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Flowering time QTL in natural populations of *Arabidopsis thaliana* and implications for their adaptive value

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Abstract

The genetic basis of phenotypic traits is of great interest to evolutionary biologists, but their contribution to adaptation in nature is often unknown. To determine the genetic architecture of flowering time in ecologically relevant conditions, we used a recombinant inbred line population created from two locally adapted populations of *Arabidopsis thaliana* from Sweden and Italy. Using these RILs, we identified flowering time QTL in growth chambers that mimicked the natural temperature and photoperiod variation across the growing season in each native environment. We also compared the genomic locations of flowering time QTL to those of fitness (total fruit number) QTL from a previous three-year field study. Ten total flowering time QTL were found, and in all cases, the Italy genotype caused early flowering regardless of the conditions. Two QTL were consistent across chamber environments, and these had the largest effects on flowering time. Five of the fitness QTL colocalized with flowering time QTL found in the Italy conditions, and in each case, the local genotype was favoured. In contrast, just two flowering time QTL found in the Sweden conditions colocalized with fitness QTL and in only one case was the local genotype favoured. This implies that flowering time may be more important for adaptation in Italy than Sweden. Two candidate genes (*FLC* and *VIN3*) underlying the major flowering time QTL found in the current study are implicated in local adaptation.

Keywords: adaptation, ecological genetics, life history evolution, phenotypic plasticity, quantitative genetics

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Introduction

Understanding the genetic architecture of adaptive traits is a goal of many evolutionary biologists. Although progress has been made in discovering the genetic basis of many phenotypic traits (Mackay *et al.* 2009; Alonso-Blanco & Méndez-Vigo 2014), whether causative QTL and/or genes have relevance to adaptation in native environments can only be addressed through studies of locally adapted populations and a demonstration of the adaptive significance of allelic variation (Feder & Mitchell-Olds 2003; Barrett & Hoekstra 2011; Anderson *et al.* 2014). Information on the genes underlying adaptation can provide insight into how commonly adaptation is associated with fitness trade-offs due to antagonistic pleiotropy at a single locus or due to adaptive alleles that are unique to each habitat (Anderson *et al.* 2013). Furthermore, it is only through knowledge of the genes underlying adaptive traits that we can address the long-standing question of whether adaptation is commonly due to a few mutations of large effect (Orr 1998) or to many mutations of small effect (Fisher 1930); a question that remains unresolved (Rockman 2012).

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The use of a model system such as *Arabidopsis thaliana* (hereafter *Arabidopsis*) has advantages for studying the genetics of adaptive traits, as information from its sequenced and extensively annotated genome increase the likelihood of identifying causal genes. In particular, the genetics of flowering time has received much attention in *Arabidopsis* (Srikanth & Schmid 2011) due partly to the fact that flowering time is expected to be subject to strong selection (Simpson & Dean 2002). Studies on other plant systems have shown that the timing of reproduction is often crucial for fitness, as flowering too early or too late could reduce reproductive success or increase mortality due to drought (Sherrard & Maherali 2006) or cold temperatures (Inouye 2008; Munguía-Rosas *et al.* 2011). Furthermore, there is evidence that divergent selection on flowering time can contribute to local adaptation among populations (Hall & Willis 2006). Studies on *Arabidopsis* demonstrate latitudinal clines in flowering time across accessions (Stinchcombe *et al.* 2004) and selection on flowering time in some environments (Korves *et al.* 2007; Scarcelli *et al.* 2007; Li *et al.* 2010; Fournier-Level *et al.* 2013). Genes in the flowering time pathway that perceive and respond to environmental stimuli have been identified in *Arabidopsis* (Srikanth & Schmid 2011), such as FLOWERING LOCUS C (*FLC*) and FRIGIDA (*FRI*), both of which are affected by cold temperatures (Michaels & Amasino 1999; Johanson *et al.* 2000).

Despite the numerous studies that investigate the genetic basis of flowering time in *Arabidopsis*, there is surprisingly little evidence that these genes contribute to adaptation in natural populations. One approach towards this aim has been to examine patterns of variation in candidate genes. Among *Arabidopsis* accessions, correlations between latitudinal variation and allelic variation in candidate genes such as *FLC* and *FRI* have been found (Caicedo *et al.* 2004; Méndez-Vigo *et al.* 2011). Although these results demonstrate striking correlational patterns, experimental studies are better able to show causative links between flowering time genes and fitness. For example, Korves *et al.* (2007) planted 136 European *Arabidopsis* accessions in a common garden in Rhode Island and found that functional *FRI* alleles increased winter survival in a fall cohort and decreased fecundity in a spring cohort, although these effects depended on an interaction with *FLC*.

Studies that investigate candidate genes are appealing as we ultimately hope to identify the genes important in natural variation and adaptation. However, they also assume a priori that these are the primary genes underlying flowering time variation in natural populations. In contrast, both genome-wide association studies and quantitative trait loci (QTL) studies use markers that are distributed across the genome and allow the identification of genomic regions that contain the causal loci due to their linkage disequilibrium with the markers. These studies therefore make no a priori assumptions about the genes important for flowering time and adaptation. While association studies have the advantage of being able to examine allelic variation across large numbers of *Arabidopsis* accessions, extensive population structure makes it difficult to distinguish adaptive allelic variation from spurious associations between markers and traits (Zhao *et al.* 2007). Atwell *et al.* (2010) performed an association study on flowering time phenotypes among 199 genotypes and found an overrepresentation of a priori candidate genes within their peaks of association. However, the authors relied heavily on the presence of these candidate genes to differentiate true associations from false, as both selection and population structure can cause linkage disequilibrium among unlinked loci. In contrast, QTL mapping studies use experimental populations such as F_2 hybrids or recombinant inbred lines (RILs) in which recombination breaks up associations among alleles. Using 117 RILs derived from five mapping populations of *Arabidopsis*, Fournier-Level *et al.* (2013) found differential selection on flowering time genomic regions across four European common gardens.

Although QTL mapping is a powerful means of detecting the genetic basis of phenotypic variation, many QTL studies of flowering time in *Arabidopsis* have used crosses involving the laboratory strains Landsberg erecta (*Ler*) or Columbia (*Col*), (see Grillo *et al.* 2013; for a comprehensive review). These strains have early flowering phenotypes due to mutations that impair FRIGIDA (*FRI*) function (Johanson *et al.* 2000), and therefore, studies using *Ler* or *Col* as a mapping parent unsurprisingly often show that *FRI* has a large effect on flowering time. While these laboratory strains have provided crucial information on the biochemical pathways involved in flowering time, only QTL studies that use natural populations will provide insight into the genes that are important for natural variation in flowering time. Further, it is rarely known whether the populations under study are adapted to their local habitats, and this is necessary for addressing questions about adaptive trade-offs and the genetic architecture of adaptive traits.

The current study takes advantage of a large mapping population created from natural populations of *Arabidopsis* from Sweden and Italy. An extensive reciprocal transplant study conducted with these populations provided the first evidence that native *Arabidopsis* are adapted to their local habitats (Ågren & Schemske 2012; Lowry 2012), and thus presents a unique opportunity to dissect the genetic variation that is relevant to local adaptation. In addition, recent studies to map

fitness QTL in RILs grown in the native environments have identified many of the genomic regions that are important for variation in fitness in the field (Ågren *et al.* 2013). Here, we use a set of 528 RILs from these two locally adapted populations of *Arabidopsis* from Sweden and Italy to map QTL for flowering time in simulated environmental conditions. We then compare the genomic location of our flowering time QTL to the location of fitness QTL from three years of field studies (Ågren *et al.* 2013) to determine whether flowering time QTL affect fitness and whether they contribute to fitness trade-offs among sites.

There are two reasons to suspect that flowering time may be involved in local adaptation between the parental populations used in the current study. First, the study populations are located near the northernmost and southernmost margins of the native range of *Arabidopsis* in Europe and experience large differences in temperature and photoperiod which may contribute to geographic differences in selection on flowering time. Second, there is substantial genotype by environment interaction for flowering time, with the Italy population flowering 33–50 days earlier in Italy but just 3 days earlier in Sweden (Ågren & Schemske 2012).

Grillo *et al.* (2013) performed a study using F_2s from these mapping parents to investigate the genetic architecture of flowering time under laboratory conditions with and without vernalization. The current study builds on those results by using a large RIL mapping population, which allows greater precision in estimating flowering time through the use of replicate genotypes and presents the opportunity to compare flowering time QTL with fitness QTL that were recently mapped using the same set of RILs in the field (Ågren *et al.* 2013). In addition, we grew plants in growth chambers programmed to mimic the natural temperature and photoperiod fluctuations found during a typical growing season in *Arabidopsis* in Sweden and Italy (Fig. 1). Many studies of the genetics and fitness effects of flowering time in *Arabidopsis* do not grow plants under the environmental conditions typical of the parental populations (Grillo *et al.* 2013; but see Li *et al.* 2006), despite ample evidence that the environment has a large effect on the identity of flowering time QTL (Weinig *et al.* 2002; Li *et al.* 2006; Brachi *et al.* 2010). Measuring flowering time under relevant environmental conditions is important for elucidating the QTL that are responsible for flowering time variation in native habitats (Zuellig *et al.* 2014). Moreover, the dynamic changes

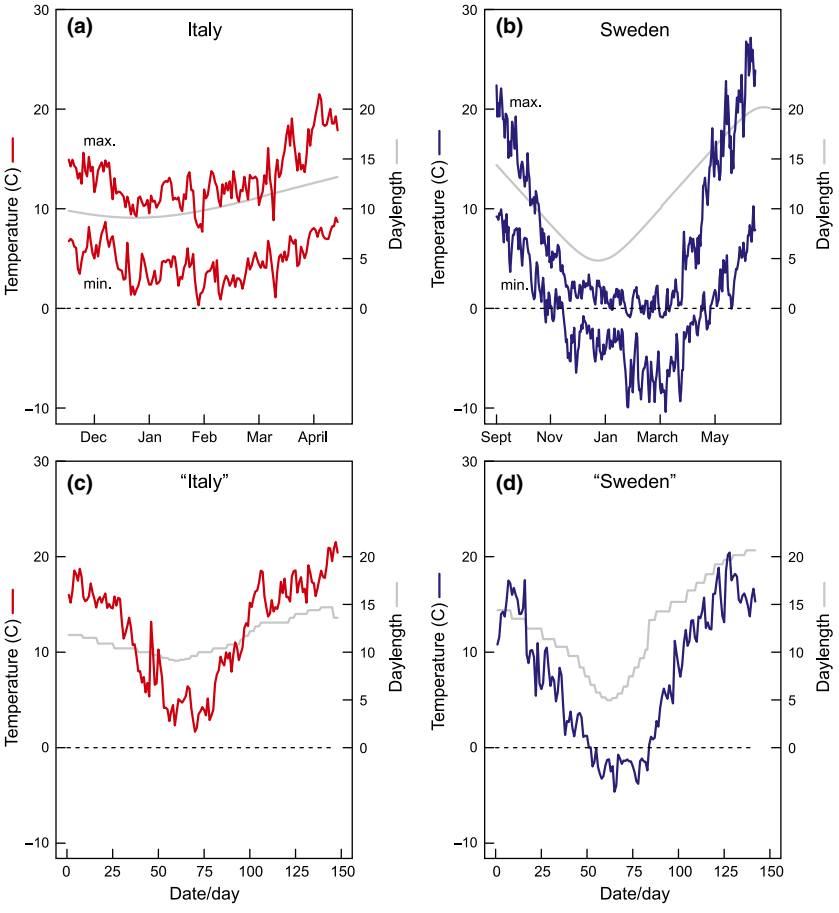

Fig. 1 A comparison of field temperatures (a,b) and growth chamber temperatures (c,d). Field data were recorded from both the air and the soil over four growing seasons at the native sites in Italy (a) and Sweden (b). The coloured lines represent the means of the absolute minimum and absolute maximum temperatures recorded from each day across the four growing seasons. Photoperiod is represented by the grey line, and data are taken from the U.S. Naval Observatory. The bottom two panels display the average temperature and photoperiod for each day in the Italy (c) and Sweden (d) chamber conditions.

among temperature and photoperiod across a growing season may be distinct from the fixed environmental conditions that are often used in laboratory studies (Li *et al.* 2006). The use of growth chambers that mimic the range of variation in temperature and photoperiod conditions experienced in the field allows us to isolate the effects of these environmental factors believed to play a large role in flowering time variation in *Arabidopsis* without the statistical noise of microhabitat variation in soil moisture, herbivores or pathogens.

We address the following questions: (i) What are the number and effect sizes of QTL underlying flowering time under simulated environmental conditions? (ii) Do these QTL colocalize with known flowering time genes? (iii) Does the identity of flowering time QTL differ between plants grown in simulated Sweden and Italy environments? (iv) Do flowering time QTL colocalize with genomic regions known to affect fitness in the field?

Methods

Field localities and RIL construction

We focus on two locally adapted populations of *Arabidopsis* (Ågren & Schemske 2012); one in north-central Sweden (Rödåsen; N 62°48′ E 18°12′) and one in central Italy (Castelnuovo; 42°07′ E 12°29′), that represent the northern and southern limits of the native range in Europe (Koornneef *et al.* 2004). Both populations exhibit a winter annual life history; seeds germinate in the autumn and overwinter as rosettes. Plants flower during March–April in Italy and May–June in Sweden (Ågren & Schemske 2012). Recombinant inbred lines (RILs, $n = 528$) were created by selfing F1 plants derived from a cross between an individual from the Swedish locality (♂) with an individual from Italy (♀) for nine generations. These RILs were genotyped for 348 SNPs that were evenly spaced across the five nuclear chromosomes of the Columbia physical map. For further details, see Ågren *et al.* (2013).

Experimental setup

Approximately 40 sterilized seeds from each RIL and parents were sown on sterilized petri dishes with media consisting of Gambog's B-5© nutrient mix, Bacto© Agar and ultrapure water. Dishes were wrapped in parafilm and cold-stratified in the dark at 4 °C for 5 days to break seed dormancy. Native populations in both Italy and Sweden experience cold periods at or below this temperature in the field during germination. Afterwards, the dishes were moved into a growth chamber with a constant temperature of 22 °C, 16 h days, and a photosynthetically active radiation (PAR) level of 125 μmol $m^{-2}s^{-1}$ using a combination of fluorescent and incandescent lights. The dishes were randomized throughout the chamber every day.

After 8–10 days in the chambers, seedlings were transplanted into 5.3-cm-long tubes filled with a 1:1:1 mixture of sure-mix, perlite and vermiculite. Seedlings were then returned to the chamber for another 8 days before randomizing replicates from each RIL across six 75 cm × 70 cm plastic trays. We programmed two specialty chambers designed to hold subfreezing temperatures (BioChambers Inc. Model# GC-20) to mimic the natural photoperiod and the range of temperatures of the Swedish and Italian sites (Fig. 1). The programmes were based on photoperiod data from the U.S. Naval Observatory and field temperatures that were recorded directly at the parental sites (see Ågren & Schemske 2012) once each hour from November 2003 to July 2008, with a HOBO Temperature Data Logger (HOBO Pro Data Logger Series® H08-031-08). We recorded air temperatures about 30 cm above the ground and soil temperatures ~1 cm below the soil surface. As *Arabidopsis* spends its early life history near the soil as a rosette, but is also exposed to air temperatures after bolting, we incorporated minimum and maximum temperatures from both the air and soil measurements to establish the chamber conditions. To simulate the pattern of variation experienced by seedlings in a typical year, temperatures in the growth chambers were varied on a 24-h cycle and were calculated by averaging the absolute minimum and maximum temperatures of the air and soil for any given day. Temperatures for each day were randomly selected among years but kept in a chronological sequence. Temperature data loggers (U14 LCD) were used to record the temperature settings in the growth chambers in the Sweden experiment to verify that the chambers were holding the programmed temperatures.

The chamber regime corresponded to the growing season of *Arabidopsis*; September–June in Sweden and October–April in Italy. This regime approximately matched the number of days of the life cycle (germination to seed production) for the Italy environment (148 days, Fig. 1). However, due to space and time constraints, and because specialty chambers routinely malfunction at subzero temperatures, the Sweden environment was shortened by compressing its natural life cycle of 284 days to 142 days in the chamber, such that every 2 days in the field became 1 day in the chamber (Fig. 1). Despite not corresponding to equal numbers of days in the field for the Sweden environment, our goal was to capture the range of variation experienced by plants across their life cycle in Sweden.

Six and eight seedlings from each RIL were used for the Italy and Sweden conditions, respectively, as well as 200 of each parent for both conditions. We used

more replicates in the Sweden experiment due to the increased mortality expected from freezing damage in Sweden conditions. To compensate for having fewer plants, extra plants were used as spacers in the Italy treatment so that the density of plants remained constant between treatments.

The trays were watered with deionized water and ½ strength Hoagland's solution as needed. Every 3 days, trays were randomized both within and between the chambers until plants began flowering. To avoid damaging inflorescences, randomization was ceased when plants began to flower and during freezing in the Sweden conditions. A preliminary analysis suggested that the effect of tray explained a relatively small amount of the variation compared to the effect of line (0.3% vs. 61.5% in the Italy environment; 7.0% vs. 30.1% in the Sweden environment) and therefore was not used as a covariate in the final analysis.

In the Swedish environment, there was high mortality and tissue damage during freezing conditions. We quantified the percentage of tissue damage for each plant using digital photographs taken before and after freezing conditions, in order to determine the extent to which variation in flowering time among genotypes was influenced by differences in tissue damage. However, an analysis suggested that damage explained a relatively minimal amount of the variation in flowering time (2%) and will not be discussed further. In both environments, plants were censused every day, and date of first flowering was recorded when the first petals became visible.

QTL analysis

For each RIL in each environment, we calculated the mean time to first flower. RILs that had fewer than three individuals survive to flower in the Swedish conditions were excluded from the analyses. Of the 528 lines planted in the Sweden experiment, 293 lines had three or more individuals survive to flower and were used in the analysis. We chose a minimum of three replicates per RIL as the best compromise between obtaining RIL mean estimates averaged over multiple trays and having a sufficient number of RILs for QTL mapping. A preliminary analysis with a minimum of two replicates per RIL surviving to flower yielded similar results to our final data set with the exception of a loss of the small-effect QTL on chromosome 2 (not shown). It was expected that the flowering time of genotypes with high survival in Sweden would, on average, flower later in Italy than genotypes that were excluded from the Sweden analysis due to low survival. However, the genotypes excluded from the Sweden analysis actually had greater average flowering times in Italy than the genotypes included in the Sweden analysis (62.1 days to 60.7 days, respectively; $P < 0.0001$). In the Italy analysis, all of the 525 lines planted were used, and this included all but three of the 293 lines (1%) used in the analysis for the Sweden conditions.

QTL mapping for mean time to first flower in each environment was conducted using R/qtl (Broman *et al.* 2003) and Haley Knott regression. To calculate thresholds for incorporating additive QTL and epistatic interactions at experiment wise $\alpha = 0.05$, 10 000 permutations were performed with an automated stepwise model selection scanning for additive and epistatic QTL at each step (Manichaikul *et al.* 2009). We then fit the refined model with ANOVA to calculate the effect size and percentage variance explained for each QTL. Because the automated stepwise procedure is sensitive to departures from normality, we first transformed the data by quantile normalization (Broman & Sen 2009). We then fitted this model with the non-normalized data to generate allelic effect sizes on the raw scale, which were subsequently multiplied by two to produce genotypic effect sizes for the alternate homozygotes.

Stepwise QTL analyses can sometimes result in spurious QTL that are artefacts of reduced recombination between adjacent markers. (Broman & Sen 2009). A manual inspection of our data revealed two QTL at adjacent markers on chromosome 1 in the Italy conditions, one of which was spurious and driven by a single recombinant genotype. In this case, we refitted a model with only a single QTL at this position. Between the two environmental conditions used in the current experiment, QTL were deemed to be the same if their 95% credible intervals were each <15.2 cM and they overlapped with each other (Ågren *et al.* 2013).

To identify likely candidate genes within the 95% credible intervals of our flowering time QTL, we used data sets of gene annotations (GOSLIM file), and genomic locations (ver. 9 GFF) downloaded from TAIR (The *Arabidopsis* Information Resource). We filtered the list of genes to those containing 'flowering' or 'vernalization' in their 'GO' terms and those for which there was experimental evidence that the gene influenced flowering (direct assay, mutant phenotypes, expression patterns, or genetic or physical interactions). Finally, we filtered this list of genes to include only those in which the start position occurred within 300 Kb (~1 cM, the average distance between markers) of the ends of the 95% credible intervals of our flowering time QTL. We did not search for candidate genes under QTL with very wide credible intervals, defined here as >1/4 of the smallest chromosome (15.2 cM).

Colocalization of flowering time and fitness QTL

We compared the genomic location of flowering time QTL found in the current study to that of fitness QTL

found in the field as reported in Ågren *et al.* (2013). In brief, in three consecutive years (2009–2011), Ågren *et al.* (2013) planted seedlings of 398 RILs and the two parents into experimental gardens located at the sites of the source populations. For each site-year combination, cumulative fitness (total fruits per plant) was quantified, and QTL mapped. They identified a total of 15 distinct QTL, of which 10 were shared between sites. See Ågren *et al.* (2013) for further details.

The genomic locations of the flowering time QTL and fitness QTL were compared to determine whether they colocalize to the same genomic position. As far as we are aware, there is no standard quantitative approach for evaluating colocalization of QTL, particularly from multiple QTL models. Weinig *et al.* (2002) considered two or more QTL to colocalize if the likelihood ratio (LR) test statistic remained above the significance threshold between the two point estimates. However, it is possible for two adjacent, large effect QTL to lead to this pattern as well. Leinonen *et al.* (2013) considered QTL overlap significant if both QTL peaks overlapped with the credible intervals of one another, although in some cases credible intervals can be quite large. Huang *et al.* (2010) conducted multiple-trait composite interval mapping to evaluate the probability that more than one trait are due to a pleiotropic locus. Power for this method requires that sufficient recombination between the point estimates of adjacent QTL has occurred in the mapping population. More work is needed to establish guidelines for statistically determining whether QTL from different studies map to the same locus.

In the absence of consistent methods, we used two different criteria for evaluating colocalization of flowering time QTL and fitness QTL. The most stringent criteria for colocalization required that the point estimate of the flowering time QTL was within the range of the point estimates of unique fitness QTL identified in different years and that the flowering time QTL credible interval was <15.2 cM (<¼ the length of the smallest chromosome) or that the range of point estimates of flowering time QTL that were shared between environments overlapped the range of point estimates of fitness QTL. The less stringent criteria required that point estimates for flowering time QTL were within the range of point estimates for fitness QTL without regard to the size of credible intervals.

Results

Flowering time phenotypes

Sweden parents flowered later than Italy parents in both environments, and flowering was delayed in the Sweden chamber relative to the Italy chamber (Fig. 2). In the Sweden conditions, the average flowering time (defined as the number of days after transplanting) was 138, 128 and 132 days for the Sweden parents, Italy parents and RILs, respectively, while in the Italy conditions, the average flowering times were 124, 104 and 118 days for the Sweden parents, Italy parents and RILs, respectively (Fig. 2). There was a significant positive correlation between RIL mean flowering times between the two chamber environments ($r = 0.50$, $P < 0.0001$; Fig. S1, Supporting Information).

Genetic basis for flowering time

A total of nine QTL contributing to variation in flowering time were found in the Italy conditions, and three QTL were found in the Sweden conditions. Two QTL were shared between environments (Figs 3 and 4), resulting in ten unique QTL (Table 1). The direction of the effect was the same in both environments for all QTL – the Italy genotype caused earlier flowering, while the Sweden genotype caused later flowering (Fig. 5).

The nine flowering time QTL found in the Italy conditions explained 61% of the difference between the parents, while the three flowering time QTL in the Sweden conditions explained 86% of the difference between the parents. The individual QTL with the largest effect on flowering time in both conditions was FlrT 5:1 (Table 1). Substitution of the Swedish genotype at this locus delayed flowering by 2.7 days in the Italy

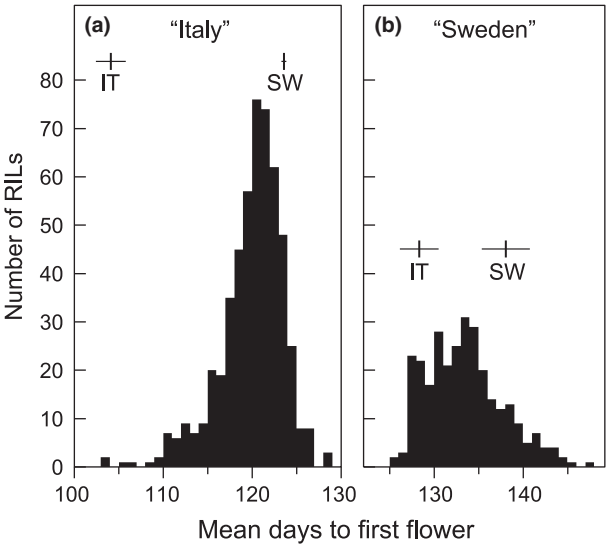

Fig. 2 The distribution of RIL means for flowering time in environmental chambers simulating the temperature and photoperiod in Italy (a) and Sweden (b). 'IT' and 'SW' represent mean flowering times and 95% confidence intervals for the two parents.

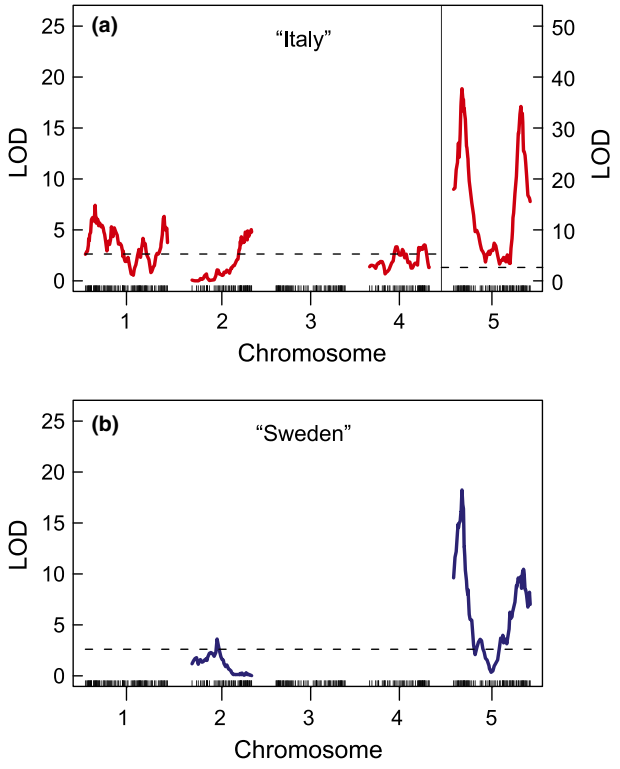

Fig. 3 Stepwise LOD profiles produced from multiple QTL models (Broman & Sen 2009) for flowering time in Italy (a) and Sweden (b). Only profiles of significant QTL are shown. Note the difference in scale for chromosome five in Italy.

conditions and 3.8 days in the Sweden conditions, which represents 14% and 39% of the parental difference in flowering times, respectively (Fig. 5). Substitution of the Swedish genotype at the QTL with the next largest effect (FlrT 5:4, Table 1) delayed flowering in the Italy conditions by 2.6 days and 3.0 days in the Sweden conditions (13% and 30% of the difference between the parents, respectively). Substitution of the Swedish genotype at any of the QTL unique to the Italy environment would delay flowering by 0.7–1.3 days in the Italy conditions or 4%–7% of the difference between the parents. A substitution of the Swedish genotype at the QTL unique to the Sweden environment delayed flowering by 1.6 days in the Sweden conditions or 17% of the difference between the parents (Fig. 5; Table 1).

No epistatic interactions among flowering time QTL were detected based on the stepwise model selection procedure. Heat-maps showing strength (LOD) of pairwise interactions among all loci do show some minor interactions, but these effects were very small when compared to the additive effects, and did not survive the model selection process (Fig. S2, Supporting Information).

G x E interactions

Although we found one QTL unique to the Sweden environment and seven unique to the Italy environment, the two QTL with the largest effects were found in both experimental conditions. The reaction norms for the two chamber environments show that the environ-

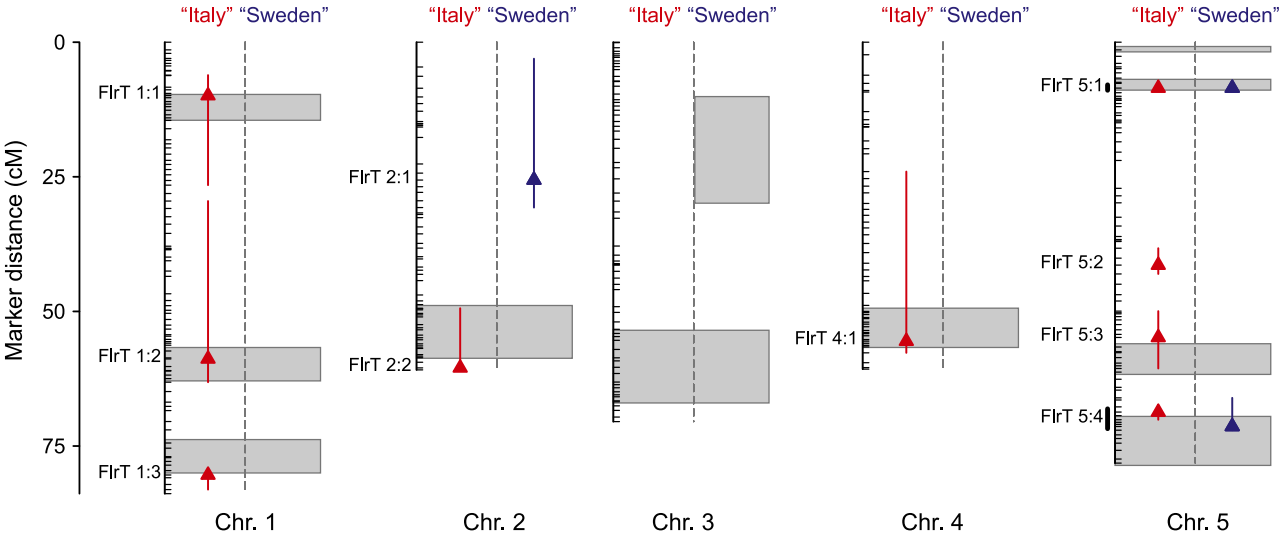

Fig. 4 Flowering time QTL detected in growth chambers and fitness QTL detected in the field. The five chromosomes are represented by vertical black lines with marker positions at horizontal ticks. Arrows indicate flowering time QTL position and the direction of the effect of the Swedish genotype (in all cases it resulted in later flowering). The 95% Bayesian credible intervals are shown by the vertical line (red = Italy chamber, blue = Sweden chamber). The dark line next to the flowering time label name indicates the range of the point estimates when flowering time QTL were found in more than one environment (FlrT 5:1 and FlrT 5:4). Shaded boxes represent the range of point estimates from fitness QTL detected in more than one site x year combination in the field (Ågren *et al.* 2013).

Table 1 Flowering time QTL and their chromosomal positions, LOD scores and effect sizes expressed as the percentage of the difference between the parental flowering times, percentage variance explained (PVE) and effect of the Swedish genotype. The Bayesian 95% confidence intervals and candidate genes that colocalize are also indicated.

Env.	QTL	Chr.	Pos.	LOD	Percentage diff. b/w parents	PVE	Swedish genotypic effect (SE)	Bayesian 95% CI	Candidate genes
IT	FlrT 1:1	1	9.9	7.41	5.3	1.87	1.02 (0.19)	6.1–26.6	
IT	FlrT 1:2	1	58.8	4.17	3.6	1.04	0.70 (0.21)	29.5–63.2	
IT	FlrT 1:3	1	80.4	6.33	5.1	1.59	1.00 (0.21)	79.6–83.1	*HAC1*
IT	FlrT 2:2	2	60.5	5.03	3.8	1.26	0.75 (0.19)	49.4–60.9	*FUS1; FES1; SRO1; ELF4; FBH4; VOZ2; CKB4; SPA1; CCA1*
IT	FlrT 4:1	4	55.5	3.51	3.9	0.87	0.76 (0.20)	24.0–57.7	
IT	FlrT 5:1	5	8.5	37.72	13.8	10.92	2.67 (0.21)	8.5–8.5	*FLC*
IT	FlrT 5:2	5	41.4	7.40	5.4	1.87	1.04 (0.24)	38.3–43.1	*TCH2*
IT	FlrT 5:3	5	54.8	5.10	6.5	1.27	1.26 (0.28)	50.0–60.6	*WNK8; U2AF35B; NUC; CUL4; APS1; LATE*
IT	FlrT 5:4	5	68.7	34.23	13.5	9.75	2.62 (0.24)	68.7–70.1	*VIN3*
SW	FlrT 2:1	2	25.6	3.61	16.7	3.71	1.64 (0.41)	3.1–30.7	
SW	FlrT 5:1	5	8.5	18.24	39.2	21.09	3.84 (0.41)	8.5–8.5	*FLC*
SW	FlrT 5:4	5	71.4	10.46	30.2	11.35	2.96 (0.41)	66.1–72.1	*VIN3; VIP4; ELF5*

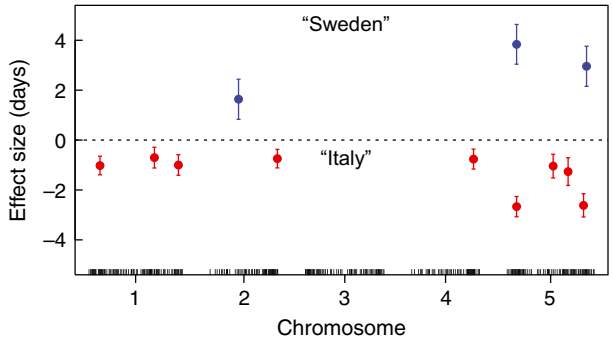

Fig. 5 Effect sizes and 95% confidence intervals of the local homozygous genotypes for flowering time QTL identified in the two experimental environments. In all cases, the Italy genotype was associated with earlier flowering and the Swedish genotype with later flowering.

ment causes a larger change in flowering time for the Italy parents relative to the Sweden parents (Fig. 6). This is consistent with results found in field studies (Ågren & Schemske 2012). Flowering time was significantly affected by the interaction between chamber environment and genotype at the marker loci closest to four of the flowering time QTL: FlrT 1:2, FlrT 1:3, FlrT 5:2 and FlrT 5:3 (Table S1, Supporting information). In all cases, individuals with alternate alleles at these loci have larger differences in their flowering times in Italy conditions than Sweden conditions.

Candidate genes

Candidate genes were found within several of the (<15.2 cM) flowering time QTL regions (Table 1). Among the largest effect QTL, the flowering time gene FLOWERING LOCUS C (*FLC*) colocalizes with FlrT 5:1. Within the QTL region FlrT 5:4, the candidate gene *VIN3* was found within the range of point estimates, and *VIP4* and *ELF5* were found within the credible interval of this QTL in the Sweden conditions (Table 1).

Colocalization with fitness QTL

There was strong evidence for colocalization between fitness QTL and two flowering time QTL (Fig. 4). Both QTL FlrT 5:1 and FlrT 5:4 were found in both environments and overlapped with the point estimates of fitness QTL. Furthermore, these QTL had the largest effects on flowering time and colocalized with the candidate genes described above. The point estimates in the two chambers for FlrT 5:1 were not only identical to each other (Table 1), they were identical to the point estimate for a fitness QTL found in the field in Sweden in 2009 and within only 1 cM of a fitness QTL found in Italy in 2010 (See Fig. 4). This does not mean that we have identified the causal loci, but simply that despite recombination among markers in this genomic region, the same marker is the most closely linked with the causal loci in all of these instances. For this fitness QTL, the Italy genotype increased fitness in both Italy and Sweden.

Although the point estimates for FlrT 5:4 differed between the Italy and Sweden chambers, a likelihood ratio test comparing a two QTL model to a single QTL model using the peak of the summed LOD profiles (e.g. Leinonen *et al*. 2013) indicated that the two QTL model did not offer a significant improvement over a

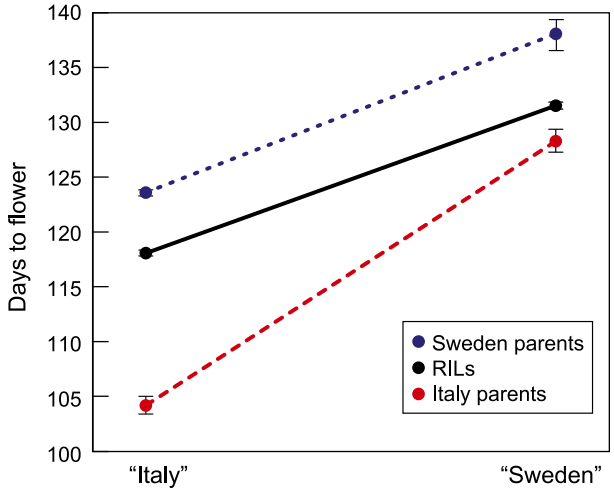

Fig. 6 The mean flowering times and 95% confidence intervals of the RILs and parents in both environments.

one QTL model ($\chi^2 = 0.71$, d.f. = 1, $P = 0.339$), so we cannot reject that they are the same QTL. The range of point estimates between chambers for FlrT 5:4 also overlaps the range of point estimates for fitness QTL found in the field (Fig. 4). For this fitness QTL, the Italy genotype increased fitness in Italy in all three years of study and the Swedish genotype increased fitness in Sweden in 2011.

Three flowering time QTL had point estimates within the range of point estimates for fitness in the field, but had confidence intervals larger than 15.2 cM: FlrT 1:1, FlrT 1:2 and FlrT 4:1 (Fig. 4; Table 1). These QTL were unique to the Italy environment and colocalized with QTL for which the Italy allele increased fitness in Italy (in all 3 years for FlrT 1:2 and 4:1 and in 2010 for FlrT 1:1). However, due to the large credible intervals of these QTL, we are less confident about their chromosomal positions.

Discussion

Number and effect sizes of flowering time QTL

We found evidence for a relatively small number of QTL controlling flowering time in both experimental environments. The two QTL with the largest effects were also shared between environments, and explained 13–14% and 30–39% of the difference between the parents in the Italy and Sweden environments, respectively. Using an F_2 population produced from the same parents as the mapping population in the current experiment, but grown under different experimental conditions, Grillo *et al.* (2013) also identified these QTL, which further supports their significant effects on flowering time in these populations. In addition to the two shared flowering time QTL, we found one QTL that was unique to the Sweden environment and explained 17% of the difference between the parents, while 7 QTL were unique to the Italy environment and explained between 4 and 7% of the difference between the parents.

The number of lines in the analysis for Italy is larger than that for Sweden due to increased mortality caused by freezing temperatures in the simulated Swedish winter. If we reanalyze the Italy data using only those lines that were included in the Sweden data set, we lose the power to detect 2 QTL that were observed in the full Italy data set and see a reduction in LOD scores. Although reducing the sample size by half reduced our power, we were still able to identify seven of the nine QTL from the original analysis. Even this smaller sample size ($n = 293$) is large relative to other studies (Huang *et al.* 2010; Fournier-Level *et al.* 2013), as many previous QTL studies for flowering time have used mapping populations with <150 individuals (Grillo *et al.* 2013). Although we believe the use of a large mapping population such as ours allows adequate power to detect QTL of moderate effect, it is likely that small-effect gene regions contributing to flowering time were not detected, and this may have inflated the estimation of the effects of QTL that were identified (Beavis 1998). Therefore, the ten QTL found in this experiment should be considered a minimum number. This is more than twice the number found on average in previous studies. Among 98 QTL experiments on flowering time in *Arabidopsis*, the average number of QTLs identified for flowering time was four, with a range of 1–10 (Grillo *et al.* 2013).

Candidate genes

Taking advantage of the well-studied flowering time pathway in *Arabidopsis* allowed the identification of several candidate genes for further investigation. The candidate gene *FLC* colocalizes with a large effect flowering time QTL found in both Italy and Sweden chamber environments (FlrT 5:1). Active *FLC* alleles repress flowering (Michaels & Amasino 1999) and vernalization reduces *FLC* expression to promote flowering (Sánchez-Bermejo *et al.* 2012). Natural variation in *FLC* has also been associated with flowering time variation in many *Arabidopsis* accessions from across its native range (Salomé *et al.* 2011; Sánchez-Bermejo *et al.* 2012). *FLC* was also implicated in flowering time both with and without vernalization in the F_2 mapping population study (Grillo *et al.* 2013).

The *FLC* protein coding region was sequenced in the Sweden and Italy parents of our mapping population, and no nonsynonymous polymorphisms were found

(Grillo *et al.* 2013). However, the cis-regulatory control of *FLC* has been supported by a number of studies. While Caicedo *et al.* (2004) identified two major *FLC* haplotypes that are differentiated by latitude among European accessions of *Arabidopsis*, no nonsynonymous polymorphisms were found between these haplotypes. Instead, it appears that vernalization induces the expression of different alternatively spliced transcripts. In addition, very low nonsynonymous diversity in *FLC* was found among 182 Iberian *Arabidopsis* accessions, and polymorphisms were located mainly in the first intron (Méndez-Vigo *et al.* 2011). The lack of nonsynonymous polymorphisms in *FLC* found across multiple studies strongly suggests that the causative allelic variation in this gene may be regulatory in nature.

Another candidate gene that colocalized with flowering time QTL in both the Italy and Sweden chamber is *VIN3*. Like *FLC*, this gene is located in the vernalization pathway and acts to repress levels of *FLC* through recognition of the length and duration of vernalization (Sung & Amasino 2004). Allelic variation in *VIN3* may cause adaptive differences in the cold conditions that are required for sufficient *FLC* repression to allow flowering to occur. In the study by Grillo *et al.* (2013), this gene also colocalized with flowering time QTL found in the vernalization treatment. Unlike *FLC*, there is evidence for nonsynonymous polymorphisms between the two parental lines in this gene. Grillo *et al.* (2013) found two single base pair substitutions as well as a three base pair indel that result in different amino acids between the parents.

We did not find evidence for the importance of *FRI* in these populations, which contrasts with many studies that have identified *FRI* as a major determinant of flowering time in *Arabidopsis* (reviewed in Grillo *et al.* 2013). Many QTL studies of the genetic basis of flowering time in *Arabidopsis* have used laboratory strains chosen for their rapid flowering and nonfunctional *FRI* alleles (Alonso-Blanco & Méndez-Vigo 2014). Although *FRI* may be an important component of the flowering time pathway, we did not find that allelic variation in *FRI* contributes to natural variation in flowering time among the populations in our study. Ultimately, understanding the genes that contribute to natural variation in flowering time across *Arabidopsis* populations can only be evaluated through studies that use natural populations, and these genes may not necessarily be the same genes found to be important for flowering time variation in laboratory strains.

G × E interactions on flowering time

Genes that regulate flowering are often involved in complex biochemical pathways that perceive environmental stimuli (e.g. vernalization and photoperiod) and initiate flowering (Simpson & Dean 2002). If different genes respond to different environmental cues, we would expect to identify unique flowering time QTL in each environment. Many QTL studies of flowering time in *Arabidopsis* have identified distinct QTL under different experimental conditions (Weinig *et al.* 2002; Li *et al.* 2006; Kover *et al.* 2009; Brachi *et al.* 2010). However, the two largest effect QTL identified in the current study were shared between environments. Therefore, the genes underlying these QTL may be involved in multiple biochemical flowering time pathways or operate independently of the environment. Interestingly, none of the candidate genes that colocalize with flowering time QTL from the Sweden chamber are part of the photoperiod pathway. This may be due to the longer duration and stronger intensity of cold temperatures in the Sweden conditions, and therefore, the signals in this treatment may override photoperiod signalling. To verify that temperature and photoperiod are more important than other, microhabitat variables in regulating flowering time, future studies will measure QTL for flowering time in the field to determine whether the same QTL are observed.

Fewer QTL were detected under Swedish conditions than Italian conditions. A reanalysis of the Italy chamber data set using the same subset of lines used in the Sweden chamber analysis found five of the seven QTL unique to the Italy conditions even with the reduced number of RILs. Therefore, the greater number of flowering time QTL in the Italy chamber does not appear to be solely an artefact of sample size. Instead, the greater range of phenotypic variation in flowering time observed in the Italy conditions may make it easier to detect minor effect QTL. Furthermore, the Sweden conditions may represent saturated vernalization conditions that could normalize flowering time among different genotypes and reduce or remove the contribution of some genes as a result. Strange *et al.* (2011) found that some QTL that had large effects on flowering time without vernalization had no effect when vernalization was saturated.

Colocalization of flowering time QTL and fitness QTL from the field

The two largest effect flowering time QTL found in both experimental conditions colocalize with fitness QTL and have tight credible intervals (Fig. 4). For one of these (FlrT 5:1), the Italy genotype is favoured at both field sites (Ågren *et al.* 2013), despite the fact that the Italy genotype decreases flowering time and the Sweden genotype increases flowering time. There are several possible explanations for why the late-flowering

local genotype may be maladaptive in Sweden. First, field studies demonstrate that differences in parental fitness in Sweden are largely attributable to differential survival between the populations, not fecundity (Ågren & Schemske 2012). Therefore, early flowering may increase fecundity in Sweden as long as individuals survive the winter. In addition, recent climate warming in Sweden (Kullman 2001) may have increased the fitness of southern genotypes. In fact, winter survival of the Italian genotype in Sweden increased with higher minimum winter temperatures (Ågren & Schemske 2012). Therefore, increased winter survival whether due to climate change or the presence of local alleles at other loci may confer fitness advantages to early flowering in Sweden. Finally, Ågren *et al.* (2013) found that the local genotype was maladaptive in Sweden for several fitness QTL and suggest that weaker selection against nonlocal genotypes or genetic drift due to small effective population sizes in Sweden may have increased the chances for maladaptive alleles to become fixed.

The other flowering time QTL found in both conditions (FlrT 5:4) colocalizes with a QTL that exhibits a fitness trade-off, with the Italy genotype increasing fitness in Italy and decreasing fitness in Sweden (Fig. 4). In a study of the mustard *Boechera stricta*, Anderson *et al.* (2013) also found evidence for a fitness trade-off that mapped to the same location as a known flowering time QTL detected in a growth chamber experiment. Flowering time genes may result in fitness trade-offs if there is differential selection on flowering time in different habitats or if flowering time has pleiotropic effects on other traits that affect fitness. There is evidence that selection on flowering time differs across the native range of *Arabidopsis* (Fournier-Level *et al*. 2013), and differences in climate between Sweden and Italy suggest that divergent selection on flowering time may be expected. However, studies of *Arabidopsis* and other taxa also find evidence that flowering time genes can have pleiotropic effects on traits such as water use efficiency *(Arabidopsis*; Lovell *et al*. 2013; *Brassica rapa;* Franks 2011), vegetative biomass (*Avena barbata*; Latta & Gardner 2009) and size at reproduction (*Brassica rapa*; Haselhorst *et al*. 2011). Scarcelli *et al.* (2007) found that the candidate flowering time gene *FRI* exerted a negative pleiotropic effect on fitness in *Arabidopsis* through a reduction in the number of branches. To further investigate whether flowering time contributes to fitness trade-offs between these populations, future studies will grow near-isogenic lines (NILs) with flowering time QTL introgressed into the parental backgrounds in native habitats. Flowering time and fitness of these NILs will be measured relative to parental lines to determine the effects of these regions alone on both flowering time and fitness in the field and to examine evidence for fitness trade-offs caused by individual loci.

There is evidence to suggest that three of the eight QTL not shared between environments (seven unique to the Italy environment), colocalize with fitness QTL (Fig. 4). In all cases, the Italy genotype increased fitness in its native environment. Between these three QTL and the two that were shared among environments, we observe a total of five instances where a flowering time QTL found in the Italy environment colocalizes with a fitness QTL in which the Italy genotype increases fitness. By comparison, we observe two instances where a flowering time QTL found in the Sweden environment colocalizes with a fitness QTL, and in only one of these does the Swedish genotype increase fitness. These results indicate that differences in flowering time may be more important for local adaptation in Italy than in Sweden. Field studies on the parental populations demonstrated that freezing tolerance likely plays a large role in local adaptation at the Swedish site, and therefore, flowering time may have a relatively smaller contribution to fitness in Sweden than in Italy (Ågren & Schemske 2012). Conditional neutrality may be expected for flowering time if it is under selection in only one environment, or if, as is observed here, some genes only affect flowering time in one environment. This was observed in *Arabidopsis lyrata*, where loci that only affected flowering time in one environment were favoured in that environment, but conditionally neutral in the other (Leinonen *et al.* 2013).

Ultimately, we hope to uncover the genes underlying flowering time as well as other adaptive traits in these populations of *Arabidopsis*. Doing so will allow us to evaluate whether individual genes contribute to fitness trade-offs between these environments (antagonistic pleiotropy) or whether they are conditionally neutral. Furthermore, knowledge of the genes contributing to adaptation in native populations provides insight into the genetic architecture of adaptation and whether adaptation is commonly a result of changes in a few genes of large effect (Orr 1998) or many genes of small effect (Fisher 1930). The current study identifies candidate flowering time genes such as *FLC* and *VIN3* that are strongly implicated in local adaptation in native populations of *Arabidopsis*. Identification of these genomic regions in conditions typical of the parental habitats, and the colocalization of the associated flowering time QTL with fitness QTL from the field are significant steps towards identifying the genetic basis of adaptation in this system.

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References

Ågren J, Schemske DW (2012) Reciprocal transplants demonstrate strong adaptive differentiation of the model organism *Arabidopsis* thaliana in its native range. *New Phytologist*, **194**, 1112–1122.

Ågren J, Oakley CG, McKay JK, Lovell JT, Schemske DW (2013) Genetic mapping of adaptation reveals fitness tradeoffs in *Arabidopsis* thaliana. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 21077–21088.

Alonso-Blanco C, Méndez-Vigo B (2014) Genetic architecture of naturally occurring quantitative traits in plants: an updated synthesis. *Current Opinion in Plant Biology*, **18C**, 37–43.

Anderson JT, Lee CR, Rushworth CA, Colautti RI, Mitchell-Olds T (2013) Genetic trade-offs and conditional neutrality contribute to local adaptation. *Molecular Ecology*, **22**, 699–708.

Anderson JT, Wagner MR, Rushworth CA, Prasad KVSK, Mitchell-Olds T (2014) The evolution of quantitative traits in complex environments. *Heredity*, **112**, 4–12.

Atwell S, Huang YS, Vilhjálmsson BJ *et al.* (2010) Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature*, **465**, 627–631.

Barrett RDH, Hoekstra HE (2011) Molecular spandrels: tests of adaptation at the genetic level. *Nature Reviews Genetics*, **12**, 767–780.

Beavis W (1998) *Molecular Dissection of Complex Traits*. CRC, New York.

Brachi B, Faure N, Horton M *et al.* (2010) Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. *PLOS Genetics*, **6**, e1000940.

Broman KW, Sen Ś (2009) *A Guide to QTL Mapping with R/qtl*. Springer, New York.

Broman KW, Wu H , Sen Ś, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. *Bioinformatics*, **19**, 889–890.

Caicedo AL, Stinchcombe JR, Olsen KM, Schmitt J, Purugganan MD (2004) Epistatic interaction between *Arabidopsis FRI* and *FLC* flowering time genes generates a latitudinal cline in a life history trait. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 15670–15675.

Feder ME, Mitchell-Olds T (2003) Evolutionary and ecological functional genomics. *Nature Reviews Genetics*, **4**, 649–655.

Fisher RA (1930) *The Genetical Theory of Natural Selection*. Oxford University Press, Oxford.

Fournier-Level A, Wilczek AM, Cooper MD *et al.* (2013) Paths to selection on life history loci in different natural environments across the native range of *Arabidopsis thaliana*. *Molecular Ecology*, **22**, 3552–3566.

Franks SJ (2011) Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. *New Phytologist*, **190**, 249–257.

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

Haselhorst MSH, Edwards CE, Rubin MJ, Weinig C (2011) Genetic architecture of life history traits and environment-specific trade-offs. *Molecular Ecology*, **20**, 4042–4058.

Huang X, Schmitt J, Dorn L *et al.* (2010) The earliest stages of adaptation in an experimental plant population: strong selection on QTLs for seed dormancy. *Molecular Ecology*, **19**, 1335–1351.

Inouye DW (2008) Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology*, **89**, 353–362.

Johanson U, West J, Lister C, Michaels S, Amasino R, Dean C (2000) Molecular analysis of FRIGIDA, a major determinant of natural variation in *Arabidopsis* flowering time. *Science*, **290**, 344–347.

Koornneef M, Alonso-Blanco C, Vreugdenhil D (2004) Naturally occurring genetic variation in *Arabidopsis thaliana*. *Annual Review of Plant Biology*, **55**, 141–172.

Korves TM, Schmid KJ, Caicedo AL *et al.* (2007) Fitness effects associated with the major flowering time gene *FRIGIDA* in *Arabidopsis thaliana* in the field. *American Naturalist*, **169**, E141–E157.

Kover PX, Rowntree JK, Scarcelli N, Savriama Y, Eldridge T, Schaal BA (2009) Pleiotropic effects of environment-specific adaptation in *Arabidopsis thaliana*. *New Phytologist*, **183**, 816–825.

Kullman L (2001) 20th century climate warming and tree-limit rise in the southern Scandes of Sweden. *Ambio*, **30**, 72–80.

Latta RG, Gardner KM (2009) Natural selection on pleiotropic quantitative trait loci affecting a life-history trade-off in *Avena Barbata*. *Evolution*, **63**, 2153–2163.

Leinonen PH, Remington DL, Leppälä J, Savolainen O (2013) Genetic basis of local adaptation and flowering time variation in *Arabidopsis lyrata*. *Molecular Ecology*, **22**, 709–723.

Li Y, Roycewicz P, Smith E, Borevitz JO (2006) Genetics of local adaptation in the laboratory: flowering time quantitative trait loci under geographic and seasonal conditions in *Arabidopsis*. *PLoS ONE*, **105**, 1–8.

Li Y, Huang Y, Bergelson J, Nordborg M, Borevitz JO (2010) Association mapping of local climate-sensitive quantitative trait loci in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 21199–21204.

Lovell JT, Juenger TE, Michaels SD *et al.* (2013) Pleiotropy of FRIGIDA enhances the potential for multivariate adaptation. *Proceedings of the Royal Society B-Biological Sciences*, **280**, 1043–1050.

Lowry DB (2012) Local adaptation in the model plant. *New Phytologist*, **194**, 888–890.

Mackay TFC, Stone EA, Ayroles JF (2009) The genetics of quantitative traits: challenges and prospects. *Nature Reviews Genetics*, **10**, 565–577.

Manichaikul A, Moon JY, Sen Ś, Yandell BS, Broman KW (2009) A model selection approach for the identification of

quantitative trait loci in experimental crosses, allowing epistasis. *Genetics*, **181**, 1077–1086.

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.

Michaels SD, Amasino RM (1999) FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell*, **11**, 949–956.

Munguía-Rosas MA, Ollerton J, Parra-Tabla V, De-Nova JA (2011) Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured. *Ecology Letters*, **14**, 511–521.

Orr HA (1998) The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. *Evolution*, **52**, 935–949.

Rockman MV (2012) The QTN program and the alleles that matter for evolution: all that's gold does not glitter. *Evolution*, **66**, 1–17.

Salomé 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.

Sánchez-Bermejo E, Méndez-Vigo B, Picó FX, Martínez-Zapater JM, Alonso-Blanco C (2012) Novel natural alleles at FLC and LVR loci account for enhanced vernalization responses in *Arabidopsis thaliana*. *Plant, Cell and Environment*, **35**, 1672–1684.

Scarcelli N, Cheverud JM, Schaal BA, Kover PX (2007) Antagonistic pleiotropic effects reduce the potential adaptive value of the FRIGIDA locus. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 16986–16991.

Sherrard ME, Maherali H (2006) The adaptive significance of drought escape in *Avena barbata*, an annual grass. *Evolution*, **60**, 2478–2489.

Simpson GG, Dean C (2002) *Arabidopsis*, the rosetta stone of flowering time? *Science*, **296**, 285–289.

Srikanth A, Schmid M (2011) Regulation of flowering time: all roads lead to Rome. *Cellular and Molecular Life Sciences*, **68**, 2013–2037.

Stinchcombe JR, Weinig C, Ungerer M *et al*. (2004) A latitudinal cline in flowering time in *Arabidopsis thaliana* modulated by the flowering time gene FRIGIDA. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 4712–4717.

Strange A, Li P, Lister C *et al*. (2011) Major-effect alleles at relatively few loci underlie distinct vernalization and flowering variation in *Arabidopsis* accessions. *PLoS ONE*, **6**, 1–11.

Sung S, Amasino RM (2004) Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature*, **427**, 159–164.

Weinig C, Ungerer MC, Dorn LA *et al*. (2002) Novel loci control variation in reproductive timing in *Arabidopsis thaliana* in natural environments. *Genetics*, **162**, 1875–1884.

Zhao K, Aranzana MJ, Kim S *et al*. (2007) An *Arabidopsis* example of association mapping in structured samples. *PLOS Genetics*, **3**, 0071–0082.

Zuellig MP, Kenney AM, Sweigart AL (2014) Evolutionary genetics of plant adaptation: insights from new model systems. *Current Opinion in Plant Biology*, **18C**, 44–50.

E.L.D., J. Å and D.W.S. designed the study, D.W.S. produced the RILs, E.L.D. collected the data, E.L.D. and C.G.O. performed the analyses, and E.L.D. wrote the manuscript with contributions from all the other authors.

Data accessibility

Flowering time phenotypes for all RILs and parents as well as conditions used for growth chamber conditions: Dryad doi:10.5061/dryad.m663t.

Genotypic data: TAIR, filename 'Castelnuovo_Rodasen_RIdata', and Dryad doi:10.5061/dryad.m663t.

Seeds for 404 RIL lines and parents available: TAIR, set #CS98760.

Supporting information

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

Fig. S1. The correlation in flowering time for each RIL genotype in the Sweden chamber (*x*-axis) and the Italy chamber (*y*-axis). The means of the parents in each environment are indicated with crossing lines showing the 95% confidence intervals.

Fig. S2. Heat-maps illustrating all pairwise (Scan-two) additive and epistatic LOD scores for each year and site combination. The LOD score for the full model (QTLi + QTLj + QTLi × QTLj) of each pairwise marker combination is plotted below the diagonal. Above the diagonal is the LOD score of the epistatic term in the model: the difference between the LOD score of the full model and the additive-only model. The legends for the full and epistatic LOD scores are plotted on the left and right side, respectively, of the vertical bar adjacent to each plot.

Table S1. Effects of chamber environment and genotype at marker loci of point estimates on flowering time for the 10 flowering time QTL examined with ANOVA. D.f. = 1 for all variables.