%
Stigma-anther separation	2	*QTL2_28*	0.0 (C1_594)	3.6	0.8, 5.7%
	8	*QTL8_38*	41.0 (C7_210)	3.4	0.1, 6.7%
	13[3]	*QTL13_25*	15.0 (C7_310)	1.6	0.3, 4.9%

Cross: *M. guttatus x M. micranthus*

Trait	Linkage group	Name of QTL	QTL map position cM (nearest marker)	LOD score	Genetic effect [1], PVE [2]
Corolla width	1	*QTL1_1*	1.0 (C1_479)	8.5	2.2, 10.1%
	8[3]	*QTL8_35*	34.0 (B5_180)	1.1	0.2, 1.6%
	9	*QTL9_19*	19.0 (B5_070)	7.7	-0.8, 10.1%
	12	*QTL12_8*	8.0 (C7_461)	4.4	-0.8, 5.6%
	13[3]	*QTL13_5*	0.0 (C7_245)	1.6	0.5, 1.6%
	14[3]	*QTL14_39*	32.0 (B4_166)	1.1	-1.5, 1.6%
Corolla length	1	*QTL1_19*	13.0 (C1_200)	5.8	0.8, 3.9%
	9	*QTL9_19*	19.0 (B5_070)	6.1	-0.7, 2.85
	12	*QTL12_8*	8.4 (C7_461)	3.8	-0.9, 3.35
	13[3]	*QTL13_25*	15.0 (C7_310)	1.3	0.6, 1.9%
Pistil length	1	*QTL1_1*	1.0 (C1_479)	17.2	1.3, 6.6%
	2	*QTL2_11*	11.0 (C1_247)	9.6	-1.6, 3.3%

	4	*QTL4_0*	0.0 (B5_535)	4.6	0.8, 4.7%
	9	*QTL9_19*	19.0 (B5_070)	10.7	-1.2, 6.9%
Stamen length	1	*QTL1_1*	1.0 (C1_479)	12.7	1.4, 2.1%
	2	*QTL2_11*	11.0 (C1_247)	6.4	-1.3, 2.1%
	9	*QTL9_19*	19.0 (B5_070)	9.4	-1.1, 4.5%
Stigma-anther separation	1	*QTL1_1*	1.0 (C1_479)	17.9	1.7, 1.2%
	2	*QTL2_11*	11.0 (C1_247)	11.1	-1.6, 7.0%
	4	*QTL4_0*	0.0 (B5_535)	7.6	0.9, 2.1%
	6	*QTL6_43*	43.0 (B5_052)	4.5	-1.1, 1.3%
	9	*QTL9_19*	19.0 (B5_070)	8.2	-0.9, 2.8%
	13	*QTL13_18*	15.0 (C7_310)	4.2	1.4, 1.2%

1. Joint estimate of additive and dominance genetic effect, all measure in mm
2. PVE, percentage of variance explained
3. The genetic locus only identified from pair-wise genome scan and tested significant in two-way ANOVA

Table 4-4. Significant interacting pairs of loci found by R/qtl pairwise genome scans. Interacting pairs were deemed significant if both the joint and interaction LOD exceeded the threshold of α = 0.05. The genome-wide LOD thresholds were generated by 1000 permutations. The nearest marker denotes the AFLP marker that appears closest to the map location of where identified the maximum LOD score. The percentage of variance explained by the pair of locus was estimated by the fit of a two-way ANOVA model

Floral Trait		Locus 1		Locus 2		Joint LOD In pairwise genome scans	LOD score in ANOVA	% of variance explained (PVE)
		Chr (Map position)	Nearest marker	Chr (Map position)	Nearest marker			
Trait: Corolla width								
Cross:								
M. guttatus* X *M. platycalyx	**(1)**	4 (32.0)	C3_341	8 (38.0)	C7_210	69.2	2.9	1.37 *
	(2)	3 (7.0)	B5_186	8 (38.0)	C7_210	69.5	2.3	2.30 *
	(3)	9 (0.0)	C7_312	12 (12.0)	C7_461	45.9	2.1	1.01 *
	(4)	10 (0.0)	C1_295	10 (26.0)	C7_224	46.6	3.2	1.53 *
Cross:								
M. platycalyx* X *M. micranthus	**(1)**	2 (28.0)	C1_522	8 (41.0)	C7_210	49.0	7.7	4.66 ***
	(2)	7 (0.0)	B3_166	12 (5.0)	C7_230	18.7	2.6	1.48 *
	(3)	12 (11.0)	C7_461	13 (25.0)	C7_310	13.9	3.2	1.85 *
	(4)	8 (41.0)	C7_210	8 (0.0)	C3_307	59.7	3.8	2.18 **
	(5)	8 (41.0)	C7_210	7 (0.0)	B3_166	27.6	2.4	2.39 **
	(6)	8 (41.0)	C7_210	12 (5.0)	C7_230	39.8	3.5	1.99 **
	(7)	8 (41.0)	C7_210	13 (25.0)	C7_310	37.7	2.6	1.48 *
Cross:								
M. guttatus* X *M. micranthus	**(1)**	1 (1.0)	C1_479	12 (8.0)	C7_461	13.2	1.9	2.65 *
	(2)	9 (19.0)	B5_070	12 (8.0)	C7_461	10.2	2.6	3.53 **
Trait: Corolla length								
Cross:								
M. guttatus* X *M. platycalyx	**(1)**	1 (20.0)	C1_404	8 (38.0)	C7_210	78.6	2.7	1.05 *
	(2)	8 (38.0)	C7_210	11 (25.0)	C4_188	89.4	2.8	1.09 *
	(3)	4 (32.0)	C3_341	11 (25.0)	C4_188	75.1	2.9	1.16 *
	(4)	4 (15.0)	B3_485	13 (25.0)	C7_310	52.1	3.2	1.28 *
	(5)	4 (32.0)	C3_341	8 (38.0)	C7_210	89.3	5.9	1.29 *
	(6)	8 (38.0)	C7_210	8 (10.0)	C3_307	89.3	5.9	2.39 ***
	(7)	10 (10.0)	C1_295	10 (26.0)	C7_224	63.1	2.9	1.15 *
	(8)	10 (10.0)	C1_295	13 (25.0)	C7_310	50.4	2.8	1.09 *

Cross:								
M .platycalyx* X *M. micranthus	**(1)**	2 (28.0)	C1_522	8 (41.0)	C7_210	67.7	6.4	2.74 ***
	(2)	6 (35.0)	B5_399	11 (14.0)	C4_174	35.3	2.9	1.20 *
	(3)	8 (41.0)	C7_210	9 (5.0)	C7_312	60.1	4.7	2.00 **
	(4)	8 (41.0)	C7_210	11 (15.0)	C4_174	64.5	4.8	2.04 **
	(5)	9 (5.0)	C7_312	11 (15.0)	C4_174	39.8	3.3	1.38 *
	(6)	9 (5.0)	C7_312	12 (12.0)	C7_461	24.1	5.0	2.10 **
	(7)	11 (15.0)	C4_174	12 (12.0)	C7_461	37.5	4.6	1.91 **
	(8)	11 (15.0)	C4_174	13 (5.0)	C7_245	38.8	3.1	1.27 **
Trait: Pistil length								
Cross:								
M. guttatus* X *M. platycalyx	**(1)**	1 (20.0)	B4_106	8 (38.0)	C7_210	78.1	2.7	1.51 **
	(2)	2 (12.0)	C4_446	8 (38.0)	C7_210	76.8	2.5	2.47 *
	(3)	2 (12.0)	C4_446	10 (26.0)	C7_224	63.4	3.3	3.32 *
	(4)	4 (32.0)	C3_341	8 (38.0)	C7_210	79.6	2.4	2.40 *
	(5)	9 (15.0)	B5_070	12 (0.0)	C7_230	54.9	2.1	2.10 *
Cross:								
M. platycalyx* X *M. micranthus	**(1)**	2 (30.0)	C1_343	8 (41.0)	C7_210	67.7	5.3	2.47 ***
	(2)	7 (0.0)	B3_166	8 (41.0)	C7_210	51.4	4.9	2.29 **
	(3)	7 (0.0)	B3_166	11 (17.0)	C4_188	33.9	2.1	1.00 *
	(4)	7 (0.0)	B3_166	12 (10.0)	C7_461	25.7	2.7	1.23 *
	(5)	8 (41.0)	C7_210	11 (17.0)	C4_188	64.2	2.8	1.29 *
	(6)	11 (17.0)	C4_188	12 (10.0)	C7_461	39.5	3.1	1.41 *
Cross:								
M. guttatus* X *M. micranthus	**(1)**	1 (1.0)	C1_479	4 (0.0)	B5_535	18.6	2.4	2.67 *
	(2)	4 (0.0)	B5_535	9 (19.0)	B5_070	16.0	2.5	2.57 *
Trait: Stamen length								
Cross:								
M. guttatus* X *M. platycalyx	**(1)**	2 (12.0)	C4_446	8 (38.0)	C7_210	62.4	4.3	2.50 **
	(2)	4 (32.0)	C3_341	8 (38.0)	C7_210	65.5	2.4	1.37 *
	(3)	4 (32.0)	C3_341	11 (24.0)	C4_188	53.8	2.6	1.47 *
	(4)	4 (32.0)	C3_341	12 (10.0)	C7_461	34.5	2.3	1.34 *
	(5)	8 (38.0)	C7_210	9 (5.0)	C7_312	68.0	2.3	1.30 *
	(6)	8 (38.0)	C7_210	11 (24.0)	C4_188	67.5	2.5	1.43 *
	(7)	10 (0.0)	C1_295	10 (26.0)	C7_224	37.5	2.7	1.57 *

Cross:								
M. platycalyx* X *M. micranthus	**(1)**	2 (28.0)	C1_343	8 (41.0)	C7_210	75.7	6.8	2.47 **
	(2)	2 (28.0)	C1_343	11 (19.0)	C4_188	60.9	3.5	1.22 *
	(3)	4 (2.0)	B5_535	10 (26.0)	C7_224	32.0	2.7	1.25 *
	(4)	8 (41.0)	C7_210	9 (3.0)	B5_070	68.7	10.6	4.03 ***
	(5)	8 (41.0)	C7_210	11 (19.0)	C4_174	71.5	3.5	1.21 *
	(6)	11 (10.0)	C4_147	11 (19.0)	C4_174	44.3	4.4	1.55 **
Trait: Stigma-anther separation								
Cross:								
M. guttatus* X *M. platycalyx	**(1)**	8 (41.0)	C7_210	5 (15.0)	B4_341	21.7	2.1	2.19 *
Cross:								
M. platycalyx* X *M. micranthus	**(1)**	8 (41.0)	C7_210	13 (15.0)	C7_310	6.5	1.8	3.41 *

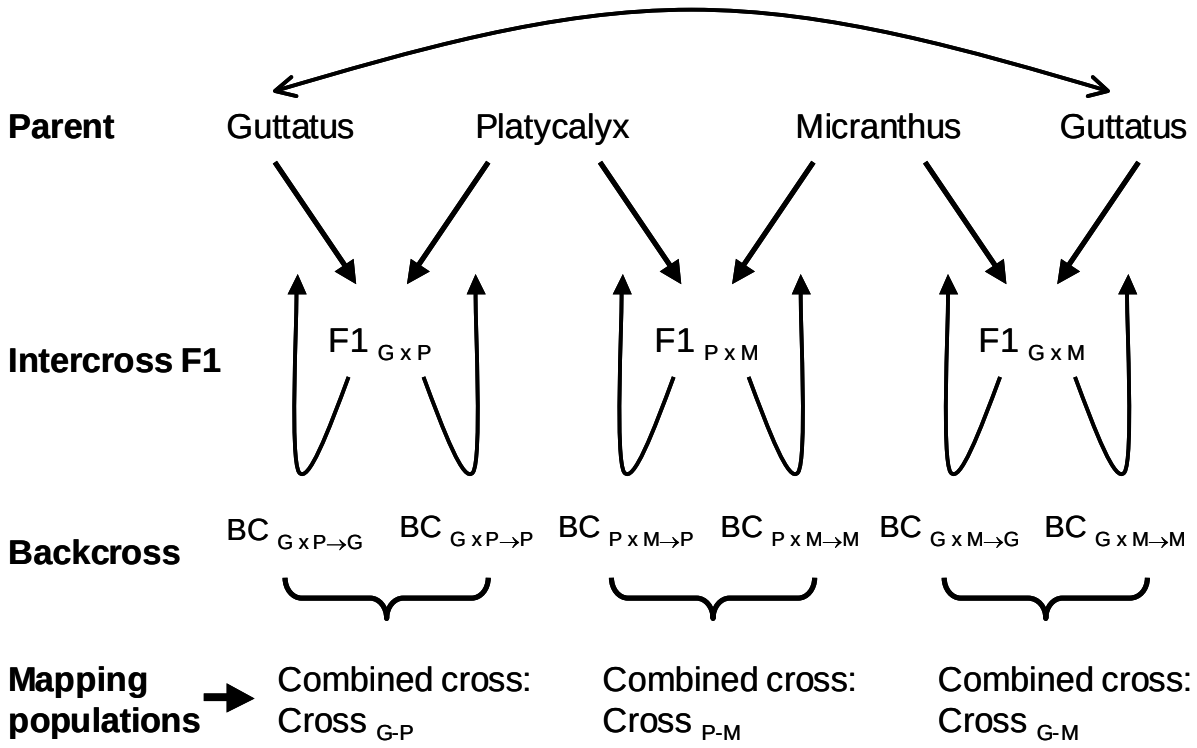

Figure 4-1. The crossing scheme and mapping populations for QTL phylogenetic analysis. In the figure, G represents *Mimulus guttatus*, P represents *M. platycalyx* and M represents *M. micranthus*. Mapping populations were generated by combining two backcrosses from the same pair of parent species.

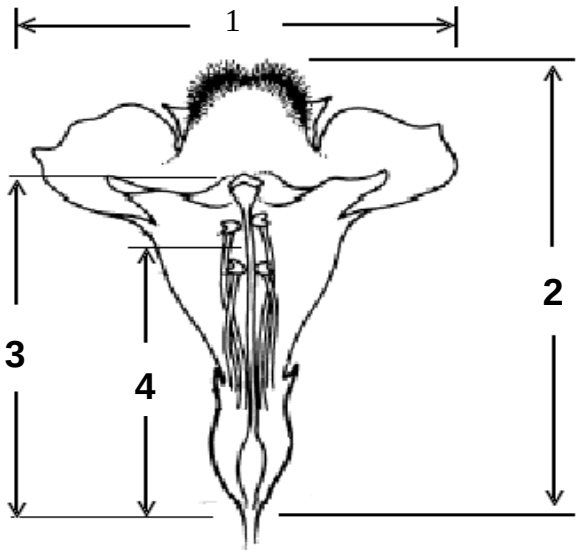

Figure 4-2. A central dissection of a *Mimulus guttatus* flower and the floral traits measured in this study. Trait 1, corolla width, shows the measure of the widest part of corolla width, trait 2, corolla length, takes the measure of the corolla tube length, trait 3 is the measure of pistil length and trait 4 is the measure of the average stamen length. Lastly, trait 5, stigma-anther separation was taken by the difference of trait 3 and trait 4.

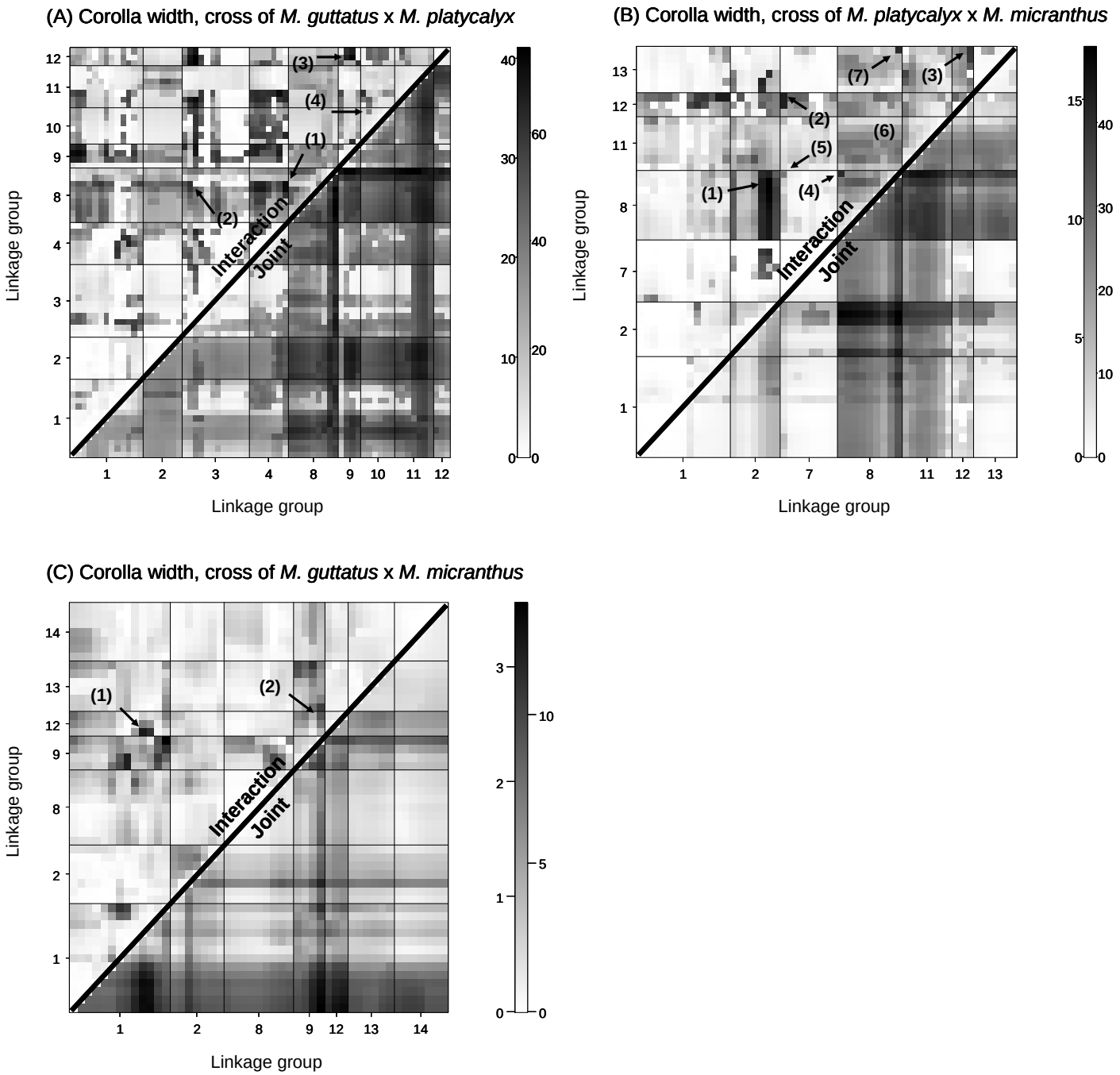

Figure 4-3. The results in pairwise genome scan for the variation of corolla width in *Mimulus* crosses. The joint and interaction LOD scores were established through 1000 permutations using R/QTL program. (A) The joint and interaction LOD scores along linkage groups of M. guttatus x *M. platycalyx* that showed significance in individual QTL genetic effect as well as the interaction between loci. (B) The joint and interaction LOD scores along linkage groups of the cross of *M. platycalyx* x *M. micranthus*. (C) The joint and interaction LOD scores along linkage group of the cross of *M. platycalyx* x *M. micranthus*. The numbers showed in the figures correspond to the genetic interaction terms in Table 4-4. The vertical bars on the left are the LOD scores for interaction LOD (left side) and joint LOD (left side).

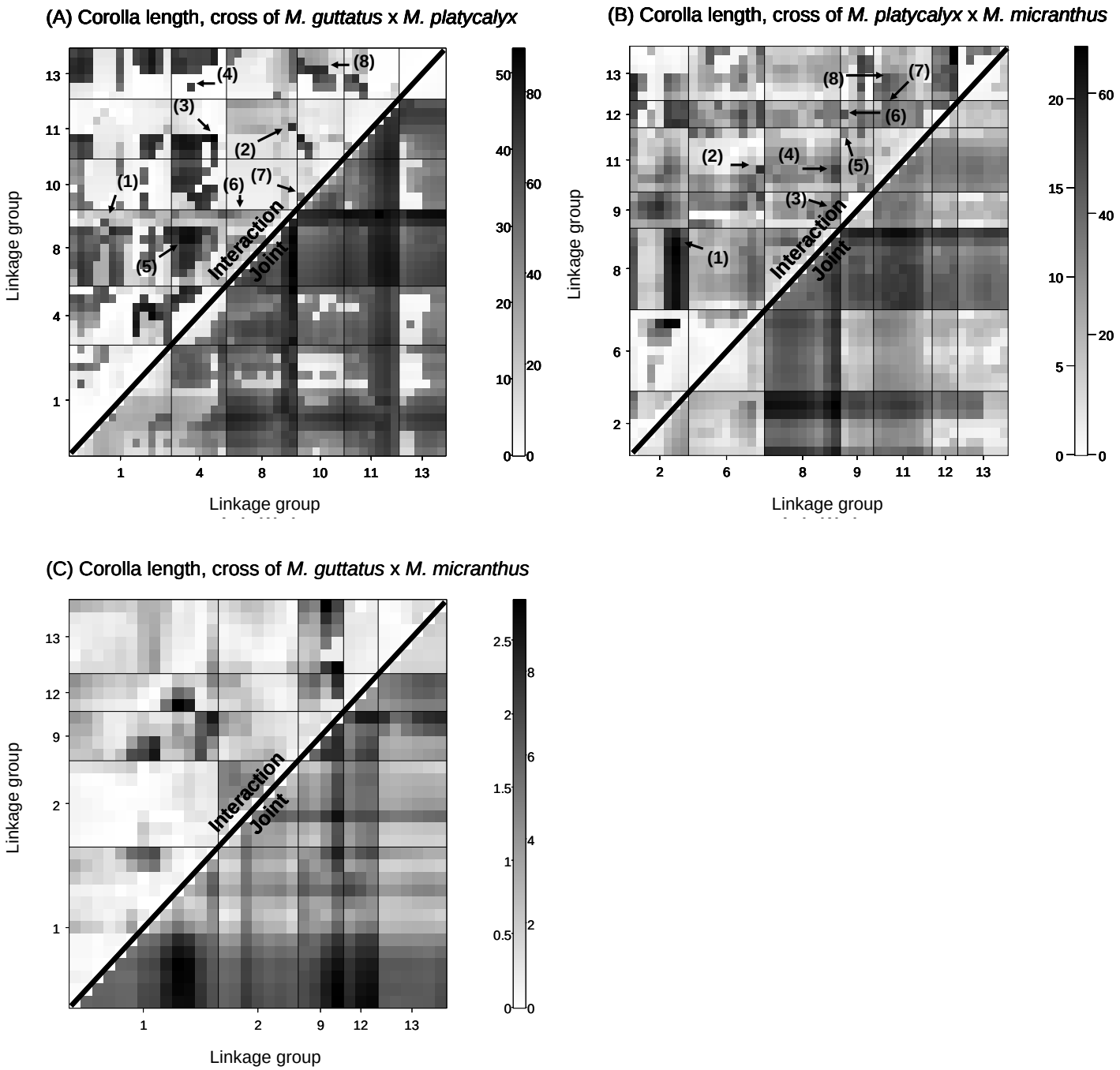

Figure 4-4. The results in pairwise genome scan for the variation of corolla length in *Mimulus* crosses. The joint and interaction LOD scores were established through 1000 permutations using R/QTL program. (A) The joint and interaction LOD scores along linkage groups of *M. guttatus* x *M. platycalyx* that showed significance in individual QTL genetic effect as well as the interaction between loci. (B) The joint and interaction LOD scores along linkage groups of the cross of *M. platycalyx* x *M. micranthus*. (C) The joint and interaction LOD scores along linkage group of the cross of *M. platycalyx* x *M. micranthus*. The numbers showed in the figures correspond to the genetic interaction terms in Table 4-4. The vertical bars on the left are the LOD scores for interaction LOD (left side) and joint LOD (left side).

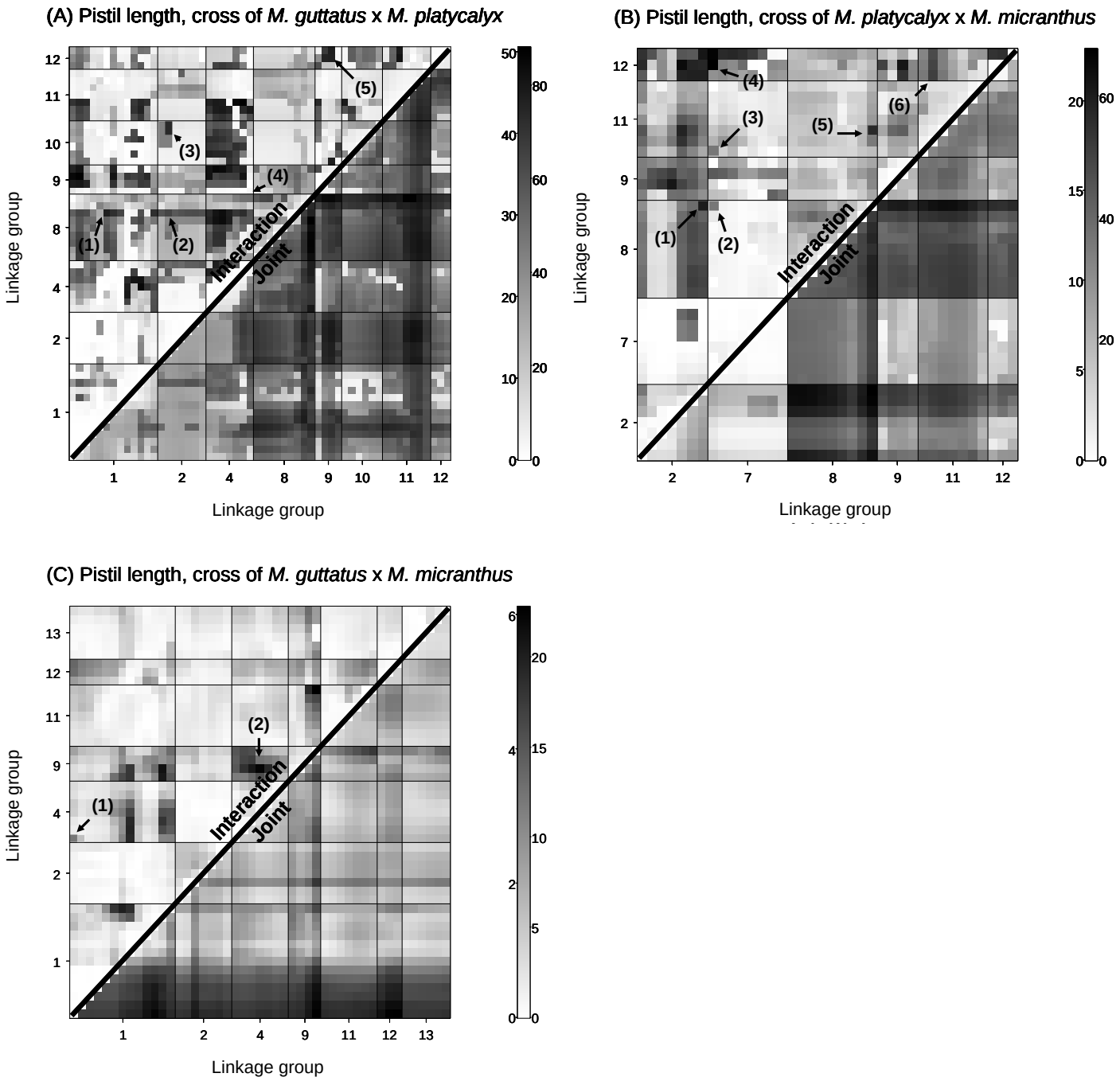

Figure 4-5. The results in pairwise genome scan for the variation of pistil length in *Mimulus* crosses. The joint and interaction LOD scores were established through 1000 permutations using R/QTL program. (A) The joint and interaction LOD scores along linkage groups of *M. guttatus* x *M. platycalyx* that showed significance in individual QTL genetic effect as well as the interaction between loci. (B) The joint and interaction LOD scores along linkage groups of the cross of *M. platycalyx* x *M. micranthus*. (C) The joint and interaction LOD scores along linkage group of the cross of *M. platycalyx* x *M. micranthus*. The numbers showed in the figures correspond to the genetic interaction terms in Table 4-4. The vertical bars on the left are the LOD scores for interaction LOD (left side) and joint LOD (left side).

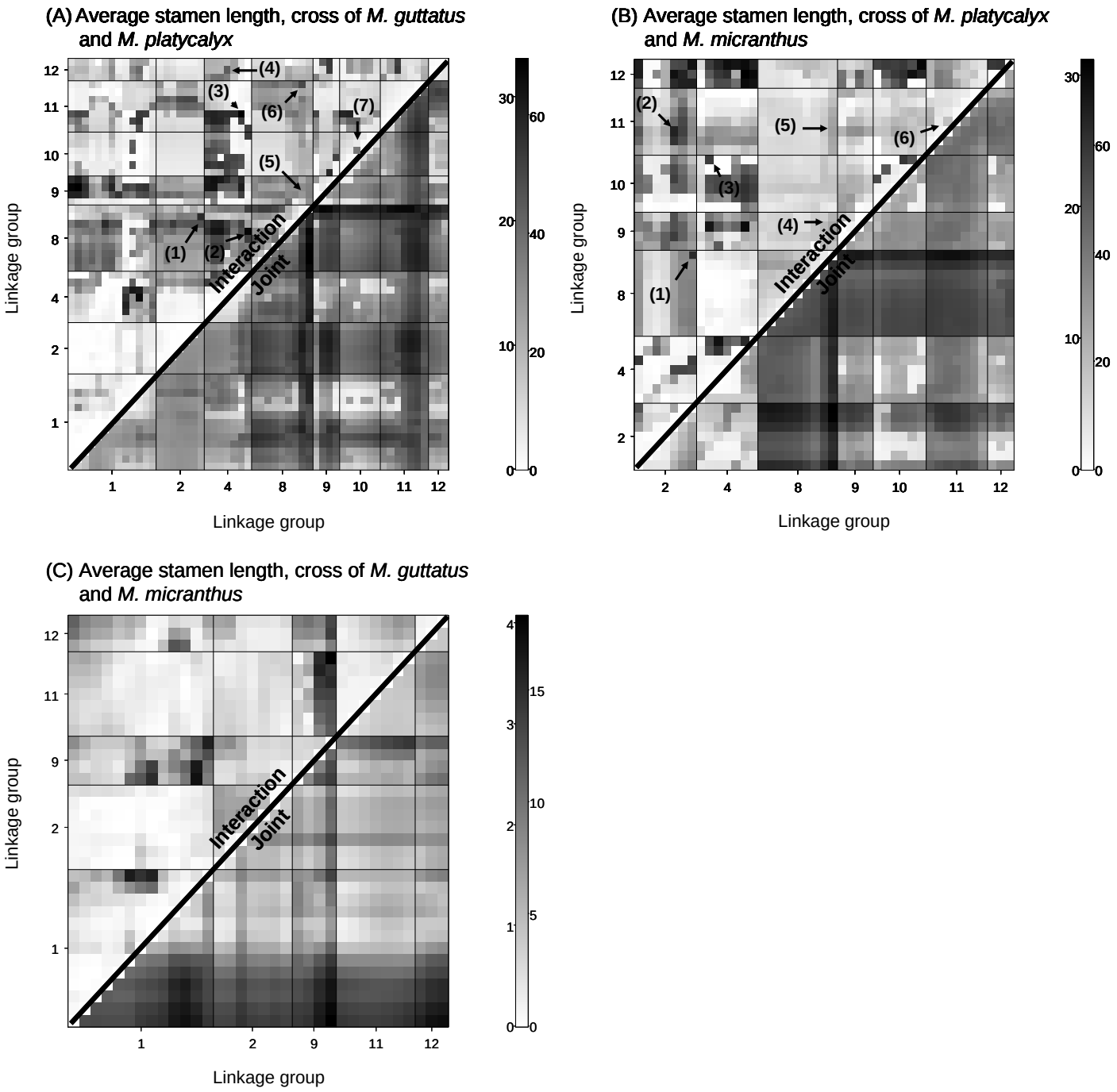

Figure 4-6. The results in pairwise genome scan for the variation of average stamen length in *Mimulus* crosses. The joint and interaction LOD scores were established through 1000 permutations using R/QTL program. (A) The joint and interaction LOD scores along linkage groups of *M. guttatus* x *M. platycalyx* that showed significance in individual QTL genetic effect as well as the interaction between loci. (B) The joint and interaction LOD scores along linkage groups of the cross of *M. platycalyx* x *M. micranthus*. (C) The joint and interaction LOD scores along linkage group of the cross of *M. platycalyx* x *M. micranthus*. The numbers showed in the figures correspond to the genetic interaction terms in Table 4-4. The vertical bars on the left are the LOD scores for interaction LOD (left side) and joint LOD (left side).

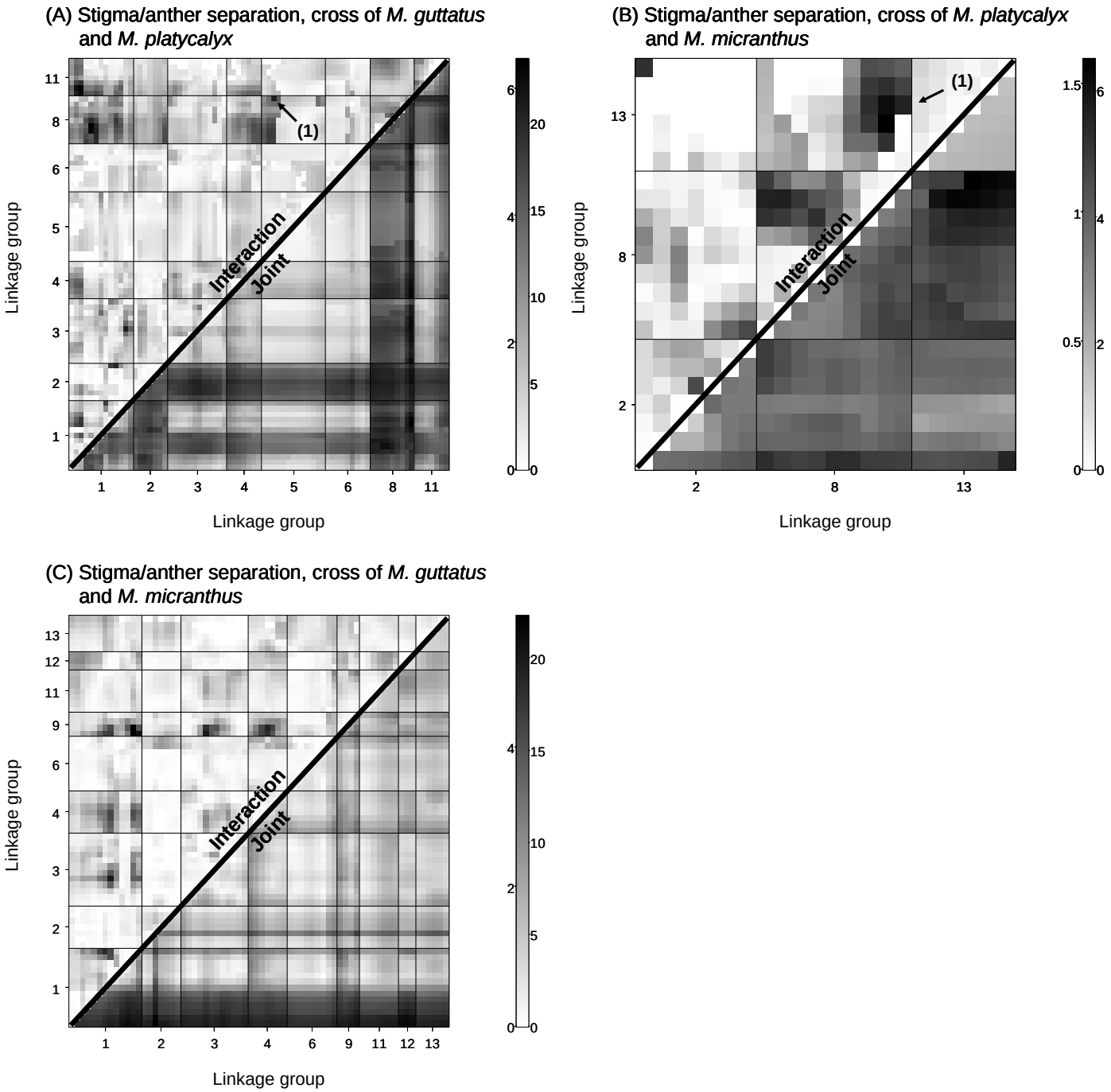

Figure 4-7. The results in pairwise genome scan for the variation of stigma-anther separation in *Mimulus* crosses. The joint and interaction LOD scores were established through 1000 permutations using R/QTL program. (A) The joint and interaction LOD scores along linkage groups of *M. guttatus* x *M. platycalyx* that showed significance in individual QTL genetic effect as well as the interaction between loci. (B) The joint and interaction LOD scores along linkage groups of the cross of *M. platycalyx* x *M. micranthus*. (C) The joint and interaction LOD scores along linkage group of the cross of *M. platycalyx* x *M. micranthus*. The numbers showed in the figures correspond to the genetic interaction terms in Table 4-4. The vertical bars on the left are the LOD scores for interaction LOD (left side) and joint LOD (left side).

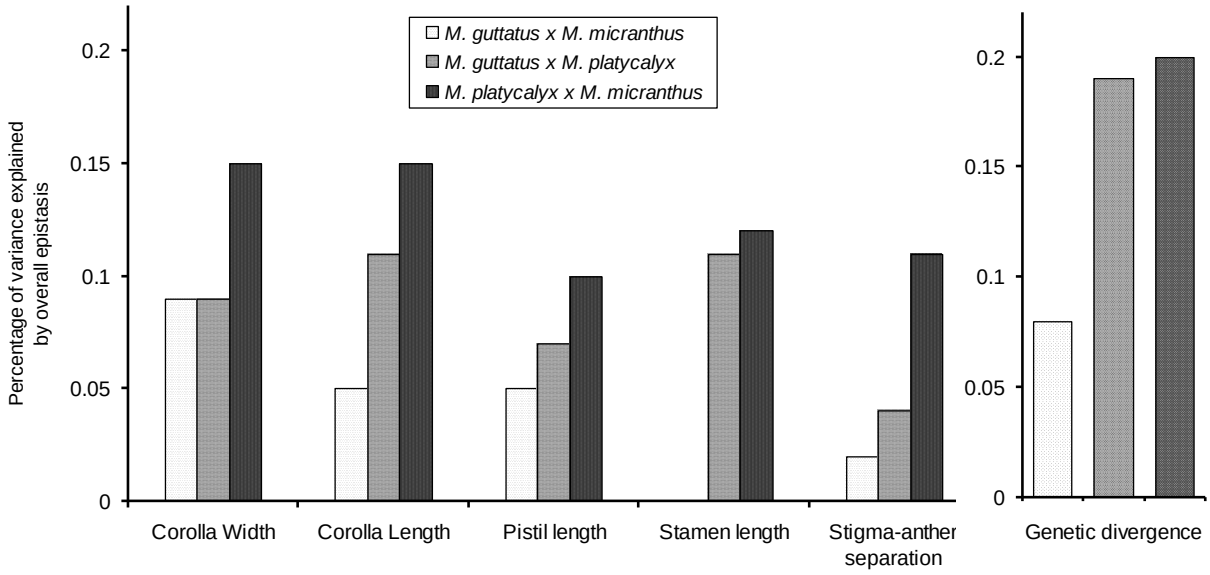

Figure 4-8. Epistasis detected in the *Mimulus* crosses, and the degree of genetic divergence between species as estimated with AFLP markers. The percentage of variance explained by overall epistasis was calculated by fitting QTL with interaction terms in a two way ANOVA model, in which all the interacting pairs detected in pairwise genome scans are included.

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CHAPTER 5. GENERAL CONCLUSION

The match between the organisms and the world they live, known as adaptation, has been the center of research interest to all evolutionary biologists (Orr 2005). The genetic study of adaptation began nearly a century ago (Risch 2000; Barton and Keightley 2002). Even before the structure of DNA was discovered, the adaptational process was seen as a movement of a population towards the phenotype that best fits the present environment (Fisher 1930).

With the development of advanced genotyping technologies and statistical methods, quantitative trait locus (QTL) mapping has become a powerful and prevailing tool to characterize the genetic architecture of adaptation (Mackay 2001). However, as stressed by (Orr 2001), results from QTL mapping studies vary greatly, the estimated number of genes ranging from few to many and the magnitude of genetic effect ranging from minor to large. There seems to be no obvious rules that would allow us to make a prediction about the genetic basis of a given trait between taxa (Barton and Keightley 2002). Such inconsistency of QTL numbers and the size of genetic effect raise the questions about the degree of genetic divergence between taxa, the strength of selection and the nature of standing variation. To further investigate these fundamental questions that surround genetic adaptation, this thesis is aimed to extend the classical QTL mapping approach to include the context of species evolutionary history, upon which I overly changes of QTL through evolutionary time among species lineages.

Using the traditional biometric method where the effective number of genetic factors are estimated among *Mimulus guttatus* species complex, in this thesis I first

identified a wide range of effective number of genetic factors that distinguish related taxa; the number ranges from as little as 0.45 to as many as 13.96. Although the biometrical comparison was made with the some tenuous assumptions of additivity and uniformity of gene action, these findings only suggest the minimal mutational steps that allow populations to reach more distant fitness peaks.

My results from both of the biometric method and the classical QTL mapping approach did not find the evidence supporting the correlation of gene numbers and species evolutionary divergence (Chapter 2 and Chapter 4). The divergence of traits driven by local adaptation could generate a covariance of alleles at the underlying QTL due to the response of all QTLs to selection. Also, the estimated numbers of QTL (or effective genetic factor) is undoubtedly smaller than the actual number, as QTL of smaller effects cannot be detected unless sample size is greatly increased. This would blur the correlation between gene number and evolutionary distance.

The degree of dominance is known to play an important role in the evolution of mating system, especially in increasing the probability and the rate of evolution for selfing (Haldane 1927). With the availability of multiple species comparisons, directional partial dominance was found prevailing towards inbreeding characters, suggesting the accelerated rate of evolution of self fertilization from cross fertilization (Chapter 2; Fenster and Ritland 1994). More interestingly, the epistasis estimated between *M. guttatus* and *M. micranthus* was, in general, the least in comparison with other two intra-specific crosses (Chapter 4). This result suggests the evolution of selfing from outcrossing in *M. micranthus* was mainly governed by QTL with additive and dominant effects, with little epistasis.

Epistasis based on QTL evidence was considerable in progeny of *Mimulus* inter-specific crosses; and notably, the percentage of variance explained by the epistasis term can exceed the amount explained by QTL loci individually. Not only was epistasis found between QTL loci identified, but also epistatic effects were uncovered between chromosome regions that showed no QTL individually (Chapter 4). These results indicate that floral traits are a co-adapted genetic complex where major functional genes as well as regulatory modifiers collectively interact. Epistasis in such a genetic complex may be a key element in population genetic differentiation and speciation, as recombination of unrelated alleles can lead to decreased fitness of hybrids, promoting genetic isolation. Moreover, epistasis was greater in crosses involving *M. platycalyx* (Chapter 4), and since this lineage is about twice as distant as the other two as estimated from AFLP markers (Chapter 3), this suggests that epistatic variance increases with evolutionary distance.

Despite the strength of QTL analysis in providing fundamental information about numbers, locations, size of QTL as well as interactions between QTLs, many important questions remain unanswered (Orr 2001). These include: does adaptation mostly involve new mutations or standing genetic variation; does the adaptation process start with mutations of small effect (Fisher 1930) or mutations with of large effect (Gillespie 1984); can the distribution of phenotypic effects of beneficial mutations involved in the adaptation process be described; do the more complicated traits take more mutations to change? To gauge these evolutionary scenarios of QTLs, a comparative method that is capable of integrating the genetic changes on organism evolutionary history is required.

Studies involving comparative genomics might be a better method to infer adaptational processes, first through the identification of functional conserved DNA sequences/genes across a range of related taxa (Kellis et al. 2003; Eddy 2005). The core principle for identifying such conserved sequences and domains is that selection has constrained variation of the nucleotides in functionally important sequences relative to those sequences that are presumed to be non-functional (Boffelli et al. 2004). Using the rice (*Oryza sativa* sp. *japonica*) genome annotation, with genomic sequences and clustered transcript assemblies from other 184 plant species, 861 rice genes were identified that are evolutionarily conserved among six diverse species from Poaceae (Campbell et al. 2007). These findings can however only exhibit the presence of significant sequence similarity across the three separate Poaceae subfamilies, and the majority of conserved-Poaceae-specific sequences (86.6%) are found encoded with no putative function or functionally characterized protein domain. As a result, the functional connection between species genetic adaptation and genetic variation is missing in these comparisons.

In this thesis, rather than inferring the genetic architecture of species divergence with the classical QTL mapping method, I went beyond this with a novel analysis for QTL, “lineage specific QTL mapping" (Chapter 3). This approach is a cross-fertilization between QTL mapping and phylogenetic analysis. The analysis of lineage specific QTL effect provides the appropriate evolutionary framework upon which the more incisive questions about genetic adaptation process can be tested. At the simplest, by adding just one additional taxon into the classical pairwise mapping routine, one can infer the QTL changes occurred along each of the lineages, specifically from the point of the most recent common ancestor to the end point of the branch. After partitioning the QTL

genetic effect on all phylogenetic branches, we can determine if QTL effects are homologous (arising in an ancestral lineage leading to two data) or arose independently in derived lineages.

To illustrate the strength of the analysis of lineage specific QTL effect, I examined the evolutionary genetic basis that underlies variation of mating system in *M. guttatus* species complex (Chapter 3). For the evolution of inbreeding in two closely related *M. platycalyx* and *M. micranthus*, it appears that non-homologous major QTLs are predominant, while independently derived QTLs "fine tune" the trait. Second, selection was evident, as the directionality of QTL genetic effects as identified from lineage specific QTL mapping was consistent with the selective maintenance of intermediate outcrossing rate. Finally, I speculate that non-homologous QTLs, e.g., those being seen commonly between *M. platycalyx* and *M. micranthus* arisen via convergent evolution, are of larger effect as compared to those that occur later in derived lineages. This as well accords with theoretical expectations, as initial evolution towards selfing is more likely to occur with few loci, because associations more easily allow the development of associations between loci affecting inbreeding depression and loci controlling selfing (Holsinger 1991; Uyenoyama and Waller 1991); the evolution of selfing is more closely coupled to the loss of inbreeding depression .

To conclude, results from this thesis showed that the genetic basis for quantitative variation of mating system in the *M. guttatus* species complex is complex. The genetic architecture involved in the species adaptation process reflects a mixture of factors including the epistatic interaction between genetic loci, strength of selection, the nature of the standing genetic variation, and evolutionary separation between taxa. Although there

is a healthy body of theoretical work about adaptation, an analytical and testable framework for empirical research is still needed (Orr 2005).

Finally, this thesis has developed the technique of detecting QTL in species lineages, which enables the distinction homology and homoplasy of QTL, and greater resolution of the pathway of QTL evolution. The challenge has now just begun. Aided by the revolution in genomic technology, we can hope that there will be much more progress towards understanding the genomic nature of QTL, though activities such as cloning of QTL, identification of the allelic variation at specific QTL and its association with gene-expression differences, and identification of the regulatory and structural changes involved with multiple allelic substitutions. With this thesis, I, hereby, once again emphasize the importance and the informativeness of phylogenetic comparisons in genomics.

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