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Major quantitative trait loci control divergence in critical photoperiod for flowering between selfing and outcrossing species of monkeyflower (*Mimulus*)

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Summary

- Divergence in flowering time is a key contributor to reproductive isolation between incipient species, as it enforces habitat specialization and causes assortative mating even in sympatry. Understanding the genetic basis of flowering time divergence illuminates the origins and maintenance of species barriers.
- We investigated the genetics of divergence in critical photoperiod for flowering between yellow monkeyflowers *Mimulus guttatus* (outcrosser, summer flowering) and *Mimulus nasutus* (selfer, spring flowering). We used quantitative trait locus (QTL) mapping of F_2 hybrids and fine-mapping in nearly isogenic lines to characterize the genomic regions underlying a > 2 h critical photoperiod difference between allopatric populations, and then tested whether the same QTLs control flowering time in sympatry.
- We identified two major QTLs that almost completely explain *M. nasutus*'s ability to flower in early spring; they are shared by allopatric and sympatric population pairs. The smaller QTL is coincident with one that differentiates ecotypes within *M. guttatus*, but the larger effect QTL appears unique to *M. nasutus*.
- Unlike floral traits associated with mating system divergence, large interspecific differences in flowering phenology depend on only a few loci. Major critical photoperiod QTLs may be 'speciation genes' and also restrict interspecific gene flow in secondary sympatry.

Introduction

The timing of reproduction is a key determinant of both individual fitness and population success. In flowering plants, the optimal time for reproduction may depend on local abiotic factors, such as seasonal water availability, and biotic factors such as the availability of pollinators or density of seed predators (reviewed in Elzinga *et al.*, 2007). Therefore, flowering phenology is an important axis for adaptive divergence among plant populations and species (Hall & Willis, 2006; Martin & Willis, 2007; Méndez-Vigo *et al.*, 2011; Leinonen *et al.*, 2012; Nakazato *et al.*, 2013). Furthermore, because divergence in peak flowering time causes assortative mating, phenological shifts can directly cause pre-mating reproductive isolation. Thus, flowering time is a potential 'magic trait' in plants (Gavrilets & Vose, 2007) and understanding the genetic basis of divergence in flowering time and flowering cues is central to a full understanding of plant speciation (Rieseberg & Willis, 2007; Rieseberg & Blackman, 2010). Nonetheless, surprisingly little is known about the genetic architecture, much less the molecular genetic basis and evolutionary history, of interspecific differences in flowering phenology.

Flowering is a complex trait requiring an integrated developmental response to both internal and external conditions. In the molecular model plant *Arabidopsis thaliana*, the regulatory network controlling flowering is generally divided into multiple cue-dependent plus autonomous pathways funneling through a handful of key integrator genes (reviewed in Amasino, 2010), although these pathways are increasingly understood to be highly complex (Andrés & Coupland, 2012), and not independent from traits such as germination (Wilczek *et al.*, 2010; Chiang *et al.*, 2012). Photoperiod is among the most important cues for flowering in many plants, as it can be a reliable predictor of future seasonal environments (Amasino, 2010). Long-day plants, such as *A. thaliana*, require daylengths greater than a critical photoperiod (e.g. 14 h) to initiate floral development, whereas short-day species such as rice (*Oryza* spp.) require daylengths less than a critical photoperiod. Other taxa, such as the yellow monkeyflower *Mimulus guttatus* (Friedman & Willis, 2013), resemble long-day plants in requiring a daylength cue greater than some minimum threshold to flower, but vary in critical photoperiod across a broad range including both short (< 12 h) and long days.

Simple mutations in known flowering time pathway genes can underlie large differences in flowering behavior among natural populations. In *A. thaliana*, for example, allelic variation at a handful of loci explains much of the variation in flowering behavior across diverse *A. thaliana* accessions (Alonso-Blanco *et al.*,

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2009; Salome *et al.*, 2011). Major quantitative trait loci (QTLs) have also been identified for inter-variety differences in flowering in rice (Yano *et al.*, 2000) and for the evolution of early flowering in domesticated sunflower (Helianthus; Blackman *et al.*, 2011) and peas (*Pisum* spp.) (Weller *et al.*, 2012). By contrast, a massive quantitative genetic analysis in maize (*Zea mays*), a diverse outbred species, found a highly polygenic basis to standing variation for flowering phenology (Buckler *et al.*, 2009). From these studies, it is clear that mutations with major effects on flowering time may be fixed by the strong selection of domestication; however, it is not yet clear whether they also contribute to the (often large) differences in flowering phenology that can reproductively isolate co-occurring species.

Here, we investigate QTLs underlying a striking difference in the critical photoperiod for flowering between the yellow monkeyflowers *Mimulus guttatus* and *Mimulus nasutus*. *Mimulus guttatus* (common yellow monkeyflower) is an extremely diverse, primarily outcrossing wildflower of wet soils across western North America (Wu *et al.*, 2008). *Mimulus nasutus*, a highly selfing species that appears to be recently derived from within *M. guttatus* (Sweigart & Willis, 2003), co-occurs with annual ecotypes of *M. guttatus* in patches throughout their shared range. In addition to being divergent in mating system and associated floral traits (Fishman *et al.*, 2002), *M. nasutus* tends to occupy drier (or more ephemerally wet) shallow-soiled microsites and to flower earlier than even annual *M. guttatus*. Hybridization does occur at some sympatric sites and there is also evidence of ongoing interspecific introgression (Sweigart & Willis, 2003), but divergence in flowering time is a key pre-mating barrier between these species (Kiang & Hamrick, 1978; Martin & Willis, 2007). In the glasshouse and field, *M. nasutus* accessions flower readily under daylengths as short as 9 h, whereas *M. guttatus* is highly variable but often requires at least 13–14 h of daylight to initiate flowering (Friedman & Willis, 2013). Thus, genetic differences in critical photoperiod are an important determinant of the realized differences in flowering phenology that contribute to reproductive isolation between *M. nasutus* and *M. guttatus*.

In addition to addressing the general question of whether a key plant speciation trait has diverged by major or minor steps, QTL mapping can begin to address fundamental questions about the repeatability of evolutionary transitions and the origins of interspecific variation. Recently, Friedman & Willis (2013) mapped major QTLs for both critical photoperiod and vernalization requirement differences between populations of *M. guttatus*. This close comparative context allows us to ask whether the genetic regions (and potentially genes) involved in the divergence of *M. nasutus* from an *M. guttatus*-like ancestor are novel or appear to be drawn from existing variation within *M. guttatus*. In addition, we can directly compare the genetic architecture of flowering time divergence to that of other species-diagnostic traits, such as highly polygenic floral traits associated with the evolution of selfing (Fishman *et al.*, 2002). Finally, by identifying strong positional candidate genes with fine-mapping, we can assess whether genes or gene families implicated in flowering time variation in other taxa are also likely to be associated with critical photoperiod divergence between species of *Mimulus*.

We focus mapping efforts on a well-characterized, highly divergent pair of lines from allopatric Oregon populations: Iron Mountain *M. guttatus* (IM62) and Sherar's Falls (SF) *M. nasutus*. The IM62 line was derived from a high-elevation annual population that flowers in a narrow window between snowmelt and summer drought (June–July); it requires 13–14 h of daylight to flower (Friedman & Willis, 2013; L. Fishman pers. obs.) By contrast, all *M. nasutus* accessions will flower under 10-h days (Friedman & Willis, 2013), consistent with early spring flowering on substrates with very ephemeral water availability (including the spray-misted basalt cliffs from which SF was collected). We first used bulk segregant analysis and targeted genotyping of large SF × IM62 F_2 populations (replicated in 2 yr) to map two major loci that together confer *M. nasutus*'s ability to flower under early spring daylengths (< 12-h days). Secondly, we used nearly isogenic lines (NILs) to confirm and fine-map those QTLs to physically contiguous scaffolds of the *M. guttatus* draft genome, allowing us to identify strong functional and positional candidate genes for the interspecific divergence. We then extended our analysis to a pair of sympatric populations, and used targeted mapping to demonstrate that the two major loci identified in our allopatric cross are also the primary cause of flowering cue divergence in sympatry.

Materials and Methods

Study system and plant material

The *Mimulus guttatus* DC species complex (Phrymaceae) is a morphologically and ecologically diverse group of partially cross-fertile taxa. The outcrossing yellow monkeyflower *M. guttatus* is found from the Aleutian Islands to Baja California and east to the Rocky Mountains. *Mimulus nasutus* Greene, which generally self-pollinates before corolla opening, is the most widespread of several selfing taxa derived from *M. guttatus*, and has a broadly overlapping range. We used inbred lines derived from collections at three sites. The high-elevation Iron Mountain, OR (IM) *M. guttatus* population consists of bee-pollinated annuals that germinate in fall (overwintering under snow) or late spring, flower at mid-summer, and die by early August. The IM62 line (5+ generations inbred) was used to generate the *M. guttatus* reference genome (www.Phytozome.org). The Sherar's Falls, OR (SF) *M. nasutus* line was collected from basalt rocks flanking a waterfall on the Deschutes River, is naturally inbred, and has been maintained by inbreeding. Hybrids of SF and IM62 lines were previously used for linkage and QTL mapping (Fishman *et al.*, 2001, 2002), and for genetic characterization of postmating prezygotic (Fishman *et al.*, 2008) and postzygotic (Fishman & Willis, 2005; Sweigart *et al.*, 2006; Case & Willis, 2008; Barr & Fishman, 2010) barriers. The Catherine Creek, WA (CAC) populations occupy seasonal seeps on the north side of the Columbia River Gorge (< 200 km from SF and IM). The two species co-occur in numerous patches throughout this area, but *M. nasutus* tends to occupy rockier microsites (A. Kenney & A. Sweigart, pers. obs.)

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Phenotypic and bulk segregant analyses (2009)

In Spring 2009, we conducted a bulk segregant analysis of an SF × IM62 F_2 population grown under short days in our glasshouse at the University of Montana. Seeds were planted into 96-well flats containing Sunshine #1 soil less potting mix (Sungro Horticulture, Agawam, MA, USA) on 10 December 2008, chilled for 4 d at 4°C to promote germination, and then placed in a glasshouse with a 12 : 12 h day : night cycle. Daytime temperatures were *c.* 20–28°C and nighttime temperatures were *c.* 13–15°C. Supplemental light (600-W high-pressure sodium Philips Son-T Agro bulbs; Philips North America, Andover, MA, USA) was provided during the 12-h day, and plants were bottom-watered daily. After germination, seedlings were thinned to 1 per well. Parental controls (n = 24 each) were randomized in a single flat and the control flat position within the bench of F_2 flats was rotated weekly. Plants were checked for floral buds weekly until 20 March 2009.

Tissue was collected from a subset of early-flowering plants (buds before 15 March; n = 96) and from nonflowering plants (no buds by 20 March; n = 356) for selective genotyping. DNA was extracted using a standard CTAB-chloroform protocol modified for 96-well format. For bulk segregant analysis, we constructed four flowering pools and four nonflowering pools (n = 4 individuals per pool). The eight pools were genotyped at 96 previously mapped MgSTS (*Mimulus guttatus* Sequence Tagged Site) markers, which are exon-primed and amplify intron-length polymorphisms (e-prefix in text and figures; for details on MgSTS markers, see www.mimulusevolution.org). Four to six MgSTS markers (forward primer 5′ fluorescent-labeled) from each of the 14 linkage groups were assembled into multiplexes and amplified using standard touchdown PCR protocols. The PCR products were run on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with an in-lane size standard and informative polymorphisms were scored using GeneMapper software (Applied Biosystems) and verified by eye. Markers were flagged as positives if two or more of the four flowering pools were homozygous for *M. nasutus* alleles, but nonflowering pools appeared heterozygous. Positive markers, plus additional linked markers on two linkage groups, were then genotyped in the F_2 hybrids (n = 452) following the same protocols.

QTL mapping (2010)

In spring 2010, we grew a second F_2 population (n = 768) for targeted QTL mapping. Growth conditions were similar (supplemental light and 12-h daylengths), but we included seven additional inbred lines from the IM *M. guttatus* population (n = 4 each) along with parental line controls (IM62, n = 12; SF, n = 8). Seeds were planted on 5 January and plants were scored as flowering if they initiated buds by 21 March, when natural daylengths reached 12 h. Supplemental light was then provided for 16 h per day and plants monitored for flowering for 100 d.

F_2 hybrids were genotyped at MgSTS markers (e-prefix) as previously, and at new markers designed in the targeted QTL regions on LG7 and LG8 (m-prefix). Briefly, we used the draft genome build of *M. guttatus* (www.Phytozome.org, V2.0), plus whole-genome resequence data for the *M. nasutus* SF line (aligned to the IM62 reference with bwa; Li & Durbin, 2009) to design exon-primed markers spanning intron indel polymorphisms between SF and IM62. These markers (Supporting Information Table S1) were amplified and genotyped using the same PCR and scoring protocols as for existing MgSTS markers. For QTL mapping, flowering versus not flowering by 21 March was coded as a binary trait (0 versus 1). We conducted QTL mapping in Windows QTL Cartographer, using the composite interval mapping module (CIM; window size 10 cM; three cofactors). We also used the categorical multiple interval mapping function in QTL Cartographer (Li *et al.*, 2006), but the results were nearly identical to those for CIM; we present the standard CIM analysis here. To test for interactions between the major QTLs, we used logistic regression in JMP 10 (SAS Institute, Cary, NC, USA), with the two markers closest to the QTL peaks and their interaction as fixed effects.

NIL fine-mapping

We previously generated two sets of NILs with *M. guttatus* (IM62) and *M. nasutus* (SF) introgressions in the opposite genetic background (Fishman & Willis, 2005; Sweigart *et al.*, 2006). Each fourth-generation NIL (*M. nasutus* background, BN_4; *M. guttatus* background, BG_4) carries a unique complement of heterozygous introgressions embedded in a genome that is expected to be 93.75% homozygous for parental alleles. To catalogue introgressed regions, we genotyped all NILs at MgSTS markers distributed throughout the genome (L. Fishman *et al.*, unpublished). NILs were then selfed to generate homozygous introgressions as necessary. We selected three NILs with introgressions spanning the LG7 and/or LG8 flowering time QTLs for further genetic analyses. For two of these NILs, *M. nasutus* is the recurrent parent: BN_4.216 is heterozygous at the LG7 QTL and BN_4.37 is homozygous for *M. guttatus* alleles at the LG8 QTL. The remaining NIL, BG_4.327, has *M. guttatus* as the recurrent parent and is heterozygous at *both* the LG7 and LG8 QTLs. For each NIL, we grew selfed progeny under the same conditions as the 2010 F_2 grow-out and scored plants for floral bud initiation. We genotyped all selfed progeny (or, for BN_4.37, a small subset of the selfed progeny) for markers linked to the LG7 and LG8 QTLs.

Targeted mapping in sympatric *M. guttatus* and *M. nasutus* hybrids

We grew reciprocal F_2 hybrids of *M. guttatus* (CAC6G, three generations inbred) and *M. nasutus* (CAC9N, naturally inbred) lines from the sympatric Catherine Creek populations. We sowed seeds directly into 96-well flats containing Fafard® 3B soilless potting mix (Sungro Horticulture) on 9 February 2012, chilled them for 7 d at 4°C to promote germination, and then placed them in the University of Georgia Plant Biology glasshouse with a 12 : 12 h day : night cycle. Daytime temperatures were *c.* 17–24°C and nighttime temperatures were *c.* 13–17°C. We provided supplemental light (400-W high-pressure sodium bulbs) during the 12-h day and bottom-watered plants daily. After germination, we

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thinned seedlings to one per well. Reciprocal F_2s were grown in separate sets of flats ($n = 368$ and 460 F_2s with the *M. nasutus* and *M. guttatus* cytoplasmic backgrounds, respectively). Parental controls (one each per flat) were randomly dispersed within each flat and in additional F_2 flats not used for mapping (total $n = 21$ *M. nasutus* and 26 *M. guttatus*).

We scored plants every other day for floral bud initiation, beginning on 17 March when natural daylength was *c.* 12 h. We considered plants that had initiated floral buds by 14 April (when natural daylength was *c.* 13 h) as flowering under short days and scored all other plants as not flowering. At Catherine Creek, *M. nasutus* begins flowering in late March/early April when daylength is *c.* 12.5 h (A. Kenney, pers. obs.). Therefore, the conditions in this experiment are relevant to variation found within this population. To test for an effect of the LG7 and LG8 QTLs on flowering under short days, we genotyped the F_2s at markers flanking each QTL. We extracted DNA and genotyped plants at a combination of MgSTS and custom-designed markers, except that PCR products were run on an ABI 3730 Genetic Analyzer at the University of Georgia, and polymorphisms scored using GENEMARKER software (Soft Genetics, State College, PA, USA). For analyses, we exclusively used F_2 individuals without recombination between the flanking markers. We performed χ^2 tests in JMP PRO 10 (SAS Institute) on the F_2 hybrids to test for associations between QTL genotype and short-day flowering.

Results

Phenotypic differentiation and bulk segregant analysis (2009)

In both the 2009 and 2010 grow-outs, all SF *M. nasutus* controls flowered under <12-h days, whereas no IM62 *M. guttatus* or other IM lines flowered under spring conditions. In 2009, two markers near the center of LG7 (MgSTS markers e34 and e251; both with three of four flowering pools homozygous for the SF allele) and two markers near the bottom of LG8 (e829 and e621; with three and two flowering pools homozygous for the SF allele, respectively) were flagged as positives, as was one marker (e779; two SF homozygous flowering pools) on LG12. The LG12 marker, however, was not associated with flowering in the larger genotyped data set (Pearson χ^2; $P = 0.49$; $n = 427$), so we focused on LG7 and LG8 markers for further genotyping. Single marker analyses of 10 markers across LG7 and four on the bottom of LG8 indicated major QTLs in each region, with e34 on LG7 (likelihood ratio; LR = 64.3) and e294 (LR = 71.8) most strongly associated with 12-h flowering. These two markers together explained 27% of the variance in the selected subset of F_2s, with 70.0% of NN double homozygotes and 0.0% of GG double homozygotes flowering.

QTL mapping (2010)

In 2010, the LG7 (LR = 177.0; $r^2 = 0.23$) and LG8 (LR = 46.8; $r^2 = 0.065$) QTLs were again highly significant (Fig. 1). At the LG7 QTL, the *M. nasutus* allele was partially recessive

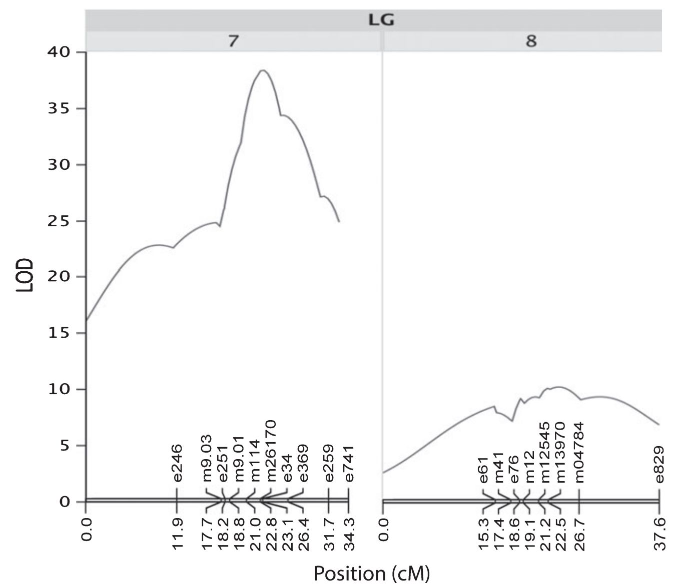

Fig. 1 Localization of *Mimulus nasutus–Mimulus guttatus* critical photoperiod quantitative trait loci (QTLs) in the 2010 SF × IM F_2 mapping population. Linkage maps of target genomic regions were constructed in JOINMAP (Kyazma BV, Wageningen, Netherlands), and QTLs were mapped in Windows QTL CARTOGRAPHER (Basten *et al.*, 2002) using CIM with three cofactors. LOD ($\log_{10}$ of odds) values > 2.5 indicate a significant genotype–phenotype association (1000 permutations). Marker names with an e- prefix are MgSTS markers (www.mimulusevolution.org); details on the custom m-prefix markers are given in Supporting Information Table S1. Proximal markers for each mapped region (e376 and e656 for LG7 and LG8, respectively) are not shown.

($a = -0.28$; $d = -0.18$; both $P < 0.01$), whereas the LG8 QTL appeared nearly additive ($a = -0.23$; $d = -0.05$). Together, the two QTLs explained > 95% of the species difference in the response to flowering (Fig. 2), but there was no evidence of epistasis (genotypic interaction $P > 0.4$ in logistic regression). That is, the ability to flower under short days depended on the additive action of alleles across the two loci, with *c.* 50% of NG (LG7, *M. nasutus*; LG8, *M. guttatus*) individuals flowering under 12-h days, but 96% of NN F_2s flowering. Conversely, *c.* 25% of plants homozygous for *M. nasutus* alleles at the LG8 QTL (but homozygous for *M. guttatus* alleles at LG7) flowered, indicating that its effects are at least partially independent from the genotype at the more major LG7 QTL (Fig. 2).

As in previous iterations of the SF × IM62 F_2 population grown under long days (Fishman *et al.*, 2002, 2008), we observed transmission ratio distortion (TRD) toward excess *M. nasutus* alleles at LG7 markers and Mendelian segregation in the focal LG8 region. In this cross, multiple gametic mechanisms of TRD, including centromere-associated meiotic drive (LG11; Fishman & Willis, 2005) and competition among haploid pollen genotypes (*c.* 10 loci; Fishman *et al.*, 2008), generally cause *M. guttatus* over-transmission. One explanation for the local TRD favoring *M. nasutus* would be differences in seed germination attributable to F_2 genotype at the LG7 flowering QTL, as the environmentally dependent genetic pathways for flowering and germination partially overlap, at least in the Brassicaceae (Chiang *et al.*, 2009). In that case, we would expect TRD to be

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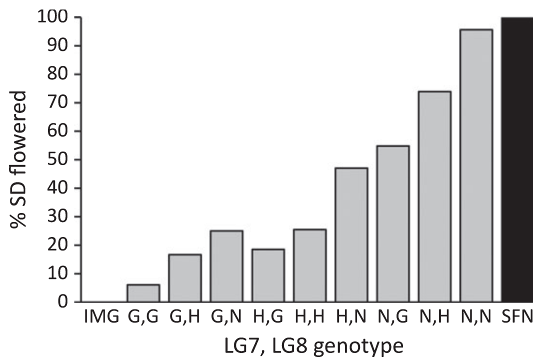

Fig. 2 Percentage of individuals that flowered under short days (SD), for Iron Mountain (IM) *Mimulus guttatus* (white; $n = 40$; 0%), Sherar's Falls (SF) *Mimulus nasutus* (black; $n = 8$), and all two-locus F_2 genotypes (gray; total $n = 623$) at LG7 and LG8 quantitative trait locus (QTL) peaks (e34 and m13790, respectively).

strongest at the QTL peak (near e34) and to exhibit a pattern consistent with zygotic selection (e.g. reduced germination of *M. guttatus* homozygotes) rather than gametic selection. Instead, the frequency of the *M. nasutus* allele was highest at e741 (distal; 0.583; $n = 644$), lowest at e376 (proximal; 0.482; $n = 443$) and intermediate at e34 (0.565; $n = 663$). In addition, genotypic frequencies (N : H : G) at e34 were virtually identical to the expected values given the skewed allele frequencies ($P > 0.5$). Together, this suggests that *M. nasutus*-biased transmission on LG7 is not a pleiotropic effect of the major critical photoperiod QTL, but instead reflects gametic distortion at a more distal locus. Notably, the distal end of LG7, beyond e741, contains the dominant nuclear restorer (*R f*) of cryptic mitochondrial male sterility in IM *M. guttatus* (Barr & Fishman, 2010).

Fine-mapping of QTLs in NILs (2011)

To refine the positions of the LG7 and LG8 QTLs and characterize their individual phenotypic effects, we grew the selfed progeny of three independent NILs under similar short-day glasshouse conditions. One of these lines, BG_4.327, is doubly heterozygous for introgressions spanning the LG7 and LG8 QTLs in an otherwise *M. guttatus* genetic background. Only one of 23 selfed progeny from this line flowered under short days. Genotyping revealed that this individual alone carried homozygous *M. nasutus* introgressions for *both* flowering time QTLs (Fig. 3), underscoring their necessary roles in critical photoperiod divergence. Moreover, the recombination breakpoints on LG7 coincided precisely with the QTL identified in the F_2 hybrids: the proximal breakpoint occurred between m9.01 and m114, whereas the distal breakpoint occurred between e369 and e259. For LG8, the proximal breakpoint in BG_4.327 was located at the edge of the QTL, so did not provide any further refinement of this QTL.

The BN_4.216 line carries a heterozygous introgression spanning the LG7 QTL in a nearly isogenic (including the LG8 QTL) *M. nasutus* genetic background. As expected, segregation of the LG7 introgression among the selfed progeny additively affected the propensity to flower under short days: 97.1% of *M. nasutus* homozygotes flowered, compared with only 56.9% of heterozygotes and 10.8% of *M. guttatus* homozygotes ($n = 941$). Because the *M. nasutus* allele is nearly completely penetrant against this isogenic background, recombinational mapping allowed us to place a distal boundary for the LG7 QTL at m110 (Fig. 3). The proximal boundary was at m9.01, consistent with mapping results above (Figs 1, 3). Despite a large mapping population ($n = 941$), there were too few recombinants to further refine the LG7 QTL. Indeed, we observed a severe reduction in recombination in this region among the BN_4.216-selfed progeny compared with our 2010 F_2 population (3.5 cM versus 30 cM between e741 and e251), consistent with previous findings for heterospecific *Mimulus* NILs (Sweigart *et al.*, 2006).

None of 84 selfed progeny of BN_4.37 – a line that carries a homozygous *M. guttatus* introgression spanning the LG8 QTL in an *M. nasutus* (including the LG7 QTL) genetic background – flowered under short days. To define the introgression breakpoints in this line, and thereby refine the QTL it contains, we genotyped eight of these selfed progeny for additional markers designed from the genome sequence assembly. This approach localized the recombination breakpoint to between m12545 and m13970, placing a distal boundary on the LG8 QTL (Fig. 3).

Candidate gene identification

The LG7 QTL spans 2.5–3 cM (1–2 LOD drop, respectively) centered near marker e34, and the NIL data also restrict it to this region. The 5.35 cM region between flanking m114 and e369 markers corresponds to *c.* 530 kb of the V. 2.0 *M. guttatus* genome, indicating that 1 cM equals *c.* 100 kb. Thus, the QTL encompasses *c.* 250–300 kb containing 36–42 annotated genes (Fig. 3). Forward searches for *Mimulus* homologs of *A. thaliana* genes with functional annotations related to flowering identified only two loci on LG7 as possible candidates; both are near the QTL region. The only *Mimulus* match to Cryptochome 2 (CRY2; *Migut.G00510*) is adjacent to the gene containing e251, making it a positional candidate as well as a strong functional candidate for shifts in critical photoperiod (El-Assal *et al.*, 2001). However, flanking markers m9.01 and m9.03 place this gene well outside of the QTL confidence interval (Fig. 1) and the NIL breakpoints exclude it (Fig. 3). The other candidate (*Migut.G00570*, a homolog of FLOWERING LOCUS T) is predicted to be physically coincident with the peak of the LG7 QTL (Fig. 3); this location was genetically confirmed by mapping an SF-IM62 intron-length polymorphism in *Migut.G00570* as a marker (m26170) in the 2010 F_2 mapping population (Fig. 1).

The one and two LOD confidence intervals of the LG8 QTL spanned *c.* 6.5 and 17 cM, respectively (Fig. 1), but we were able to truncate the distal edge of the QTL with the BN4.37 NIL, a nonflowering introgression line that is *M. nasutus* homozygous for LG8 markers beyond m12545. This limited the LG8 QTL to the 300–500 kb interval between m41 or m12 (1 or 2 LOD drop) on the proximal side and the NIL breakpoint between m12545 and m13970 on the distal side (Fig. 3). This region contains *c.* 60–70 genes, including a homolog of GIBBERELLIC ACID INSENSITIVE (GAI; *Migut.H02266*) in the larger

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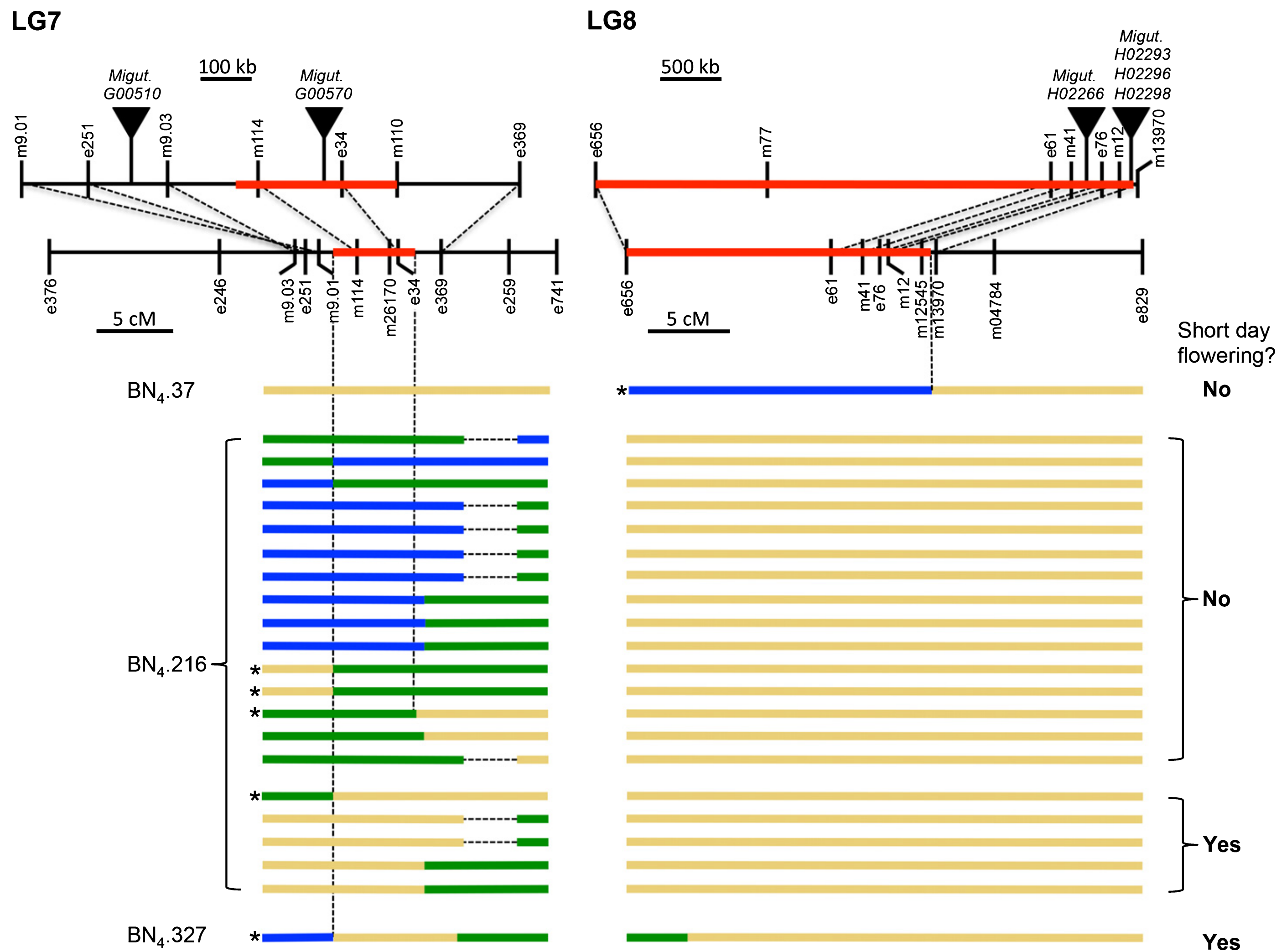

Fig. 3 Fine-mapping of *Mimulus nasutus–Mimulus guttatus* critical photoperiod quantitative trait loci (QTLs) using nearly isogenic lines (NILs). At the top are physical positions of genetic markers and candidate genes from the IM62 reference genome assembly (www.Phytozome.org) with genetic distances underneath taken from our F_2 linkage maps in Fig. 1 (note that the genome assembly flips the orientation of markers m9.01 to m9.03 relative to our genetic map). For both LGs, left is proximal and right is distal. NIL genotypes are represented with colored bars: yellow, *M. nasutus* homozygotes; green, heterozygotes; blue, *M. guttatus* homozygotes (dashed lines, missing genotypes). Key recombinants are indicated by asterisks; together, their breakpoints refine the QTLs to the regions indicated in red. All NILs were scored for the ability to flower under short days (phenotypes are given at the right).

interval and, directly under the QTL peak, a cluster of three genes with homology to the MADS-box gene SHORT VEGETATIVE PHASE/AGAMOUS-LIKE 24 (SVP/AGL24; *Migut.H02293*, *Migut.H02296*, and *Migut.H02298*).

Assessment of LG7 and LG8 QTLs in hybrids of sympatric *M. guttatus* and *M. nasutus*

Catherine Creek *M. nasutus* and *M. guttatus* parental lines strongly differed in their ability to flower under short days (90.4% versus 19.2% flowered, respectively), although less so than the allopatric inbred parental lines. Some early flowering by the *M. guttatus* parent, in particular, may reflect the later assay for flowering in this experiment relative to the SF × IM62 experiments. Consistent with the 2010 SF × IM62 F_2 results, 39% (324/828) of sympatric F_2s initiated flower buds under short days. The reciprocal F_2 groups flowered at the same overall rate (40% versus 38% for *M. nasutus* and *M. guttatus* cytoplasmic backgrounds, respectively; $\chi^2 = 0.185$; $P = 0.67$) and showed the same expression of our focal QTL effects. In the full data set, both the LG7 and LG8 QTLs significantly affected floral bud initiation under short days (Fig. 4; LG7, $\chi^2 = 53.5$; $P < 0.0001$; LG8, $\chi^2 = 19.2$; $P < 0.0001$). Together, the two QTLs almost recapitulate the Catherine Creek parental phenotypes – 18% of the F_2s homozygous for the *M. guttatus* allele at both QTLs flowered under short days and 73% of the individuals homozygous for *M. nasutus* at both QTLs flowered (Fig. 4); the pattern of inheritance is also very similar to that of the allopatric line cross (Fig. 2). These results demonstrate that the same two large-effect QTLs are important for flowering time divergence between both allopatric and sympatric populations of *M. guttatus* and *M. nasutus*.

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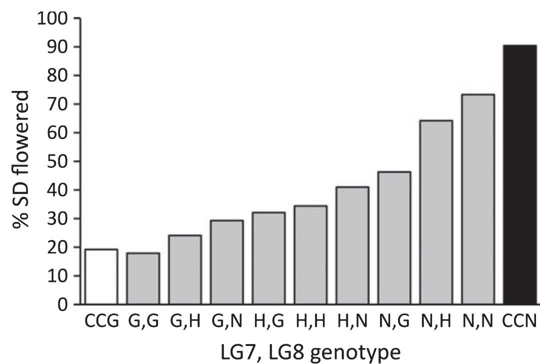

Fig. 4 Percentage of individuals that flowered under short days (SD), for Catherine Creek (CC) *Mimulus guttatus* (white; $n = 26$), CC *Mimulus nasutus* (black; $n = 21$), and all two-locus F_2 genotypes (gray; total $n = 757$) at LG7 and LG8 quantitative trait loci (QTLs). LG7 QTL flanking markers: distal, e251 or m114; proximal, m110. LG8 QTL flanking markers: distal, e829 or e96; proximal, m11.

Discussion

We identified two major QTLs that together explain divergence between outcrosser *M. guttatus* and selfer *M. nasutus* in the ability to flower under short days, a key adaptive trait that also contributes to reproductive isolation. We fine-mapped the most important critical photoperiod QTL, on LG7, to a < 300-kb region containing a single candidate gene. This genomic region was not associated with critical photoperiod variation within *M. guttatus* in a previous study (Friedman & Willis, 2013), suggesting that the genetic basis of early spring flowering in *M. nasutus* may be novel. The smaller QTL, on the bottom of LG8, was fine-mapped to a region of *c.* 500 kb containing multiple candidates. In contrast to the LG7 QTL, this region also contains QTLs for critical photoperiod and vernalization requirement (Friedman & Willis, 2013), as well as flowering time under long days and other life-history traits within *M. guttatus* (Hall *et al.*, 2006, 2010; Lowry & Willis, 2010). Analysis of *M. guttatus*-background NILs suggests that *M. nasutus* alleles at both loci are necessary and sufficient for the switch to obligate short-day flowering, and thus may act as major speciation genes. Nonetheless, the additive nature of both loci indicates that intermediate genotypes could still have been visible to natural selection for early flowering during the divergence of *M. nasutus*. Finally, the same QTLs identified in our cross of allopatric populations also fully account for differences in photoperiodic responses between *M. nasutus* and *M. guttatus* from a sympatric site, indicating that these QTLs may be important for adaptation species-wide and act as species barriers in areas of contact.

Genetic architecture of interspecific differences in critical photoperiod

QTL mapping can both characterize broad patterns of genetic architecture of complex traits and identify genomic regions containing candidate molecular variants for further evolutionary analysis. Our bulk segregant and targeted mapping approach primarily focused on the former goal, as it can only detect major QTLs; however, our results do rule out a polygenic architecture for critical photoperiod divergence. This contrasts with the genetic architecture of floral traits in the same SF *M. nasutus* and IM62 *M. guttatus* cross (Fishman *et al.*, 2002). In that study, only three of 24 QTLs underlying floral traits accounted for more that 20% of the species difference in a given trait and F_2 hybrids did not recapitulate parental values, suggesting that *M. nasutus*'s transition to obligate self-fertilization was gradual, characterized by a 'moving optimum', and/or drawn from the abundant standing variation for mating system traits present within *M. guttatus* (Bodbyl Roels & Kelly, 2011). Our finding of a relatively small set of major mutations underlying short-day flowering suggests that *M. nasutus*'s ability to occupy habitats with transient water availability may have been a relatively abrupt transition.

Despite being measured as a discrete trait and only involving two major QTLs, short-day flowering appeared surprisingly quantitative. That is, although the *M. nasutus* allele at the larger LG7 QTL is partially recessive, the total dose of *M. nasutus* alleles cumulatively affected the probability that an individual crossed the threshold and initiated flowering (Figs 2, 4). This inheritance pattern is very similar to that seen for a pair of critical photoperiod QTLs (LG8a and LG8b) distinguishing annual and perennial *M. guttatus* populations from northern California (Friedman & Willis, 2013). The additivity and lack of epistasis we see in F_2 hybrids suggest that alleles promoting short-day flowering could have been readily recruited by natural selection. However, genetic background effects may also have been important during initial spread, as *M. nasutus* homozygosity at both loci was necessary for short-day flowering in the *M. guttatus* background BG_4.327 progeny. In addition, in both SF × IM experiments, all *M. nasutus* controls flowered before even the fastest flowering hybrids, indicating that loci beyond our critical photoperiod QTLs may modulate the speed of flowering under short-day conditions.

Candidate genes in the context of environmental cues

The network of genes associated with photoperiod-dependent flowering in *A. thaliana* often also affect flowering phenology in other taxa, providing a clear pool of initial functional candidates (Hayama & Coupland, 2004). However, the traditional divisions of the flowering time gene network into discrete, cue-specific pathways (e.g. photoperiod and vernalization) are beginning to blur. For example, the flowering behavior of mutants (Wilczek *et al.*, 2010) and mapping populations (Anderson *et al.*, 2012a) assayed in growth chambers is not always replicable in the field. Our experiments were conducted in a glasshouse under naturally fluctuating day–night temperature conditions similar to those experienced by plants growing under short days in the field (i.e. cool nights and warm days). Thus, although we cannot pin interspecific differences in flowering time on photoperiod sensitivity *independent* of temperature, our results may better capture natural flowering time variation. Most notably, Friedman & Willis (2013) reported little or no difference in critical photoperiod between *M. guttatus* and *M. nasutus* lines from Catherine Creek grown in growth chambers at constant (21°C) temperature.

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However, these populations flower at distinct times in the field (A. Kenney & A. Sweigart, pers. obs.) and our experiment, and hybrids segregated for major critical photoperiod QTLs (Fig. 4). Because Catherine Creek populations are potentially hybridizing, introgression may contribute to genetic differences between the lines used in the two studies. However, the constant temperatures used in the earlier study may also have masked some ecologically relevant divergence in critical photoperiod that our experiment revealed.

For each QTL, we identified strong positional and functional candidates that will be accessible to targeted molecular characterization. The 35–45 genes under the major LG7 QTL included only one flowering time network candidate (*Migut.G00570*), which is annotated as the first two (of four expected) exons of a phosphatidylethanolamine-binding protein (PEBP) in the FLOWERING LOCUS T/TERMINAL FLOWER 1 (FT/TFL1) family. In *A. thaliana,* FT integrates signals to promote flowering, whereas TFL1 represses flowering (Pin & Nilsson, 2012); however, the expression patterns and interactions of FT/TFL1 family members can transcend simple repressor/promoter dichotomies (Ballerini & Kramer, 2011). Members of the FT/TFL1 family have been implicated in natural variation in flowering phenology, including photoperiod- and temperature-dependent responses, in diverse taxa (Blackman *et al.*, 2010; Pin *et al.*, 2010; Anderson *et al.*, 2012b; Koskela *et al.*, 2012; Grillo *et al.*, 2013; Nitcher *et al.*, 2013). The *M. guttatus* reference genome contains eight full FT/TFL1 family sequences (all four exons), as well as multiple, unlinked, partial sequences. *Migut.G00570* may be only a partial PEBP sequence because it is misassembled, but appears to be expressed as annotated in *M. guttatus* (V2.0 annotation; www.Phytozome.org). Even if it does not encode a complete FT/TFL1 sequence, however, *Migut.G00570* may actively regulate flowering. For example, an FT paralog with a frameshift mutation was recently shown to underlie a major photoperiod QTL in sunflower, indicating that apparently 'nonfunctional' FT-like genes can affect flowering phenology by interfering with other FT-like loci (Blackman *et al.*, 2010). Molecular population genetic analyses of the FT/TFL1 family in *Mimulus*, as well as detailed characterization of polymorphism and expression patterns of the LG7 QTL candidate, hold great promise for understanding flowering time evolution in yellow monkeyflowers and beyond.

The LG8 QTL contained two functional candidates, a homolog of GAI under the left edge of the 2-LOD drop confidence interval and a cluster of three SVP/AGL24 family genes under the peak. In *A. thaliana*, GAI and related proteins block gibberellic acid signaling and repress flowering under short days (Wilson *et al.*, 1992). SVP is a floral signal integrator that interacts with genes across flowering time pathways and directly regulates FT. Recently, a single amino acid substitution causing loss of SVP functionality was pinpointed as the source of a dramatic shift to early flowering under short-day conditions in some Asian accessions of *A. thaliana* (Méndez-Vigo *et al.*, 2013). Thus, the cluster of three SVP-like genes under our LG8 QTL, which also overlaps with vernalization and photoperiod QTLs mapped within *M. guttatus* (Friedman & Willis, 2013), is an attractive target for further investigation of within- and among-species flowering time variation in the *M. guttatus* complex.

Evolutionary history and consequences for gene flow

To understand the role of our flowering time QTLs in speciation, we must consider both their evolutionary history within the *M. guttatus* species complex and their consequences for gene flow on secondary contact. In the first case, it is particularly important to understand the directionality of genetic and phenotypic divergence. That is, where did the *M. nasutus* LG7 and LG8 QTL alleles come from, and what (if any) role did they play in the initial divergence and reproductive isolation of *M. nasutus*? Phylogeographic data suggest that *M. nasutus* was derived once from within highly diverse and paraphyletic *M. guttatus* (Sweigart & Willis, 2003; Modliszewski & Willis, 2012). Its ancestor is likely to have been an annual, as *M. nasutus* is collinear with IM62 and other widespread annual *M. guttatus* ecotypes rather than perennials (Fishman *et al.,* 2013). However, the geographical and elevational origin of *M. nasutus* is not yet clear. One plausible scenario is that *M. nasutus* evolved from a spring-flowering annual *M. guttatus* found at lower latitudes and altitudes than IM *M. guttatus.* In that scenario, the short critical photoperiod of *M. nasutus* could pre-date the origin of the species, and the causal QTLs would be shared with early-flowering *M. guttatus.* For the major LG7 QTL, however, this scenario is unlikely: Friedman & Willis (2013) found no evidence of LG7 involvement in critical photoperiod differences between LMC (a northern California annual *M. guttatus* with the same 10-h critical photoperiod as SF *M. nasutus*) and SWB (a northern California perennial with a > 13-h critical photoperiod). Therefore, the *M. nasutus* early-flowering LG7 QTL allele does not appear to be the common source of *M. guttatus* short-day flowering, and thus may be a unique adaptation to the extremely ephemeral habitats occupied by *M. nasutus.* Identification of the underlying molecular polymorphism, and characterization of its evolutionary history across the species complex, will be necessary to test this hypothesis. By contrast, the LG8 QTL is shared with within-*M. guttatus* QTLs for both vernalization requirement and critical photoperiod (Friedman & Willis, 2013). This co-localization may be coincidental, as multiple candidate genes lie under this broad QTL and the causal polymorphisms have not yet been identified. However, it is possible that the LMC-SWB LG8b photoperiod QTL of Friedman & Willis (2013) and our LG8 QTL share a common genetic basis. In that case, the more powerful *M. nasutus*-specific LG7 allele may have initially arisen in a relatively LMC-like *M. guttatus* background (e.g. one fixed for the early-flowering allele at the shared LG8 QTL), and thus could have spread rapidly when and where earlier spring flowering was favored.

Regardless of their origins, our QTLs probably contribute to current reproductive isolation between *M. nasutus* and *M. guttatus.* This may occur most importantly, but cryptically, by dictating habitat suitability on both regional and local scales (Sobel *et al.,* 2010). By initiating flowering under < 12-h days, *M. nasutus* can reproduce in shallow-soil sites that dry out by late spring. By contrast, our *M. guttatus* accessions delay flowering

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and are thus restricted to less ephemeral sites that remain moist through midsummer. Thus, a short critical photoperiod is a key component of *M. nasutus*'s drought avoidance strategy (Wu *et al.*, 2010; Ivey & Carr, 2012). By facilitating habitat specialization, as well as by promoting asynchronous flowering where the two species do co-occur, *M. nasutus*'s early flowering may be as important an isolating mechanism as autonomous self-fertilization. Ongoing investigations of phenotypic variation and genome-wide introgression patterns at Catherine Creek have the potential to reveal how the QTLs found in this study contribute to phenological behavior in nature, and whether (and under what environmental conditions) they promote assortative mating.

This work on the genetic basis for early spring flowering in selfer *M. nasutus* relative to congener *M. guttatus* provides insight into mechanisms of plant adaptation and speciation, particularly in the context of work on other traits in the same taxa (Fishman *et al.*, 2002) and parallel transitions in the same trait (Friedman & Willis, 2013). *Mimulus nasutus* appears to have taken a novel genetic path to adapt its flowering time to extremely early-drying habitats, and it will be interesting to explore whether this pattern is repeated in other parallel transitions. For example, we recently described a population of *M. guttatus* from extreme thermal soils in Yellowstone National Park that appears to have independently evolved short-day flowering, as well as annuality and self-pollination, from the local perennial ecotype (Lekberg *et al.*, 2012). Further study of the genetic and molecular basis of flowering variation across the *M. guttatus* complex holds great promise for understanding the causes and consequences of change in this evolutionarily labile and ecologically important, but genetically simple, trait.

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References

Alonso-Blanco C, Aarts MGM, Bentsink L, Keurentjes JJB, Reymond M, Vreugdenhil D, Koornneef M. 2009. What has natural variation taught us about plant development, physiology, and adaptation? *Plant Cell* **21**: 1877–1896.

Amasino R. 2010. Seasonal and developmental timing of flowering. *Plant Journal* **61**: 1001–1013.

Anderson JT, Inouye DW, McKinney AM, Colautti RI, Mitchell-Olds T. 2012a. Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. *Proceedings of the Royal Society B: Biological Sciences* **279**: 3843–3852.

Anderson JT, Lee C-R, Rushworth CA, Colautti RI, Mitchell-Olds T. 2012b. Genetic trade-offs and conditional neutrality contribute to local adaptation. *Molecular Ecology* **22**: 699–708.

Andrés F, Coupland G. 2012. The genetic basis of flowering responses to seasonal cues. *Nature Reviews Genetics* **13**: 627–639.

Ballerini ES, Kramer EM. 2011. In the light of evolution: a reevaluation of conservation in the CO–FT regulon and its role in photoperiodic regulation of flowering time. *Frontiers in Plant Science* **2**: 81.

Barr CM, Fishman L. 2010. The nuclear component of a cytonuclear hybrid incompatibility in *Mimulus* maps to a cluster of pentatricopeptide repeat genes. *Genetics* **184**: 455–465.

Basten C, Weir BS, Zeng Z-B. 2002. *QTL Cartographer: a reference manual and tutorial for QTL mapping.* Raleigh, NC, USA: Statistical Genetics, North Carolina State University.

Blackman BK, Rasmussen DA, Strasburg JL, Raduski AR, Burke JM, Knapp SJ, Michaels SD, Rieseberg LH. 2011. Contributions of flowering time genes to sunflower domestication and improvement. *Genetics* **187**: 271–287.

Blackman BK, Strasburg JL, Raduski AR, Michaels SD, Rieseberg LH. 2010. The role of recently derived FT paralogs in sunflower domestication. *Current Biology* **20**: 629–635.

Bodbyl Roels SA, Kelly JK. 2011. Rapid evolution caused by pollinator loss in *Mimulus guttatus. Evolution* **65**: 2541–2552.

Buckler ES, Holland JB, Bradbury PJ, Acharya CB, Brown PJ, Browne C, Ersoz E, Flint-Garcia S, Garcia A, Glaubitz JC *et al.* 2009. The genetic architecture of maize flowering time. *Science* **325**: 714–718.

Case AL, Willis JH. 2008. Hybrid male sterility in *Mimulus* (Phrymaceae) is associated with a geographically restricted mitochondrial rearrangement. *Evolution* **62**: 1026–1039.

Chiang GCK, Barua D, Dittmar E, Kramer EM, de Casas RR, Donohue K. 2012. Pleiotropy in the wild: the dormancy gene DOG1 exerts cascading control on life cycles. *Evolution* **67**: 883–893.

Chiang GCK, Barua D, Kramer EM, Amasino RM, Donohue K. 2009. Major flowering time gene, *FLOWERING LOCUS C*, regulates seed germination in *Arabidopsis thaliana. Proceedings of the National Academy of Sciences, USA* **106**: 11661–11666.

El-Assal S, Alonso-Blanco C, Peeters A. 2001. A QTL for flowering time in Arabidopsis reveals a novel allele of *CRY2. Nature Genetics* **29**: 435–440.

Elzinga JA, Atlan A, Biere A, Gigord L, Weis AE, Bernasconi G. 2007. Time after time: flowering phenology and biotic interactions. *Trends in Ecology & Evolution* **22**: 432–439.

Fishman L, Aagaard J, Tuthill JC. 2008. Toward the evolutionary genomics of gametophytic divergence: patterns of transmission ratio distortion in monkeyflower (*Mimulus*) hybrids reveal a complex genetic basis for conspecific pollen precedence. *Evolution* **62**: 2958–2970.

Fishman L, Kelly AJ, Morgan E, Willis JH. 2001. A genetic map in the *Mimulus guttatus* species complex reveals transmission ratio distortion due to heterospecific interactions. *Genetics* **159**: 1701–1716.

Fishman L, Kelly AJ, Willis JH. 2002. Minor quantitative trait loci underlie floral traits associated with mating system divergence in *Mimulus. Evolution* **56**: 2138–2155.

Fishman L, Willis JH. 2005. A novel meiotic drive locus almost completely distorts segregation in *Mimulus* (monkeyflower) hybrids. *Genetics* **169**: 347–353.

Fishman L, Willis JH, Wu CA, Lee Y-W. 2013. Comparative linkage maps suggest that fission, not polyploidy, underlies near-doubling of chromosome number within monkeyflowers (*Mimulus*; Phrymaceae). *Heredity*, in press.

Friedman J, Willis JH. 2013. Major QTLs for critical photoperiod and vernalization underlie extensive variation in flowering in the *Mimulus guttatus* species complex. *New Phytologist* **199**: 571–583.

Gavrilets S, Vose A. 2007. Case studies and mathematical models of ecological speciation. 2. Palms on an oceanic island. *Molecular Ecology* **16**: 2910–2921.

Grillo MA, Li C, Hammond M, Wang L, Schemske DW. 2013. Genetic architecture of flowering time differentiation between locally adapted populations of *Arabidopsis thaliana. New Phytologist* **197**: 1321–1331.

Hall M, Lowry D, Willis J. 2010. Is local adaptation in *Mimulus guttatus* caused by trade-offs at individual loci? *Molecular Ecology* **19**: 2739–2753.

Hall MC, Basten CJ, Willis JH. 2006. Pleiotropic quantitative trait loci contribute to population divergence in traits associated with life-history variation in *Mimulus guttatus. Genetics* **172**: 1829–1844.

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Hall MC, Willis JH. 2006. Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. *Evolution* **60**: 2466–2477.

Hayama R, Coupland G. 2004. The molecular basis of diversity in the photoperiodic flowering responses of *Arabidopsis* and rice. *Plant Physiology* **135**: 677–684.

Ivey CT, Carr DE. 2012. Tests for the joint evolution of mating system and drought escape in *Mimulus*. *Annals of Botany* **109**: 583–598.

Kiang YT, Hamrick JL. 1978. Reproductive isolation in the *Mimulus guttatus–M. nasutus* complex. *American Midland Naturalist* **100**: 269–276.

Koskela EA, Mouhu K, Albani MC, Kurokura T, Rantanen M, Sargent DJ, Battey NH, Coupland G, Elomaa P, Hytonen T. 2012. Mutation in TERMINAL FLOWER1 reverses the photoperiodic requirement for flowering in the wild strawberry *Fragaria vesca*. *Plant Physiology* **159**: 1043–1054.

Leinonen PH, Remington DL, Leppälä J, Savolainen O. 2012. Genetic basis of local adaptation and flowering time variation in *Arabidopsis lyrata*. *Molecular Ecology* **22**: 709–723.

Lekberg Y, Roskilly B, Hendrick MF, Zabinski CA, Barr CM, Fishman L. 2012. Phenotypic and genetic differentiation among yellow monkeyflower populations from thermal and non-thermal soils in Yellowstone National Park. *Oecologia* **170**: 111–122.

Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**: 1754–1760.

Li J, Wang S, Zeng Z-B. 2006. Multiple-interval mapping for ordinal traits. *Genetics* **173**: 1649–1663.

Lowry DB, Willis JH. 2010. A widespread chromosomal inversion polymorphism contributes to a major life-history transition, local adaptation, and reproductive isolation. *PLoS Biology* **8**: e1000500.

Martin NH, Willis JH. 2007. Ecological divergence associated with mating system causes nearly complete reproductive isolation between sympatric *Mimulus* species. *Evolution* **61**: 68–82.

Méndez-Vigo B, Martínez-Zapater JM, Alonso-Blanco C. 2013. The flowering repressor *SVP* underlies a novel *Arabidopsis thaliana* QTL interacting with the ggenetic background. *PLoS Genetics* **9**: e1003289.

Méndez-Vigo B, Picó FX, Ramiro M, Martínez-Zapater JM, Alonso-Blanco C. 2011. Altitudinal and climatic adaptation is mediated by flowering traits and *FRI*, *FLC*, and *PHYC* genes in *Arabidopsis*. *Plant Physiology* **157**: 1942–1955.

Modliszewski JL, Willis JH. 2012. Allotetraploid *Mimulus sookensis* are highly interfertile despite independent origins. *Molecular Ecology* **21**: 5280–5298.

Nakazato T, Rieseberg LH, Wood TE. 2013. The genetic basis of speciation in the *Giliopsis* lineage of *Ipomopsis* (Polemoniaceae). *Heredity* **111**: 227–237.

Nitcher R, Distelfeld A, Tan C, Yan L, Dubcovsky J. 2013. Increased copy number at the HvFT1 locus is associated with accelerated flowering time in barley. *Molecular Genetics and Genomics* **288**: 261–275.

Pin PA, Benlloch R, Bonnet D, Wremerth-Weich E, Kraft T, Gielen JJL, Nilsson O. 2010. An antagonistic pair of FT homologs mediates the control of flowering time in sugar beet. *Science* **330**: 1397–1400.

Pin PA, Nilsson O. 2012. The multifaceted roles of *FLOWERING LOCUS T* in plant development. *Plant, Cell & Environment* **35**: 1742–1755.

Rieseberg LH, Blackman BK. 2010. Speciation genes in plants. *Annals of Botany* **106**: 439–455.

Rieseberg LH, Willis JH. 2007. Plant speciation. *Science* **317**: 910–914.

Salome PA, Bomblies K, Laitinen RAE, Yant L, Mott R, Weigel D. 2011. Genetic architecture of flowering-time variation in *Arabidopsis thaliana*. *Genetics* **188**: 421–433.

Sobel JM, Chen GF, Watt LR, Schemske DW. 2010. The biology of speciation. *Evolution* **64**: 295–315.

Sweigart AL, Fishman L, Willis JH. 2006. A simple genetic incompatibility causes hybrid male sterility in *Mimulus*. *Genetics* **172**: 2465–2479.

Sweigart AL, Willis JH. 2003. Patterns of nucleotide diversity are affected by mating system and asymmetric introgression in two species of *Mimulus*. *Evolution* **57**: 2490–2506.

Weller JL, Liew LC, Hecht VFG, Rajandran V, Laurie RE, Ridge S, Wenden B, Vander Schoor JK, Jaminon O, Blassiau C *et al.* 2012. A conserved molecular basis for photoperiod adaptation in two temperate legumes. *Proceedings of the National Academy of Sciences, USA* **109**: 21158–21163.

Wilczek AM, Burghardt LT, Cobb AR, Cooper MD, Welch SM, Schmitt J. 2010. Genetic and physiological bases for phenological responses to current and predicted climates. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**: 3129–3147.

Wilson RN, Heckman JW, Somerville CR. 1992. Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. *Plant Physiology* **100**: 403–408.

Wu CA, Lowry DB, Cooley AM, Wright KM, Lee YW, Willis JH. 2008. *Mimulus* is an emerging model system for the integration of ecological and genomic studies. *Heredity* **100**: 220–230.

Wu CA, Lowry DB, Nutter LI, Willis JH. 2010. Natural variation for drought-response traits in the *Mimulus guttatus* species complex. *Oecologia* **162**: 23–33.

Yano M, Katayose Y, Ashikari M. 2000. Hd1, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS*. *Plant Cell* **12**: 2473–2483.

Supporting Information

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

Table S1 Information on additional markers used in targeted mapping of *Mimulus nasutus* × *Mimulus guttatus* critical photoperiod QTLs

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