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SPECIAL ISSUE: THE MOLECULAR MECHANISMS OF ADAPTATION AND SPECIATION: INTEGRATING GENOMIC AND MOLECULAR APPROACHES

The genetic architecture of local adaptation and reproductive isolation in sympatry within the *Mimulus guttatus* species complex

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Abstract

The genetic architecture of local adaptation has been of central interest to evolutionary biologists since the modern synthesis. In addition to classic theory on the effect size of adaptive mutations by Fisher, Kimura and Orr, recent theory addresses the genetic architecture of local adaptation in the face of ongoing gene flow. This theory predicts that with substantial gene flow between populations local adaptation should proceed primarily through mutations of large effect or tightly linked clusters of smaller effect loci. In this study, we investigate the genetic architecture of divergence in flowering time, mating system-related traits, and leaf shape between *Mimulus laciniatus* and a sympatric population of its close relative *M. guttatus*. These three traits are probably involved in *M. laciniatus*' adaptation to a dry, exposed granite outcrop environment. Flowering time and mating system differences are also reproductive isolating barriers making them 'magic traits'. Phenotypic hybrids in this population provide evidence of recent gene flow. Using next-generation sequencing, we generate dense SNP markers across the genome and map quantitative trait loci (QTLs) involved in flowering time, flower size and leaf shape. We find that interspecific divergence in all three traits is due to few QTL of large effect including a highly pleiotropic QTL on chromosome 8. This QTL region contains the pleiotropic candidate gene TCP4 and is involved in ecologically important phenotypes in other *Mimulus* species. Our results are consistent with theory, indicating that local adaptation and reproductive isolation with gene flow should be due to few loci with large and pleiotropic effects.

Keywords: flowering time, gene flow, genetic architecture, local adaptation, mating system, *Mimulus guttatus* species complex

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Introduction

The genetic architecture of adaptation has long been of central interest to evolutionary biologists. One of the earliest theoretical treatments of the effect size distribution of adaptive mutations, R.A. Fisher's geometric model (Fisher 1930), proposed that adaptation took place through fixation of many alleles of infinitely small and additive effect and allowed little room for large-effect changes. Fisher's model reigned supreme for 53 years (Robertson 1967) until modified by Kimura (1983) with the addition of mutations of moderate effect, and then further altered 15 years later with Orr's (1998a) adaptive walk. Orr's model, which predicts an exponential distribution of effect sizes with large-effect mutations occurring early in an adaptive walk and

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effect size decreasing as the phenotype moves closer to the optimum, has been accepted as a biologically realistic one (reviewed in Dittmar *et al.* 2016). This conclusion is based on numerous quantitative trait locus (QTL) mapping and candidate gene studies of adaptive phenotypes that have found mutations of both large and small effects (Bradshaw *et al.* 1998; Frary *et al.* 2000; Nachman *et al.* 2003; Colosimo *et al.* 2005; Frankel *et al.* 2012; Hung *et al.* 2012; Kronforst & Papa 2015) .

While the above models have provided a solid theoretical foundation for the investigation of the genetic basis of adaptation, none of them consider the effects of gene flow. As a strong homogenizing force, gene flow is especially important when examining the genetic architecture of local adaptation. Local adaptation occurs among closely related populations occupying different habitats. These populations are often in close geographic proximity to each other and are therefore probably still exchanging genes. Recent theory by Yeaman & Whitlock (2011) predicts that local adaptation in the presence of gene flow will be due to primarily large-effect mutations or groups of tightly linked small-effect loci. Their model, based upon work by Yeaman & Otto (2011), finds that a large-effect mutation has a larger selection coefficient and is therefore more likely to be fixed by natural selection despite the homogenizing effects of gene flow. This theory is complimentary to the inversion literature wherein chromosomal inversions that capture multiple locally adaptive alleles possess a large selective advantage (Dobzhansky 1970; Balanyà *et al.* 2003; Etges & Levitan 2004; Kirkpatrick & Barton 2006; Lowry & Willis 2010) . An inversion that captures several locally adaptive alleles acts as a single large-effect locus and will be better detected by selection in the face of significant gene flow.

Pleiotropy, when a single mutation affects multiple traits, also influences the genetic architecture of adaptation. One of the reasons Fisher hypothesized that adaptation would be due to many small-affect mutations is that larger effect mutations were more likely to have deleterious pleiotropic effects and been selected against (reviewed in Dittmar *et al.* 2016). There is now empirical evidence demonstrating that larger effect mutations are more pleiotropic (Albert *et al.* 2008; Wagner *et al.* 2008; Wang *et al.* 2010), and recent theory predicts that many mutations of small effect will predominate when adaptation involves selection on many phenotypes (Tenaillon 2014). However, synergistic pleiotropy, where all effects of a mutation are in an advantageous direction, should facilitate adaptation (Wang *et al.* 2010) and there are empirical examples of beneficially pleiotropic loci contributing to stickleback (Mills *et al.* 2014), floral (Smith 2016) and viral adaptation (McGee *et al.* 2016). Beneficial pleiotropy should be particularly effective at circumventing fitness valleys when organisms are moving through a multimodal fitness landscape. Synergistically pleiotropic loci, much like inversions, should be especially advantageous for the evolution of local adaptation with gene flow.

In addition to influencing adaptation, a mutation's effect size and degree of pleiotropy shape the tempo and mode of speciation when it contributes to reproductive isolation. The genetic architecture of reproductive isolating barriers is especially important in the context of ongoing gene flow. When species come into secondary contact in sympatry, or diverge in the presence of gene flow, increased linkage between reproductive isolating loci is predicted (reviewed in Feder *et al.* 2012a; Via 2012). This has been discussed most extensively in the inversion literature. Inversions that link loci involved in reproductive isolation will better maintain species boundaries in the face of gene flow by suppressing recombination between those loci (Noor *et al.* 2001; Rieseberg 2001; reviewed in Hoffmann & Rieseberg 2008). By the same logic, pleiotropic or tightly physically linked loci outside inversions that affect multiple aspects of reproductive isolation should also be favoured between species exchanging genes in sympatry (Feder *et al.* 2012a,b; Via 2012). We can therefore predict that adaptation and reproductive isolation should be due to few, pleiotropic loci of large effect when the homogenizing force of gene flow is present.

In plants, local adaptation between adjacent populations occurs frequently, particularly with adaptation to different edaphic environments such as serpentine (Kruckeberg 1985, 1986; Macnair & Gardner 1998), heavy metal-contaminated mine tailings (Antonovics & Bradshaw 1970; Antonovics *et al.* 1971; Macnair 1983; Wright *et al.* 2013), limestone (Raabová *et al.* 2007; reviewed in Baskin & Baskin 1988, Rajakaruna 2004) and granite outcrops (Burbanck & Platt 1964; Wyatt & Fowler 1977; Burgman 1987; Peterson *et al.* 2013). These populations may also become reproductively isolated either through ecological divergence (Martin & Willis 2007; Lowry *et al.* 2008a,b) or hitchhiking of intrinsic postzygotic incompatibilities (Wright *et al.* 2013). Despite being locally adapted to different soil types, geographic proximity facilitates gene exchange between these neighbouring plant populations. The *Mimulus guttatus* species complex, a closely related group of wildflowers, is an excellent system with which to investigate the genetic architecture of local adaptation and reproductive isolation with gene flow. Species in the complex occupy a myriad of disparate habitats such as old copper mine tailings, serpentine soils, coastal dunes, moist seeps and granite outcrops, and often co-occur within metres of each other (Wu *et al.* 2008). In addition, members of the species complex are interfertile and ongoing

introgression has been documented between several taxa (Sweigart & Willis 2003; Brandvain *et al.* 2014).

Mimulus laciniatus is a small self-fertilizing annual member of the complex that occurs in dry, exposed granite outcrop habitat in the Sierra Nevada, CA. The closely related *M. guttatus* grows in moist streams and seeps from Mexico to Alaska and Colorado to the Pacific Ocean. In the region of range overlap between these species, *M. guttatus* is often found in mesic habitat adjacent to *M. laciniatus's* rocky outcrops. Granite outcrops are harsh environments (Burbanck & Platt 1964). They are drier, more light intensive and more extreme in temperature than the seep habitat occupied by *M. guttatus* (Peterson *et al.* 2013; Ferris *et al.* 2014; K. Ferris, unpublished data). The two species are locally adapted to their different microhabitats (Peterson *et al.* 2013; K. Ferris unpublished data), but naturally occurring hybrids between *M. guttatus* and *M. laciniatus* have been reported by multiple investigators in sympatric populations since the 1960s (Vickery 1964; K. Ferris personal observation). In addition, there is preliminary genetic evidence of introgression between the species from maximum-likelihood gene trees created using populations of *M. laciniatus*, *M. guttatus* and *M. nasutus* from across each species' range (Ferris *et al.* 2014). At several loci, a *M. laciniatus* population clusters with *M. guttatus* populations rather than with other *M. laciniatus*, which is suggestive of recent gene flow.

Across their respective geographic ranges, the two species diverge in several phenotypes commonly involved in plant local adaptation: flowering time (Kiang & Hamrick 1978; Stinchcombe *et al.* 2004; Hall & Willis 2006; Franks *et al.* 2007; Lowry *et al.* 2008b; Willis *et al.* 2008; Anderson *et al.* 2012), mating system (Jarne & Charlesworth 1993; Fishman & Willis 2008) and leaf shape (Wyatt & Antonovics 1981; Hopkins *et al.* 2008; Campitelli & Stinchcombe 2013). *Mimulus laciniatus* flowers earlier than both sympatric and allopatric *M. guttatus* populations in the field and in the greenhouse (Peterson *et al.* 2013; Friedman & Willis 2013; K. Ferris, personal observation). Flowering time is a classic adaptation to dry habitats as early flowering allows plants to reproduce before the onset of seasonal drought (Fox 1989; Dudley 1996; Hall & Willis 2006; Franks *et al.* 2007; Willis *et al.* 2008; Anderson *et al.* 2012). A reciprocal transplant study found that *M. laciniatus* had higher fitness in its granite outcrop habitat than *M. guttatus* largely because of the ability to flower early before the severe summer drought (Peterson *et al.* 2013). *Mimulus laciniatus* has small flowers and a highly self-fertilizing mating system (Ferris *et al.* 2014) whereas *M. guttatus* is large flowered and highly outcrossing (Ritland & Ritland 1989; Willis 1993). Self-fertilizing taxa tend to occupy dry, marginal habitats (Stebbins 1957). This could be due to increased colonization ability and reproductive assurance (Wyatt 1986; Jarne & Charlesworth 1993), or because self-fertilizing taxa tend to have small flowers which lose less water than the large flowers necessary for attracting pollinators (Galen *et al.* 1999; Galen 2000; Carroll *et al.* 2001). In addition, *M. laciniatus* has a highly lobed leaf shape, while *M. guttatus* has a rounded entire leaf. Lobed leaves should be adaptive in exposed, dry habitats because of their thinner boundary layer and reduced hydraulic resistance in comparison with round, entire leaves (reviewed in Nicotra *et al.* 2011). There is an association between occupation of dry rocky habitat and lobed leaf shape within the *M. guttatus* species complex (Ferris *et al.* 2014, 2015).

In addition to being locally adaptive, flowering time and mating system divergence contribute to prezygotic reproductive isolation (Coyne & Orr 2004). Within the *M. guttatus* species complex flowering time can provide up to 90% reproductive isolation between close relatives, while mating system has been demonstrated to be an even stronger barrier producing almost complete isolation (Martin & Willis 2007). Their involvement in both adaptation and reproductive isolation in the species complex make flowering time and mating system examples of 'magic traits' (Servedio *et al.* 2011). Based on the repeated observation of hybrids across years in multiple sympatric populations (Vickery 1964; K. Ferris, personal observation) and preliminary genetic evidence of introgression (Ferris *et al.* 2014), *M. laciniatus* and *M. guttatus* seem to be incompletely reproductively isolated. Therefore, prezygotic isolating barriers such as flowering time and mating system-associated traits are of primary importance in sympatric populations of these species (Martin & Willis 2007) as strong postzygotic barriers may not have had time to fix between them (Lowry *et al.* 2008a).

To investigate the genetic architecture of local adaptation and reproductive isolation in a system likely experiencing gene flow, we dissected the genetic basis of flowering time, mating system, and leaf shape differences between sympatric populations of *M. laciniatus* and *M. guttatus* in Shaver Lake, CA. Parental lines derived from each population were crossed in a common greenhouse environment to create an F_2 population, and a genotyping-by-sequencing QTL mapping approach was used to map loci involved in phenotypic differentiation (Andolfatto *et al.* 2011). At the Shaver Lake locality, *M. guttatus* and *M. laciniatus* occur within a metre of each other, but occupy distinct microhabitats: granite outcrop vs. grassy seep (Fig. 1). Advanced generation phenotypic hybrids have been observed in this population over multiple years (K. Ferris, personal observation), indicating the existence of interspecific

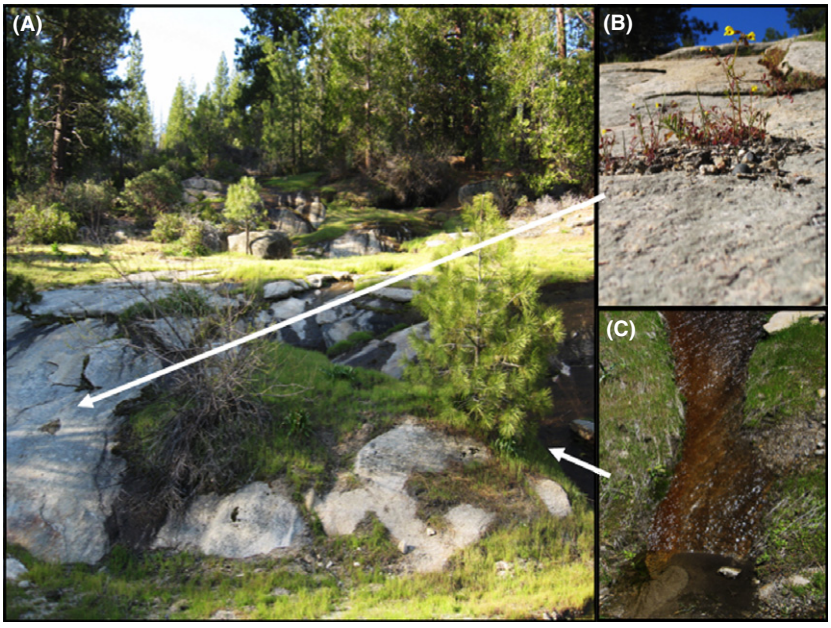

Fig. 1 Image of the Shaver Lake locality (A) with sympatric *M. laciniatus* (B) and *M. guttatus* (C) populations growing within a metre of each other.

gene flow. We hypothesize that these hybrids are advanced generation rather than F_1 or F_2 because they are large *M. guttatus*-like plants with large flowers, but with a distinctive *M. laciniatus*-like lobed leaf (K. Ferris, personal observation). The phenotypic hybrids usually occur in intermediate habitat between the *M. guttatus* and *M. laciniatus* populations. Future genetic studies will be necessary to confirm the hybrid status of these individuals, and whether the clustering of *M. laciniatus* sequences with *M. guttatus* clades in Ferris *et al.* (2014) is due to gene flow or incomplete lineage sorting. This study empirically tests the theoretical prediction that few, large-effect loci underlie local adaptation (Yeaman & Whitlock 2011) and reproductive isolation (Feder *et al.* 2012a) in the presence of gene flow.

Materials and methods

Construction of the mapping population and phenotypic analyses

To examine the genetic architecture of ecologically relevant differences between *Mimulus guttatus* and *Mimulus laciniatus* in a common environment, we created an F_2 mapping population between *M. guttatus* (SHG) and *M. laciniatus* (SHL) inbred lines. Seeds or live plants were collected from 20 wild individuals from each species at the Shaver Lake location (N 37.08.682, W 119.18.388, 5321 ft., Sierra National Forest, CA) and shipped back to Durham, North Carolina. Seeds from wild-collected SHL8 and SHG16 plants were grown and self-fertilized in the Duke University greenhouse for four generations before being reciprocally cross-pollinated. We did not observe signs of inbreeding depression such as much reduced viability or fertility in SHG16 after four generations of inbreeding. Although we did not measure the wild population phenotypic mean for these traits, the SHG16 line closely resembled the other 15 inbred lines generated from the Shaver Lake population. First-generation hybrids (F_1's) were grown in the greenhouse, and a single F_1 with SHL8 as the maternal parent was self-fertilized to produce a recombinant F_2 population. While all F1's were phenotypically similar, we chose this particular F_1 individual to create the F2 population because it was close to the F1 population phenotypic mean.

One thousand F_2, 67 SHL8 and 133 SHG16 individuals were grown in 2.5-inch pots in Fafard 4P potting soil in the Duke University Greenhouse and phenotyped for leaf shape, flower size, node of first flower and flowering time in February of 2012. F_2 and parental line seeds were cold stratified at 4 °C for 10 days and then germinated in the greenhouse on benches that were flooded twice a day under 18-h days (21 °C day/18 °C night). Plants were thinned following germination to a single seedling per pot. Flowering time was measured as the number of days between placement in the greenhouse postcold stratification and the day of first flower. To further explore divergence in developmental progress at flowering, the vegetative node containing the first flower was also recorded. Using a small metal ruler, flower size was measured in two dimensions – corolla width and lower corolla length (see Fishman *et al.* 2002 for diagram) – to the nearest 100th of an inch on the first flower of each plant. Leaf shape was also measured on the day of first flower by taping the second true leaf

of each plant to sheets of white paper and digitally scanning them. Leaf shape was quantified by performing a convex hull analysis of each leaf image in IMAGEJ v2 (Schneider *et al.* 2012) as described in previous work (Ferris *et al.* 2015). Briefly, the convex hull analysis consists of comparing the area of each leaf's convex hull (the shape created by connecting the outermost points of a leaf) to the leaf's true area and dividing this difference in area by the convex hull area to control for size. Approximately 0.01 g of fresh leaf and bud tissue was collected from each F_2 in the mapping population and frozen at −80 °C for DNA extraction. Broad-sense heritability was calculated for each trait using the formula $H^2 = V_G/V_P$. V_G was determined by subtracting the average variance in the parental lines (V_E) from the variance in the F_2 (V_P; Falconer & MacKay 1996). Phenotypic correlations among traits were estimated using the restricted maximum-likelihood method in JMP v10 (SAS, Cary, NC, USA).

QTL mapping approach

To map QTL contributing to interspecific divergence in the ecologically relevant traits of flowering time, leaf shape and flower size, we used the multiplexed shotgun genotyping (MSG) approach (Andolfatto *et al.* 2011) combined with a novel bioinformatic pipeline. DNA was extracted from each F_2 in our SHL8 × SHG16 mapping population using a modified CTAB protocol (Kelly & Willis 1998). The DNA concentration of each F_2 sample was quantified using Quant-IT picogreen and a microplate reader. Fifty nanograms (ng) of DNA from each of 424 SHL8 × SHG16 F_2s randomly chosen from our larger grow out and from eight SHL8 individuals was digested with the restriction enzyme MseI for 3 h at 37 °C. Subsequently, nine sets of 48 barcoded adaptors were ligated onto the ends of the digested DNA samples. We then pooled each set of 48 barcoded samples to create nine libraries. Libraries were cleaned with Agencourt® AMPure PCR Purification beads and then size selected to contain fragments between 250 and 400 bp using gel extraction. After size selection, an adaptor for sequencing containing a unique index was added to each of the nine pooled libraries by amplification with the Phusion® High Fidelity PCR kit (New England Biolabs). The indexed libraries were again bead purified, and the DNA concentration of each library was determined using a Qubit® fluorometer. Finally, the nine pooled libraries were combined in equimolar concentration into one sample, which was submitted for two lanes of 50-bp single-end read sequencing on an Illumina Hiseq2000 at the Duke Sequencing and Genomic Technologies Shared Resource. Additionally, 1ug of DNA from a single *M. guttatus* parent, SHG16, underwent standard Illumina library prep and sequencing on a single lane of Illumina Hiseq2000 at the above sequencing centre.

Our sequencing efforts yielded 550 million reads. We aligned reads to the *Mimulus guttatus* reference genome (PHYTOZOME v10.2) using BOWTIE v.2.1.0 (Langmead & Salzberg 2012) and called SNPs and developed imputed haplotype maps using the GBS functions in TASSEL v.3.0 (Glaubitz *et al.* 2014). As our data exhibited a high level of missing data and a limited ability to reliably score heterozygous genotypes at a given SNP, qualities typical of MSG libraries built with frequent cutters (Andolfatto *et al.* 2011), we developed and applied a custom Perl script to rescore genotypes for fixed windows of 75 SNPs with assignable ancestry. From this point forward, we refer to each of these bins of 75 SNPs as an individual MSG marker. We created 452 markers total. Ancestry assignment was achieved by combining information from SHL parental DNA included in MSG library construction and from the 31× coverage shotgun genomic sequence of the SHG parent. If all SHL MSG reads or all SHG shotgun reads matched one of the two segregating alleles, then ancestry was assigned, and those SNPs were included in MSG marker calling.

A genetic linkage map was created in JOINMAP 4.0 (© Kyazma, Van Ooijen 2006) using regression mapping with default settings. QTLs were identified using the *scanone* function in RQTL (Broman *et al.* 2003) which performs single-QTL analysis using standard interval mapping (Lander & Botstein 1989). A genomewide significance threshold was determined separately for each phenotype at the level of $\alpha = 0.05$ with 1000 permutations. For traits where more than one significant QTL was identified, we performed multiple QTL mapping using the *stepwiseqtl* function. We determined effect sizes, additive (*a*) and dominance (d) effects, degree of dominance, peak LOD scores and 1.5-LOD score intervals for each significant QTL in the final model. QTL effect size was measured in two ways: first using the R^2 term from a one-way analysis of variance (ANOVA) in R (R Development Core Team 2008) and second by calculating the proportion of the mean parental difference that each QTL explained (Fishman *et al.* 2002). Additive (*a*) and dominance (*d*) effects were calculated from the mid-point between the homozygote genotypic values at each locus, and the degree of dominance was calculated as *d*/*a* (Conner & Hartl 2004). Peak and 1.5-LOD score intervals were determined using Rqtl (Broman *et al.* 2003). The presence of epistasis between QTLs was detected by looking for significant interaction terms in a series of pairwise factorial ANOVAs in R (R Development Core Team 2008).

Results

Species differences are genetically based and genetically simple

From the common garden experiment in the Duke University greenhouse, we see that flowering time, flower size, and leaf shape divergence between Shaver Lake *Mimulus guttatus* and *Mimulus laciniatus* are genetically based with broad-sense heritabilities ranging from moderate to high (H^2 = 0.25–0.74). The *M. guttatus* parent SHG16 flowers 2 weeks later (44.42 days, SE = 0.498), and at a later node (5.534, SE = 0.119) than the *M. laciniatus* parent SHL (31.17 days, SE = 0.95; 3.12, SE = 0.153; Fig. 2). SHG also has larger flowers than SHL in terms of both corolla width (SHG = 23.46 mm, SE = 0.4 mm; SHL = 5.26 mm, SE = 0.05 mm) and length (SHG = 28.6 mm, SE = 0.33 mm; SHL = 9.86 mm, SE = 0.06 mm; Fig. 2), and SHL (0.327, SE = 0.011) possesses more highly lobed leaves than SHG (0.128, SE = 0.007; Fig. 2). The phenotypic distributions of all five traits indicate that they are genetically simple according to the Castle–Wright effective factor estimator because the variance of the F_2 distribution is large compared to the difference between the parental means for each trait (Lynch & Walsh 1998; Figs 2 and 3). All six phenotypes were significantly and positively correlated in the F2 population (Table 1), but the strongest correlations were between flowering time and node of first flower (corr = 0.88) and between corolla width and corolla length (corr = 0.94). Lobed leaf shape was positively correlated with both flowering time and flower size (Table 1). This was unexpected given the parental distributions of these traits; for example, *M. laciniatus* has highly lobed leaves, but small flowers and early flowering time (Figs 2 and 3). Strong phenotypic correlations in the segregating population provided an initial indication that single pleiotropic or multiple tightly linked loci may underlie trait divergence between *M. laciniatus* and *M. guttatus*.

Genomewide linkage map

We created a genomewide linkage map in the Shaver Lake *M. laciniatus* and *M. guttatus* cross using a binned genotyping-by-sequencing approach. After applying our

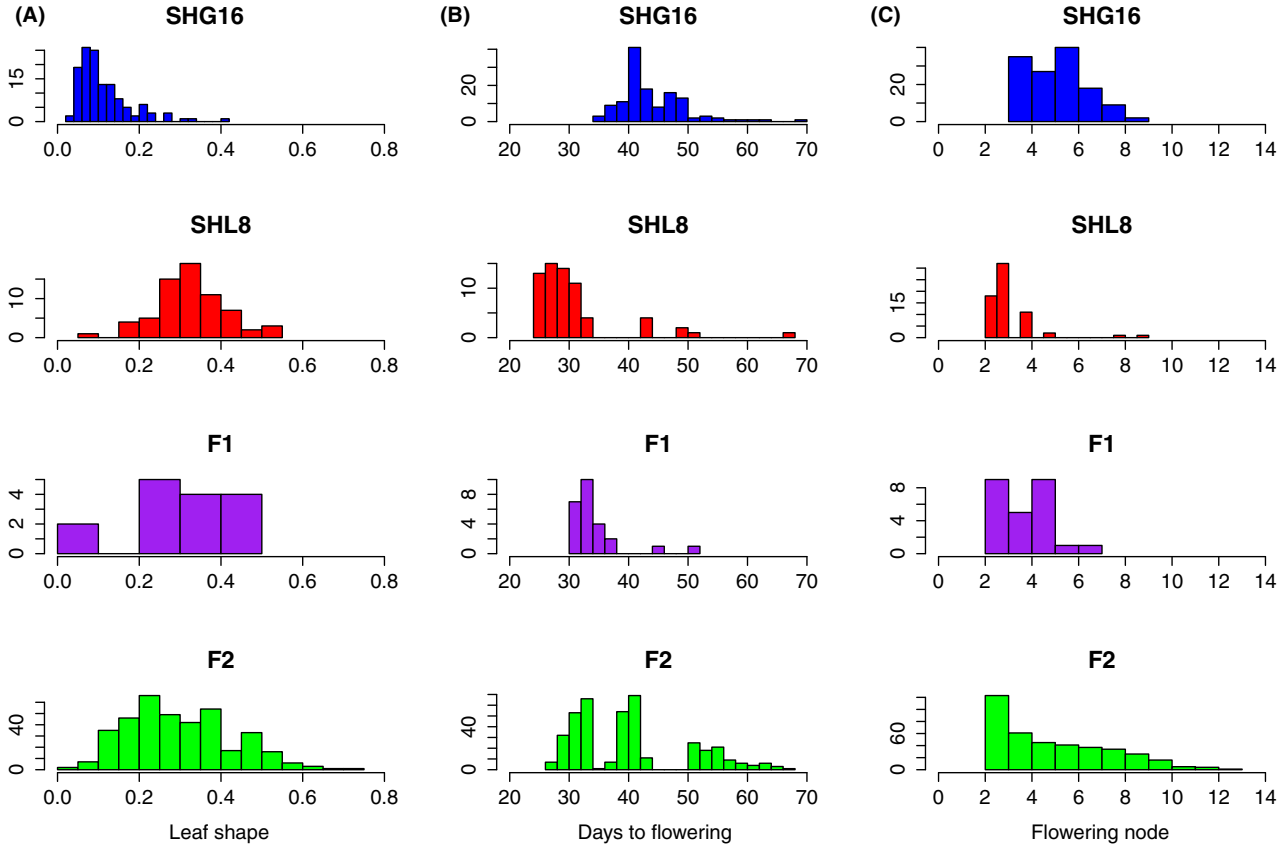

Fig. 2 Phenotypic distributions of leaf shape (A), flowering time (B), and node of first flower (C) in the *M. guttatus* SHG16 and *M. laciniatus* SHL8 parental lines, the F_1 hybrids, and F_2 mapping population.

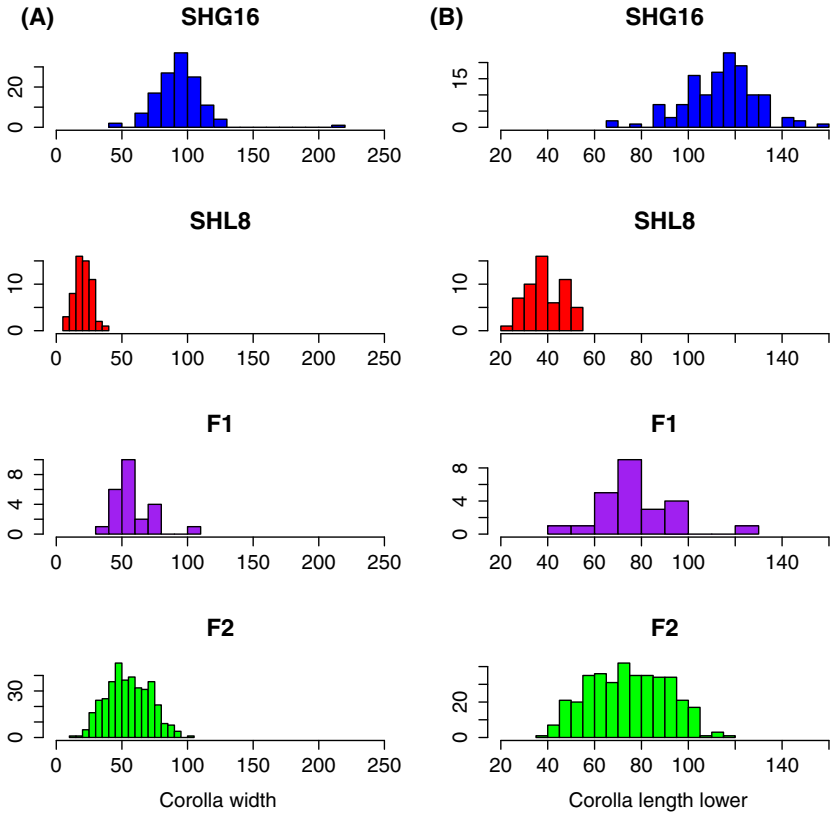

Fig. 3 Phenotypic distributions of corolla width (A) and corolla length (B) both measured in 100ths of an inch in the *M. guttatus* SHG16 and *M. laciniatus* SHL8 parental lines, the F_1 hybrids, and F_2 mapping population.

Table 1 A phenotypic correlation matrix displaying the correlations among all five morphological and life history traits across 1000 F_2 individuals in our common garden experiment. All correlations are significant at the level of $\alpha = 0.05$

	Leaf shape	Flowering time	Flower node	Corolla *W*	Corolla *L*
Leaf shape	1				
Flowering time	0.4311	1			
Flower node	0.4343	0.8846	1		
Corolla *W*	0.3516	0.6403	0.7411	1	
Corolla *L*	0.3654	0.6461	0.7411	0.9438	1

approach that incorporated shotgun genomic sequence information from the SHG parent to generate high-quality genotypes, we binned 33 318 SNPs with assignable ancestry into 75 SNP windows that were on average 0.64 ± 0.35 Mb in length, similar to the genotyping method described in Glazer *et al.* (2015). This resulted in the generation of 452 genomewide markers. After marker filtering, 422 markers were used to genotype 399 F_2 plants for map construction. The genetic map spans 1264.61 cM over 14 linkage groups that correspond to *M. guttatus's* 14 chromosomes and has an average interval length of 2.95 cM. The SHL–SHG genetic map differs from the *M. guttatus* genome assembly in several ways including large areas of marker inversion and recombination suppression on chromosomes 5, 8, 10, 11 and 14 (Fig. 4).

Common garden QTL mapping results

To examine the genetic basis of putatively adaptive phenotypic differences between Shaver Lake *M. laciniatus* and *M. guttatus*, we mapped QTLs involved in flowering time, node of first flower, flower size and leaf shape divergence in a common garden (Figs 5 and 6). Using a combination of standard interval and multiple QTL mapping, we detected five flowering, five flower size and four leaf shape QTLs that explain between 26% and 64% of the segregating variance (R^2) and 21 and 130% of the parental difference in each trait (Table 2). Two flowering (LG5 and LG7) and two leaf shape QTLs (LG5 and LG14) were only marginally significant, but we include them here as putative QTLs. Five QTLs affected more than one trait including a single large-effect locus (R^2 = 0.08–0.26) on the right arm of chromosome 8 (LG8b) that was common to all five: leaf shape, flowering time, node of first flower, corolla width and lower corolla length (Tables 2 and 3, Figs 5 and 6). There were also two significant QTLs of moderate effect, one on the left arm of chromosome 8 (LG8a, R^2 = 0.08–0.12) and another on LG10 (R^2 = 0.07–0.09),

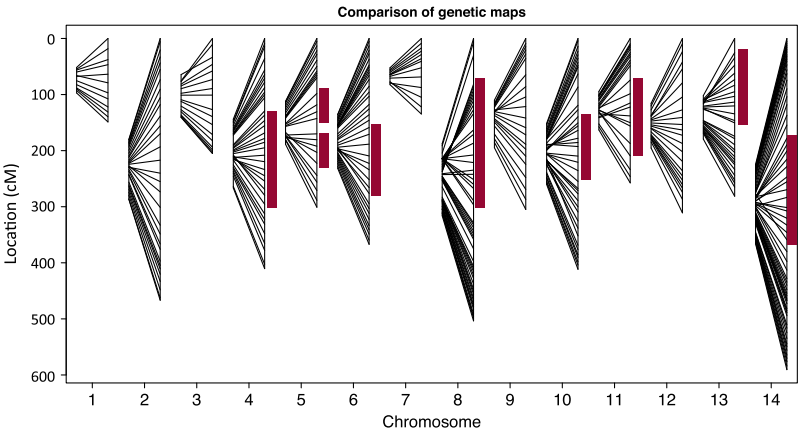

Fig. 4 Comparison of the Shaver Lake *M. laciniatus* × *M. guttatus* genetic map (left) with the physical map of *M. guttatus* line IM62 (right). These two maps share the same markers. Inverted and collapsed regions in the genetic map are highlighted with linear bars on the physical map.

that were common to all traits except leaf shape. A smaller effect, and marginally significant, QTL on LG5 was common to all traits except node of first flower (R^2 = 0.05–0.07, Tables 2 and 3).

We also found several trait-specific QTLs. A large-effect QTL on the right arm of chromosome 2 (LG2, R^2 = 0.08) and a smaller effect QTL on the left arm of chromosome 14 (LG14, R^2 = 0.04) were unique to leaf shape. In addition, there was a nonoverlapping QTL on the right arm of chromosome 14 involved in corolla width (R^2 = 0.08, Fig. 6). A QTL on chromosome 7 was shared by flowering time (R^2 = 0.08) and node of first flower (R^2 = 0.06). Several flowering time QTLs (LG8a, LG8b and LG7) overlap with QTLs that underlie differences in flowering time and critical photoperiod both within *M. guttatus* and between *M. guttatus* and *M. nasutus* (reviewed in Zuellig *et al.* 2014). The LG2QTL overlaps with one found in our previous study of leaf shape in a different *M. laciniatus* × *M. guttatus* cross (Ferris *et al.* 2015).

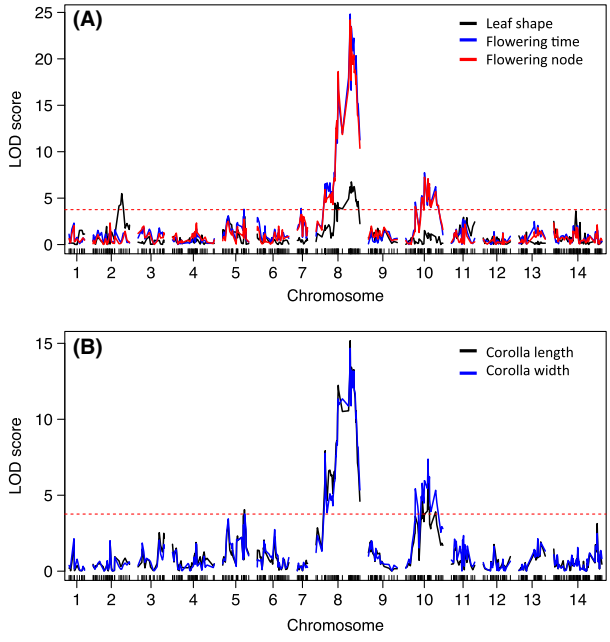

Fig. 5 Panel (A) depicts plots of genome wide LOD score for leaf shape (black line), flowering time (blue line) and node of first flower (red line). Panel (B) depicts plot of the genome wide LOD score for corolla width (black line) and corolla length (blue line). The dotted red line indicates the genome-wide significance level of $\alpha = 0.05$ at the highest LOD score cut-off of all five traits, 3.82.

We found evidence of epistasis for all six traits (Table 2). Flowering time had the highest number of significant epistatic interactions in the ANOVA models. Four out five of these interactions involved the pleiotropic QTLs on LG8. Flowering node had three significant QTL interactions between LG7 and LG8b, LG7 and LG10, and LG8a and LG8b. The pleiotropic QTL on LG5 interacted with loci involved in leaf shape (LG14), flowering time (LG7, LG8a) and corolla width (LG8a).

While most QTLs were positive in direction, meaning the effects were in the direction of what would be expected given the parental phenotypes, several were negative. The flowering time and flowering node QTL on chromosome 7 and the corolla width QTL on LG14 are negative in direction. Two of the five QTLs for leaf shape are negative in direction (Table 3), indicating that at these QTLs *M. guttatus* genotypes have more highly lobed leaves than *M. laciniatus* genotypes.

Discussion

Phenotypic divergence has a simple genetic architecture in sympatry

In our QTL mapping experiment, we found that all five morphological and life history traits had a relatively simple genetic basis. Each trait was controlled by 4 to 5 QTL of large-to-moderate effect, and these loci explained up to 64% of the segregating variance in the F_2 population (Table 3). The effect and number of QTL

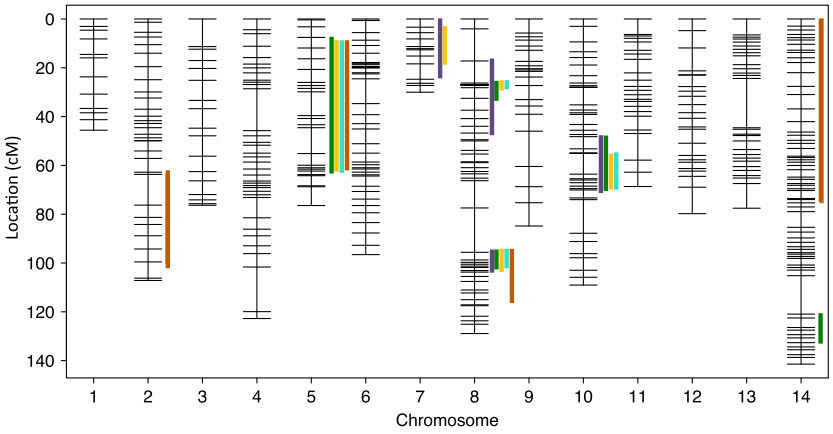

Fig. 6 Genetic map of the Shaver Lake *M. laciniatus* × *M. guttatus* F_2 population built in JoinMap. QTL location for each trait is marked by rectangular boxes: flowering time (purple), node of first flower (yellow), corolla width (green), corolla length (cyan) and leaf shape (burnt orange).

Table 2 Broad-sense heritability (H^2), QTL position, 1.5-LOD score intervals given in both cM and Mb, peak position, peak LOD score and significance level are described for each trait in the table below. Significant epistatic interactions were detected with factorial ANOVAS

Trait	H^2	Chromosome	1.5 LOD interval (cM)	1.5 LOD interval (Mb)	Peak position (cM)	Peak LOD score	*P*-value
Corolla length	0.2897	5	7.55–68.74	0.729–19.53	64	3.87	0.058*
		8a	26.25–28.16	0.872–1.67	27	7.59	<0.0001***
		8b	98.76–103.09	18.05–19.45	100.5	14.66	<0.0001***
		10	55.00–68.5	5.65–11.17	65.47	7.39	<0.0001***
		8a × 10					0.058*
		8b × 10b					0.085*
Corolla width	0.2478	5	7.55–68.36	0.729–19.53	64	4.06	0.04**
		8a	26.25–32.52	0.872–1.86	27	7.86	<0.0001***
		8b	98.76–103.09	18.05–19.45	100.5	15.16	<0.0001***
		10	47.35–67.09	5.15–9.38	65.47	6.03	0.001***
		14	120.95–129.36	23.41–24.49	127.5	3.18	0.19
		5 × 8a					0.078*
Flowering node	0.7391	7	3.36–30.01	0.323–5.44	12.64	3.24	0.17
		8a	17.21–49.48	0.405–3.58	26.25	5.9	<0.0001***
		8b	98.76–103.09	18.05–19.45	100.5	24.2	<0.0001***
		10	53.22–70.07	5.65–12.02	55	7.25	<0.0001***
		7 × 8b					0.087*
		7 × 10					0.052*
		8a × 8b					0.048**
Flowering time	0.4733	5	7.55–64.22	0.729–15.48	16.31	3.24	0.073*
		7	5.62–15.19	0.476–2.34	11.3	3.9	0.058*
		8a	26.25–28.16	0.872–1.67	27	6.56	<0.0001***
		8b	98.75–105.51	18.05–20.20	100	24.9	<0.0001***
		10	53.22–67.09	5.65–9.39	56	8.03	<0.0001***
		5 × 7					0.061*
		5 × 8a					0.049**
		7 × 8b					0.001***
		8a × 8b					0.011**
		8b × 10					0.061*
Leaf shape	0.4025	2	63.69–94.26	15.02–18.03	84.21	5.33	0.002**
		5	7.55–68.36	0.729–19.53	64	3.37	0.137
		8b	95.61–117.14	17.76–23.0	103.73	6.73	<0.001***
		14	0–78.95	0.009–18.69	64.8	3.62	0.08*
		5 × 14					0.042**

Significance levels are represented as *P*-value *<0.1, **<0.05, ***<0.01, ****<0.001.

Table 3 QTL position, R^2, effect size as proportion of parental difference, along with the mean phenotype of the *M. guttatus* homozygote (GG), the heterozygote (GL) and the *M. laciniatus* homozygote (LL), and the additive (*a*) and dominance (*d*) effects of each locus are described in the table below. Flower size was measured in 100ths of an inch. Flowering time is measured in number of days to first flower. The mean phenotype for each genotype was determined in a one-way ANOVA at each locus

Trait	Chromosome	R^2	Proportion parental difference	Mean GG phenotype	Mean GL phenotype	Mean LL phenotype	QTL direction	*a*	*d*
Corolla length	5	0.061	0.269	90.73	76.96	70.53	+	10.1	3.67
	8a	0.089	0.222	82.8	76.65	66.14	+	8.33	2.18
	8b	0.173	0.208	82.7	76.72	67.13	+	7.785	1.805
	10	0.091	0.197	81.67	75.7	66.89	+	7.39	1.42
Corolla width	5	0.075	0.270	70.91	58.05	51.22	+	9.845	3.015
	8a	0.106	0.231	63.82	57.8	46.99	+	8.415	2.395
	8b	0.195	0.233	64.17	57.15	47.24	+	8.465	1.445
	10	0.094	0.180	61.74	56.16	48.66	+	6.54	0.96
	14	0.081	0.093	50.13	56.82	56.9	−	3.385	3.465
Flowering node	7	0.059	0.601	4.69	5.81	6.14	−	0.725	1.055
	8a	0.097	1.074	6.76	5.7	4.17	+	1.295	0.235
	8b	0.251	1.340	7.02	5.46	3.79	+	1.615	0.055
	10	0.07	0.738	6.15	5.33	4.37	+	0.89	0.07
Flowering time	5	0.069	0.835	49.11	41.7	38.04	+	5.535	1.875
	7	0.081	0.356	38.03	43.35	42.75	−	2.36	1.76
	8a	0.115	0.763	46.76	41.82	36.65	+	5.055	0.115
	8b	0.256	0.891	46.98	40.65	35.18	+	5.9	0.43
	10	0.079	0.449	43.49	40.72	37.54	+	2.975	0.205
Leaf shape	2	0.081	0.568	0.253	0.303	0.375	+	0.061	0.133
	5	0.051	0.507	0.408	0.303	0.299	−	0.0545	0.0505
	8b	0.093	0.572	0.359	0.304	0.236	−	0.0615	0.0065
	14	0.039	0.381	0.259	0.295	0.341	+	0.041	0.087

detected in a mapping study depend on sample size (Beavis 1998; Lynch & Walsh 1998), and therefore, it is probably that with 424 F2s we have missed some loci of very small effect. This could lead to overestimation of our QTL effect sizes, but according to simulations by Beavis (1998), effect size of individual QTLs is only significantly overestimated in small mapping populations (e.g. 100 individuals). While flowering time (Hall *et al.* 2006, Blackman *et al.* 2010; Friedman & Willis 2013; Fishman *et al.* 2014) and leaf shape (Kimura *et al.* 2008; Vlad *et al.* 2014; Ferris *et al.* 2015) have a simple genetic basis in many plant species, the genetic simplicity of our flower size traits is surprising. Flower size is controlled by many (16–20) QTLs of small effect in other studies of intra- and interspecific floral divergence with similar power, due to similar sample sizes, within the *M. guttatus* species complex (Fishman *et al.* 2002; Hall *et al.* 2006; Fishman *et al.* 2015).

This difference in the genetic architecture of flower size in our study may be due to several factors. First, we looked at differences in floral morphology between species whereas Hall *et al.* (2006) studied flower size variation within *M. guttatus*. It has been observed in the literature that the genetic architecture of phenotypic differences between species is often due to large-effect loci, while divergence within a single species is more likely due to many loci of small effect (Remington 2015). In support of this, differences in floral size between two species of Capsella that differ in mating system, *C. rubella* and *C. grandiflora*, are also controlled by few loci of large effect (Slotte *et al.* 2012). Second, our study maps QTLs between incompletely reproductively isolated sympatric populations that are most likely experiencing ongoing gene flow. Previous QTL studies of floral morphology in the *M. guttatus* species complex intercrossed allopatric populations of *M. guttatus* and *M. nasutus* (Fishman *et al.* 2002; Hall *et al.* 2006). Ours is therefore the first study to map flower size differences in sympatric populations where there is evidence of hybridization between species.

Our QTL results from all five traits are consistent with theory predicting that when populations are subject to ongoing gene flow the genetic architecture of local adaptation should consist of few large-effect loci (Yeaman & Whitlock 2011), and with theory predicting that gene flow between differentially adapted species will increase linkage between loci involved in adaptation and reproductive isolation (Feder *et al.* 2012a,b; Via 2012). This is because single loci with large phenotypic effects or groups of tightly linked smaller effect loci

have larger selection coefficients than single loci with small phenotypic effects (Yeaman & Otto 2011). Gene exchange between populations and species acts as a homogenizing force across the genome preventing divergence. Only loci protected from this force by strong selection will remain differentiated and potentially contribute to local adaptation or reproductive isolation. Therefore, if we were to quantify the amount of gene flow across the genome, as in Kenney & Sweigart (2016), we would expect to find genomewide signatures of introgression with only a few regions of differentiation representing loci involved in adaptation and reproductive isolation. Future studies are necessary to test this prediction and confirm that our results support the theoretical predictions of Yeaman & Whitlock (2011) as other models could also explain our findings.

A large-effect pleiotropic QTL controls species differences

In addition to all traits in our study being genetically simple, we found that interspecific differences in flowering time, flower size, node of first flower and leaf shape were largely controlled by a major-effect pleiotropic QTL. This QTL, LG8b, explained the largest proportion of the variance in the F_2 population for all six characters we measured. We define a pleiotropic QTL as a genomic region that affects multiple traits. We do not know whether this region consists of a single truly pleiotropic locus or many tightly linked loci. Three other highly pleiotropic QTLs were also found in our analysis: LG8a and LG10 contributed to differences in flowering and flower size traits, while LG5 was involved in divergence in all traits except the node of first flower.

Previous studies have found a major pleiotropic QTL controlling life history and morphological characters between inland and coastal forms of *M. guttatus* (Hall *et al.* 2006). This pleiotropic QTL, also on chromosome 8, turned out to be a widespread chromosomal inversion between *M. guttatus* ecomorphs (Lowry & Willis 2010). Our largest effect pleiotropic QTL, LG8b, is on the opposite end of the chromosome from the *M. guttatus* inversion, but LG8a is in the same region. There is a region of recombination suppression and marker order reversal in the vicinity of LG8a in our genetic map (Fig. 4); however, it is difficult to say whether this is a chromosomal inversion without further experimental evidence. From our current analysis, it does not seem like LG8b is in an inverted region. The LG8b QTL region is large, but there is a definite peak in LOD scores (Fig. 5) and it is not an area of uniformly suppressed recombination or marker order reversal in the genetic map (Figs 4 and 6). From this, we conclude that the pleiotropic effects of LG8b are truly due to one locus, or several very tightly linked independent loci not in an inverted region.

The genetic architecture of species differences in flowering time and flower size is particularly interesting as these traits are also involved in prezygotic reproductive isolation. Divergent selection would have therefore simultaneously increased local adaptation and reproductive barriers between *M. laciniatus* and *M. guttatus*. The genetic architecture of reproductive isolating barriers in sympatric populations has often been found to be genetically complex (Feder *et al.* 2012a,b). For example, Rieseberg *et al.* (1999) found that 16 loci controlled pollen sterility in a *Helianthus* hybrid zone, while studies from *Drosophila* species have found that both differences in courtship behaviour and reproductive morphology are highly polygenic (Wu & Ting 2004). These studies differ significantly from our findings; however, traits such as pollen sterility and animal reproductive morphology are unlikely to be involved in local adaptation. Therefore, we might expect the genetic architectures to be different from adaptive traits such as flowering time. A single large-effect locus that affects multiple adaptive and reproductively isolating traits would be the most efficient way for selection to maintain differentially adapted species in the face of gene flow. Our work adds to the growing list of examples of synergistic pleiotropy contributing to adaptation (reviewed in Dittmar *et al.* 2016).

Flowering and flower size QTLs colocalize with QTLs from other Mimulus species

Many of our QTLs colocalize with loci detected in previous genetic mapping experiments of flowering time, flower size and leaf shape in the *M. guttatus* species complex. The unique leaf shape locus on LG2 overlapped with a previous leaf shape QTL between different, allopatric *M. laciniatus* and *M. guttatus* populations (Ferris *et al.* 2015), demonstrating that this QTL is responsible for leaf shape differences across the species range. The other leaf shape QTLs (Table 2) do not overlap with our previous analysis, which is expected for the two negative QTLs as they do not contribute to leaf lobing in the *M. laciniatus* parent. A lack of overlap between the positive QTL on LG14 and previous analyses may indicate the presence of interpopulation genetic variation in leaf shape. This is interesting as leaf shape differs slightly between populations of *M. laciniatus* (K. Ferris, unpublished data).

The pleiotropic LG8a colocalizes with the *M. guttatus* DIV2 inversion which controls divergence in numerous traits, including flowering time and flower size, between coastal and inland *M. guttatus* (Hall *et al.* 2006; Lowry *et al.* 2010). The LG8b QTL region, although not

the 1.5-LOD peak, overlaps with flower size QTLs in a *M. guttatus* × *M. nasutus* population (Fishman *et al.* 2002) and QTLs involved in critical photoperiod differences between perennial and annual *M. guttatus* (Friedman & Willis 2013) and between *M. guttatus* and *M. nasutus* (Fishman *et al.* 2014). There is also an overlap between the pleiotropic LG10a and QTL controlling intraspecific variation in flowering time within *M. guttatus* (Friedman *et al.* 2015) and flower size differences between *M. guttatus* and *M. nasutus* (Fishman *et al.* 2002). The flowering time locus on LG7 overlaps with QTL from flowering time studies in *M. guttatus* (Hall *et al.* 2006; Friedman *et al.* 2015) and *M. nasutus* (Fishman *et al.* 2014). The overlap between the genetic architecture of flowering time and flower size in *M. laciniatus*, *M. guttatus* and *M. nasutus* on chromosomes 7, 8 and 10 from multiple independent studies strongly suggests that variation at these loci was segregating in an ancestral *M. guttatus*-like population. Early flowering and small-flowered variants could have then independently fixed in the diverging lineages of the two geographically restricted self-fertilizing species: *M. laciniatus* and *M. nasutus*.

Direction of QTL effects supports parental differences being due to selection

The direction of QTL effects can provide information about the action of natural selection on an individual trait. Specifically, if the majority of loci are in a positive direction and the difference between parental lines is large, then it is most likely that directional natural selection has caused the parental trait difference (Laurie *et al.* 1997; True *et al.* 1997; Orr 1998b; Muir *et al.* 2014). The majority of QTLs (60–100%) for all six of our traits were in a positive direction, and in the case of flowering time and flower size, more than 80% of QTLs were positive. While our study does not have enough power to formally test for the action of directional selection on our traits using Orr's sign test (Orr 1998b), the proportion of positive QTLs indicates that flowering time and flower size differences between our *M. laciniatus* and *M. guttatus* parents are most likely due to natural selection.

Despite the overwhelming positive effects of our QTLs, we did find a few negative flowering time, flower size and leaf shape QTLs. The presence of loci with negative effects could indicate the presence of segregating variation maintained by balancing selection within *M. guttatus* for these traits. There is previous evidence of balancing selection acting on flower size variation within *M. guttatus* (Mojica *et al.* 2012). Given the variety of edaphic habitats annual *M. guttatus* occupies (Hall *et al.* 2006; Lowry *et al.* 2008b; Friedman & Willis 2013), it seems likely that variation in flowering time is maintained through a similar mechanism. This could explain the presence of a negative flowering time QTL on LG7. However, the existence of negative leaf shape loci is more perplexing as the *M. guttatus* parental line did not possess lobed leaves. The large-effect pleiotropic QTL, LG8b, is positive for flowering and floral traits, but negative for leaf shape (Table 3). In other words, at LG8b the *M. guttatus* allele increases flowering time, node of first flower, and flower size as we would expect, but the *M. guttatus* allele also increases leaf lobing. We find the same pattern in the phenotypic correlation analysis (Table 1).

The positive genotypic and phenotypic correlation between later flowering, large flowers and lobed leaves is perplexing. As the parental *M. guttatus* line does not exhibit a lobed leaved phenotype, the most likely scenario is that this negative QTL results from an epistatic interaction between a *M. guttatus* allele at LG8b and a *M. laciniatus* allele elsewhere in the genome. One possibility is that the LG8b QTL affects organ growth and size, which could impact all of the traits we measured. In our Shaver Lake F2 population, larger plants flowered later, had larger petals, and more lobed leaves. In addition to influencing overall plant and organ size, changes in plant cell size have been shown to affect leaf shape in *Arabidopsis thaliana* (Tsukaya 2003). In *M. laciniatus*, leaf size seems to affect the amount of lobing with larger leaves being more lobed (K. Ferris, personal observation). Thus, an interaction between a *M. guttatus* allele increasing leaf size at LG8b and a *M. laciniatus* allele increasing lobing could produce the negative phenotypic direction of this QTL. Further studies are needed to identify the link between negative leaf shape and positive flowering and flower size association at LG8b.

Candidate genes for flowering time, flower size and leaf shape

The QTLs in our study span large physical genomic intervals and consequently each one contains hundreds of genes (for complete list see Table S1, Supporting information). However, within the 1.5-LOD intervals there are candidates for each trait. The most compelling candidate loci are those with pleiotropic effects. Under the peak of the largest effect pleiotropic QTL, LG8b, there is a candidate locus, *TCP DOMAIN PROTEIN 4 (TCP4)*, which represses petal growth (Nag *et al.* 2009), is involved in leaf cell differentiation, and the transition to flowering in *A. thaliana* (Sarvepalli & Nath 2011). Hyperactivation of the TCP4 transcription factor leads to smaller leaves and an early vegetative to floral transition (Sarvepalli & Nath 2011). This is an exciting candidate gene given its potential to significantly affect all traits in the expected directions. TCP4 could also

account for the negative direction of the LG8b leaf shape QTL because of its involvement in leaf size. A *M. guttatus* allele of this gene could cause plants to be larger, flower later, have bigger petals and bigger leaves which could account for the increased lobing of F2s that were large flowered and flowered later. The smallest effect pleiotropic QTL, LG5, also contains several candidates that affect multiple traits. Two of these genes, *AGAMOUS-LIKE 42* (*AGL42*) and *TCP DOMAIN PROTEIN 12* (*TCP12*), are involved in both the flower size and the flowering time pathways in *A. thaliana*. *GROWTH REGULATING FACTOR 8* (*GRF8*) and *KNAT6*, a KNOX gene which regulates the shoot apical meristem, have effects on both floral and leaf architecture (Krizek & Anderson 2013).

The location of candidate genes in our QTL regions also supports the possibility that instead of a single locus affecting all five traits, pleiotropic QTLs contain a series of tightly linked loci that affect each trait individually. LG8b contains several candidate genes for multiple traits in close physical proximity. The first flowering time candidate is *FLOWERING LOCUS D* (*FD)* which interacts with *FLOWERING LOCUS T* (*FT*) as a positive regulator of flowering. *FD* mutants are late flowering in *A. thaliana* in both *Col* and *Ler* genetic backgrounds (Abe *et al.* 2005). *SPA1-RELATED 4* (*SPA4*) is also within the 1.5 LOD score interval of LG8b and functions in suppressing photomorphogenesis during noninductive photoperiods in *A. thaliana* (Laubinger *et al.* 2006). The *COP1-INTERACTING PROTEIN 7* (*CIP7*) is positively regulated by light and interacts with COP1 which represses flowering under short days in *A. thaliana* (Liu *et al.* 2008). LG8b also contains *CUP-SHAPED COTELYDON2* (*CUC2*) which is involved in leaf serration in *A. thaliana* (Koenig & Sinha 2010) and mutants of CUC genes in *Antirrhinum* cause fusion of neighbouring leaves (Townsley & Sinha 2012). The moderate-effect QTL on chromosome 10 contains two copies of *FRIGIDA-LIKE PROTEIN* (LG5). *FRIGIDA* regulates the floral repressor *FLC* (Shindo *et al.* 2005; Deng *et al.* 2011) and controls natural variation in flowering along an environmental cline in *A. thaliana* (Stinchcombe *et al.* 2004). This same region also contains a flower size candidate, *DA1*, which is a negative regulator of floral organ growth (Krizek & Anderson 2013). Further fine mapping will be necessary to determine whether the pleiotropic QTLs (LG8a, LG8b, LG5, LG10) are in fact due to a single locus like TCP4 or composed of several smaller effect loci.

Conclusions

Historically, there has been much debate in the literature about the genetic architecture of species differences. Are species differences controlled by many loci of small effect (Fisher 1930) or by a few large-effect genetic changes (Eldredge & Gould 1972; Gottlieb 1984)? In recent years, special attention has been paid to the genetic architecture of species or population differences in the presence of gene flow, particularly during speciation (reviewed in Nosil & Feder 2012). Recent theoretical models incorporating drift, selection and migration find that when populations diverge with gene flow there should be few genetic loci of large effect or few regions of many tightly linked loci involved (Yeaman & Whitlock 2011; Feder *et al.* 2012b). Consistent with this theory, we have found that a few large-effect pleiotropic QTLs underlie divergence in life history and morphological traits involved in adaptation and reproductive isolation between sympatric *M. laciniatus* and *M. guttatus* populations. The degree of pleiotropy impacts the efficacy of selection on individual traits (Lande 1979). While antagonistic pleiotropy may slow the response to selection, synergistic pleiotropy can instead facilitate rapid adaptive divergence (Lande 1979, reviewed in Dittmar *et al.* 2016). Future studies (i) quantifying the extent of gene flow in this population, (ii) comparing these results to the genetic architecture of the same traits in a cross between allopatric *M. laciniatus* and *M. guttatus* populations not exchanging genes and (iii) assessing the adaptive significance of our traits and alleles in each species' native habitat will be necessary to more thoroughly test specific predictions about the maintenance of adaptive divergence with gene flow.

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K.G.F. conducted the field work, greenhouse work, lab work, & QTL mapping analysis. L.L.B. measured flower size phenotypes. B.K.B. performed next generation sequence analysis. J.H.W. assisted with experimental design and funding. All authors contributed to the writing and editing of this manuscript.

Data accessibility

Whole-genome re-sequencing data from the *M. laciniatus* × *M. guttatus* hybrid population of 424 F2s has been uploaded to NCBI's Short Read Archive (SRA) in the form of fastq files: Project PRJNA317270, Accession SAMN04604733. Leaf shape, flowering time and flower size measurements, a spreadsheet with genotype and phenotype information, and the custom perl script used to rescore genotypes for windows of 75 SNPs have been uploaded to Dryad (http://dx.doi.org/10.5061/dryad.6jg7g).

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1. Table of candidate genes in QTL intervals.