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Paths to selection on life history loci in different natural environments across the native range of *Arabidopsis thaliana*

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Abstract

Selection on quantitative trait loci (QTL) may vary among natural environments due to differences in the genetic architecture of traits, environment-specific allelic effects or changes in the direction and magnitude of selection on specific traits. To dissect the environmental differences in selection on life history QTL across climatic regions, we grew a panel of interconnected recombinant inbred lines (RILs) of *Arabidopsis thaliana* in four field sites across its native European range. For each environment, we mapped QTL for growth, reproductive timing and development. Several QTL were pleiotropic across environments, three colocalizing with known functional polymorphisms in flowering time genes (*CRY2*, *FRI* and *MAF2-5*), but major QTL differed across field sites, showing conditional neutrality. We used structural equation models to trace selection paths from QTL to lifetime fitness in each environment. Only three QTL directly affected fruit number, measuring fitness. Most QTL had an indirect effect on fitness through their effect on bolting time or leaf length. Influence of life history traits on fitness differed dramatically across sites, resulting in different patterns of selection on reproductive timing and underlying QTL. In two oceanic field sites with high prereproductive mortality, QTL alleles contributing to early reproduction resulted in greater fruit production, conferring selective advantage, whereas alleles contributing to later reproduction resulted in larger size and higher fitness in a continental site. This demonstrates how environmental variation leads to change in both QTL effect sizes and direction of selection on traits, justifying the persistence of allelic polymorphism at life history QTL across the species range.

Keywords: adaptation, angiosperms, life history evolution, natural selection and contemporary evolution, phenotypic plasticity, quantitative genetics

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Introduction

Understanding the molecular targets of selection in different environments is critical for elucidating the mechanisms of adaptation to varying ecological conditions.

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In particular, geographically varying selection at specific loci may allow persistence of genetic variation across a species range (Levene 1953; Gillespie & Turelli 1989; Hall 2006). In plants, fitness results from the cumulative selection on multiple traits such as growth and reproductive timing, which are sequentially expressed throughout the life cycle and are often correlated (Roach 1986; Arntz *et al.* 1998). Natural selection acting on the loci underlying life history and developmental variation may vary across environments in several ways. First, the quantitative trait loci (QTL) controlling traits under selection may differ across environments (change in allelic effect and constant selection on trait). Such QTL by environment interaction (Q × E) for a specific trait may take the form of conditional neutrality, with allelic variation at a locus showing an effect only in certain environments, or antagonistic pleiotropy, with allelic effects reversing across environments (Mackay 2001). Second, the optimal value for a trait related to fitness may differ across environments (constant allelic effect and change in the direction of selection on trait, Lechowicz 1988). Environment-specific selection may then operate directly on this trait, so the loci underlying it are the primary targets of selection, or on other correlated traits (Lande & Arnold 1983), leading to indirect selection on the loci underlying those traits (Scheiner 1993). The fundamental challenges are thus to identify the loci underlying life history traits and trace the causal paths from allelic variation to fitness in order to understand how selection acts on molecular variation in different natural environments.

Life history traits often exhibit plastic responses to environmental variation (Scheiner 1993; Dorn *et al.* 2000; Haselhorst *et al.* 2011). Such plasticity can alter the expression of heritable variation and the architecture of trait correlations across environments (Falconer 1952; Bennington & McGraw 1996; Hoffmann & Parsons 1997; Donohue & Schmitt 1999). Life history theory predicts strong selection for an optimal combination of traits to maximize the reproductive fitness of an individual in a given environment (Fisher 1930; Roff 1992), but the optimal life history may differ across environments. Often the same quantitative trait locus (QTL) affects multiple traits, suggesting pleiotropic effects between life history and fitness traits, although it may also be that a QTL spans multiple linked loci affecting different traits (Kato *et al.* 1999; McKay *et al.* 2003; Lifschitz *et al.* 2006). However, it is often unclear whether the observed effect of a QTL on a fitness trait reflects a direct genetic effect on that trait or an indirect effect via correlated life history traits expressed earlier in life. Recent studies have addressed this issue using structural equation models (SEMs) to dissect indirect QTL effects on complex trait variation (Remington 2009; Wolf *et al.* 2011), but few have focused on selection in natural environments (Gove *et al.* 2012). Separately, several studies have extended the selection gradient approach of Lande & Arnold (1983) into SEMs, dissecting fitness through causal networks of correlated traits (Scheiner *et al.* 2000; Latta & McCain 2009; Milla *et al.* 2009), but these have not included specific loci in this framework. Here, after mapping QTL for life history and development, we use SEMs to measure patterns of selection at specific loci underlying fitness variation in different natural environments in the annual plant *Arabidopsis thaliana*.

Arabidopsis thaliana is an ideal system for this study because it is a model organism in which the genetic basis of life history traits has been extensively studied. The genetic control of flowering time is particularly well understood (Amasino 2010; Jarillo & Pineiro 2011; Srikanth & Schmid 2011). Flowering time has been the subject of extensive QTL mapping experiments (Alonso-Blanco *et al.* 2009) and association studies (Ehrenreich *et al.* 2009; Atwell *et al.* 2010; Brachi *et al.* 2010). QTL mapping combined with functional experiments have identified natural allelic variation affecting flowering time for many candidate genes (El-Assal *et al.* 2001; Werner *et al.* 2005; Wang *et al.* 2007; Caicedo *et al.* 2009; Schwartz *et al.* 2009). *A. thaliana* growth and development have also been the subject of numerous QTL studies, although no functional tests were performed to demonstrate the mechanistic involvement of specific candidate genes (Ungerer *et al.* 2002; El-Lithy *et al.* 2004, 2010; Reymond *et al.* 2006).

To test for geographic differences in natural selection at specific QTL, we planted a subset of Recombinant Inbred Lines (RILs) from five related mapping populations in four field sites spanning the native range of *A. thaliana*. RILs are particularly useful for the study of differential selection because the crosses disrupt potential coadapted gene complexes and make it possible to test multiple genetic combinations using the same set of genotypes replicated across multiple environments. Our goal was not to identify the specific loci contributing to adaptation of local populations to these sites (Turner *et al.* 2010; Anderson *et al.* 2011; Fournier-Level *et al.* 2011). Rather, we sought to dissect geographic variation in selection by replicating a genetically diverse population with known functional polymorphisms across different climates. In particular, we aimed to show how the mechanisms relating life history to fitness can change across environments and promote geographic balancing selection. We first characterized the genetic and environmental components of fitness variation in four environments (Halle and Cologne, Germany; Norwich, United Kingdom; Valencia, Spain); mapped QTL associated

with these components, and finally summarized the contribution of QTL and covarying traits to reproductive fitness with a single SEM for each environment. We demonstrate that differences in environmental conditions modulated both the effect of specific QTL and the correlations among life history traits and fitness, modifying the strength and direction of selection on reproductive timing across field sites. These results suggest a potential mechanism for geographical balancing selection in different natural environments and explain the maintenance of polymorphism through the differential selection of alleles at specific loci.

Materials and methods

Plant material

A total of 117 genotypes of selected RILs from five well-characterized mapping populations were tested in natural environments, including 19 genotypes from *BayxSha* (Loudet *et al.* 2002), 21 from *LerxAn1* (El-Lithy *et al.* 2006), 17 from *LerxCol* (Jansen *et al.* 1995), 27 from *LerxCvi* (Alonso-Blanco *et al.* 1998) and 33 from *LerxSha* (El-Lithy *et al.* 2004) and the parental lines. These lines were selected to test the effect of specific life history candidate genes in natural conditions. A preprocessing program constructed a design matrix for RIL subset selection. We used published data to classify each RIL according to parental genotypes at the polymorphic markers flanking nine candidate loci: *CRYPTOCHROME 2* (*CRY2*, El-Assal *et al.* 2001), *FLOWERING LOCUS C* (*FLC*, Koornneef *et al.* 1994; Lee *et al.* 1994), *FRIGIDA* (*FRI*, Johanson *et al.* 2000), *FRIGIDA-LIKE 1* (*FRL1*, Schlappi 2006), *HUA2* (Doyle *et al.* 2005), *PHYTOCHROME C* (*PHYC*, Balasubramanian *et al.* 2006; Sharrock & Quail 1989), *MADS-BOX AFFECTING FLOWERING 2-5* (*MAF2-5*, Caicedo *et al.* 2009; Rosloski *et al.* 2010), *FLOWERING LOCUS M* (*FLM*, Werner *et al.* 2005) and a previously reported QTL for flowering time on the top of chromosome 3 (El-Lithy *et al.* 2004). We then used the SAS OPTEX procedure with the A-optimality criterion to select subsets of RILs maximizing power to detect potential effects of the candidate genes. We checked whether the sampling strategy, which induced segregation distortion, decreased our power to detect QTL at loci that were not included as target candidate in the OPTEX procedure. For that purpose, we applied a simplification of the method of Zhang *et al.* (2010) to measure whether for each family, the segregation distortion led to bias in the QTL detection (Data S1 and Fig. S1). We obtained seeds of the selected RILs from collections bulked under 16-h photoperiod in standard greenhouse conditions at the Max Planck Institute for Plant Breeding Research, Cologne, Germany. To validate specific candidate QTL, we also grew a set of near isogenic lines (NILs) at candidate flowering time loci. For all tests on NILs, we used the background donor genotype as wild-type controls bulked under the same maternal conditions.

Field experiment and phenotyping

Plants were grown in fall 2007 in common gardens in four European sites (Wilczek *et al.* 2009), including oceanic to continental climates (Norwich, UK; Cologne, Germany and Halle, Germany), as well as a Mediterranean site (Valencia, Spain). A summary description of planting sites is provided in Table S1. Protocols were identical to previous field experiments in the same sites (Wilczek *et al.* 2009; Fournier-Level *et al.* 2011). Seeds were stratified at 4 °C in agar for 4 days, germinated on peat-based plugits and grown under natural photoperiod in a greenhouse at field temperatures. Seedlings were transplanted within 2 weeks to the field in 15 randomized blocks in 10 cm × 10 cm grids, each genotype replicated once per block. Plants were watered for a week after transplant. Seeds were sown in Norwich, between 3 September and 6 September 2007, in Cologne, between 24 September and 27 September, in Halle, between 1 October and 4 October, and in Valencia, between 12 November and 15 November, corresponding to the germination date observed for natural population of *Arabidopsis thaliana* in these locations.

We censused plants 2–3 times weekly and scored mortality, date of bolting (first appearance of the inflorescence meristem), maximum leaf length at bolting and date of flowering. To express bolting and flowering time in developmental units comparable across sites (e.g. Brachi *et al.* 2010), we used hourly microenvironmental data recorded by on-site weather stations to calculate photothermal units (PTUs) accumulated from germination to bolting and flowering for each plant following the model of Wilczek *et al.* (2009). The *Ler* genotype was the most recurrent parent in our experimental population and exhibited an intermediate bolting time compared with the other parents (Fig. S2). We therefore used the parameters estimated for the *Ler* genotype by Wilczek *et al.* (2009) as a common currency for calculations of PTU accumulation for all plants in the experiment. Plants were harvested at senescence for counts of total branch number and silique number. Plants that died before 30 days were considered to have died of transplantation shock and counted as missing, while plants that survived 30 days but died before producing fruits were assigned zero branches and siliques. Branch number included all branches longer than 1 cm. We

computed broad-sense heritabilities for each trait ($H^2 = \sigma^2_G/\sigma^2_P$) from the following models: within each site:

$$P_{ij} = \mu + G_i + \varepsilon_{ij}, \qquad (eqn1)$$

and across sites:

$$P_{ijk} = \mu + G_i + E_k + \varepsilon_{ijk}, \qquad (eqn2)$$

Where i is the genotype, k is the environment and j is the replicate nested within genotype and environment. All random effects were modelled as normally distributed with $G \sim N(\mu_G, \sigma^2_G)$ the genotype effect, $E \sim N(\mu_E, \sigma^2_E)$ the macroenvironmental effect and $\varepsilon \sim N(\mu_\varepsilon, \sigma^2_\varepsilon)$ the residual error. Variance components estimates of linear models were computed using the lmer() function of the R\lme4 package. Models including block effects, tested as random effects, did not explain the data significantly better than models with no block effects. Climate data interpolated from 1950 to 2000 at the four field locations and reported in Table S1 were found with detailed calculation at WorldClim (http://www.worldclim.org/download). The soil analyses were performed at the Kansas State University Soil Laboratory (Manhattan, KS, USA) using three to five haphazard samples of soil collected in each site.

Linkage map and QTL detection

A consensus genetic map was built using markers from previously published genetic maps of each cross. Markers were integrated according to their physical position on the *Arabidopsis thaliana* reference sequence (TAIR v.9, http://www.arabidopsis.org). Colinearity between physical and genetic positions was tested in each family using the Kolmogorov–Smirnov test for uniformity; markers deviating from the expected distribution by more than two standard deviations were discarded. QTL mapping was performed independently for the four locations in a multi-cross analysis using the iterative QTL approach as implemented in the MCQTL software (Jourjon *et al*. 2005). QTL analysis was performed for: (i) the best linear unbiased predictors of the genetic effects (BLUPs) of bolting time and flowering time in PTU, leaf length at bolting, branch number at senescence and silique number at senescence, and (ii) survival (number of successfully reproducing plants out of the 15 replicates having survived 30 days). BLUPs were computed for each trait in each environment as the solution for RIL genotype effect from the within site models. MCQTL performs QTL mapping using a linear regression model in multiple families of line crosses with fixed intrafamily QTL effects following the framework of Haley & Knott (1992) to test a single QTL at a time. Multiple QTL models are then built using the iterative QTL mapping algorithm (Charcosset *et al.* 2001). QTL mapping was performed both using the 'single' locus option for individual QTL detection and using the 'epistasis' option to allow for QTL × QTL interaction detection. QTL were declared significant when LOD scores were above the threshold defined through 1000 permutations with an associated risk $\alpha = 0.05$. Since for each QTL, MCQTL analysis estimates the effect of each parental allele, an allelic effect was declared significant at the QTL when the P-value associated with the z-test of the specific allelic effect was smaller than 0.05. To distinguish truly pleiotropic QTL from regions spanning multiple cosegregating loci, we tested the correlation between the effects of the six alleles for each pair of QTL using Pearson's correlation test. A pair of QTL was declared pleiotropic if the maximum LOD score positions were located within 10 cM from each other and the result of the Pearson's correlation test was significant with P-value <0.05.

Structural equation modelling

Structural equation models were developed to calculate selection gradient from QTL or traits to the number of siliques produced by each plant as a proxy for fitness. Survival/Reproductive success was not included in the SEM because this trait (a proportion measured from 15 replicates) was measured over a genotype, not for each individual plant. Alternatively, inclusion of survival as a binary trait would lead to the presence of missing data for life history traits, which cannot be handled in the SEM framework. Prior to developing SEMs, we tested nonlinear relationships between bolting time, leaf length at bolting and silique number. The data showed no significant quadratic influence of bolting time or leaf length on silique number with the presence of either a maximum or a minimum for fitness within the phenotypic variation observed (which indicate the presence of stabilizing or disruptive selection). This indicated that the relationships between variables could be modelled through linear effects only. SEMs were computed using the R\sem package using individual plant data to preserve any source of variance, as opposed to BLUPs. Nested model selection was based on minimizing the Bayesian Information Criterion (BIC) comparing the initial model with a model to which one additional path was tested. Adjusted Goodness of Fit (AGFI), Comparative Fit Index (CFI), Normed Fit Index (NFI), Root Mean Squared Error (RMSEA) and standardized maximum normalized residual (SRMR) were computed for model evaluation. For each i plant genotype, the vector Q_i of the allelic effects of the q QTL (trait and location specific) were modelled as:

$$Q_i = \sum_q a_{qip1} + p(G_{qi}|m_{1i}, m_{2i}) \times (a_{qip2} - a_{qip1}) \quad (eqn3)$$

Where G is the probability of the genotype of QTL q given the genotype of the individual i for the marker m_1 and m_2 flanking the QTL calculated using the calc.genoprob() function of the R\qtl package (0 if the individual shares the genotype of the parent p_1) and a is the additive effect of QTL q of either p parents of the genotype i estimated during the QTL detection step with the MCQTL software (Jourjon *et al.* 2005). The traits were modelled as random variables, while the QTL were modelled as fixed variables to avoid overestimation of their effect by fitting covariance with residual error or with other nonincluded QTL. Paths were considered significant when: *P-val* (z-test)< 0.05 for paths between traits and <0.01 for paths between QTL and traits; only significant paths were reported. Although the SEM framework handles false-positive QTL effects well (Remington 2009), we decided to be more conservative with respect to the inclusion of QTL effects. This ensured that our conclusions about selection at specific loci were robust. The rationale to establish the most suitable model within locations was to: (i) define the best topology of a simple model including the three most biologically upstream traits (bolting time, leaf length at bolting and branch number), the model with the smallest BIC was kept; (ii) include flowering time and silique number to this model. For each variable, unidirectional (single regression) and two-way paths (covariance) were tested, and the ones significant with P-value of 0.05 through z-test, kept; and (iii) on each previously added variable, include the QTL for each trait if the z-test P-value was smaller than 0.01. Standardized coefficients were finally computed to compare paths across models.

Results

Plasticity and genetic variation within environments

Plants showed very different life history strategies in the German planting sites compared with Norwich and Valencia, where milder fall and winter temperatures induced faster bolting (Fig. S2). In Norwich, plants germinating in early September all bolted rapidly between late September and mid-October. In Cologne and Halle, where plants germinated between late September and early October, the slow overwinter accumulation of photothermal time resulted in disrupted life histories with a few plants bolted in fall, including most plants bearing *Cvi* alleles leading to no vernalization requirement, but the majority did in spring. In Valencia, plants germinated in early November and all bolted in winter. Transformation of days to bolting and flowering into *Ler* photothermal units resulted in more continuous distributions (Fig. S3) which were used for all subsequent analyses (Table 1), and henceforth 'bolting time' and 'flowering time' refer to photothermal units. Mean photothermal time to bolting and flowering, leaf length, branch number, and silique number varied widely across sites (Figs. S4 and S5). Survival to reproduction also varied substantially, ranging from 15.8% in Norwich to 66.7% in Halle (Table S2).

Broad-sense heritabilities were relatively high for bolting time, flowering time and leaf length at bolting within each of the four environments, particularly Halle (Table 1). Heritabilities for branch and silique number were lower, especially in Valencia, reflecting greater microenvironmental variation in those traits. The models combining all environments showed that for the reproductive development traits (branch and silique number), macroenvironmental variances among sites (σ^2_E) were greater than genetic variances, while for life history traits, genetic variances were greater.

Specific genetic variation influencing life history and fitness across environments

To decipher the molecular basis of the genetic variation observed, we extracted BLUPs of each genotype for each trait and environment and performed QTL detection on these values and also on survival. The QTL mapping identified 56 QTL (Fig. 1 and Table S3) and 30 of these QTL collocated with the nine candidate loci for which the sample was optimized. The global R^2 of QTL models for each trait was positively correlated with heritability calculated for that trait (Pearson's $\rho = 0.75$, P-value $<10^{-16}$). No QTL were detected for branch or silique number in Valencia, in accordance with their low heritability (Table 1). Each parent contributed alleles with a significant genetic effect. *Cvi* contributed to 24 allelic contrasts and *Ler*, the most recurrent parent, only to eight contrasts while the four other parents contributed to between 13 and 18 (Table S3). Only three QTL corresponding to two loci were due to differences between more than two alleles: on chromosome three position 44 (QTL3.44) and on chromosome five around position 95 (QTL5.95).

We found a strong overlap between QTL for different traits in the different sites, suggesting strong cross-environment pleiotropy. 23 QTL in six different positions overlapped and showed correlated genetic effects for the six different alleles tested (Fig. S6). We consistently identified six QTL across environments: on the top of chromosome 1 (QTL1.04), on the top and around 43 cM of chromosome 4 (QTL4.01 and QTL 4.43), and on top,

Table 1 Summary statistics and variance components for the five traits with individual measures in the four environments

	Halle	Cologne	Norwich	Valencia	All Environments
Bolting date (PTU)					
Mean (SD)	2027 (483.5)	2173.8 (459.9)	2377.9 (264.3)	2447.2 (566.6)	
H^2	0.94	0.77	0.60	0.36	0.45 (σ^2_E/σ^2_P = 0.20)
Mean leaf length (mm)					
Mean (SD)	24.1 (5.5)	21.9 (*6.0*)	15.1 (*4.8*)	37.0 (14.4)	
H^2	0.88	0.47	0.44	0.61	0.28 (σ^2_E/σ^2_P = 0.58)
Flowering date (PTU)					
Mean (SD)	2875.1 (731.4)	4026.9 (1307.3)	2670.7 (251.9)	3615 (863.6)	
H^2	0.94	0.56	0.56	0.33	0.29 (σ^2_E/σ^2_P = 0.29)
Branch number					
Mean (SD)	42.7 (36.5)	24.2 (20.3)	0.7 (*1.3*)	38.1 (23.4)	
H^2	0.25	0.36	0.24	0	0.06 (σ^2_E/σ^2_P = 0.39)
Silique number					
Mean (SD)	911.9 (743.7)	483.3 (485.4)	3.5 (*9*)	730.3 (520.3)	
H^2	0.28	0.49	0.17	0	0.08 (σ^2_E/σ^2_P = 0.38)

H, Broad-sense heritability; σ^2_E/σ^2_P, proportion of phenotypic variance explained by the random effect of environment; PTU, photothermal units.

around 25 cM and on the bottom of chromosome 5 (QTL5.08, QTL5.25 and QTL5.95).

We used multitrait and multienvironment QTL detection using principal components of BLUP to test for QTL × environment effects (Mangin *et al.* 1998). This analysis provided inconsistent results given the low variance explained by the first eigenvector (data not shown). We also tested 2-way epistatic QTL interactions between: (i) the already detected QTL, and (ii) one already detected QTL vs. the rest of the genome, and no significant interaction was detected.

Pattern of differential mortality across sites

For the five traits for which heritability was measured with variance components models, heritabilities inversely correlated with levels of mortality across sites, from Halle to Norwich and Valencia (GLM, *P*-value = 0.00534). However, it was not possible to estimate heritability for survival as for other traits because it was already measured at the genotype level, not per individual. The proportion of variance explained by the QTL model was therefore the best proxy for measuring the genetic basis of survival to reproduction across sites. This QTL detection identified two QTL in total for every site and explained 37% of survival in Halle and 44% in Norwich, the sites contrasting the most in terms of survival rate. This showed that survival did not have significantly stronger genetic basis in high mortality sites compared with others. Moreover, different QTL were associated with survival in each site, except for QTL 5.06, detected in both Halle and Valencia (Fig. 1). Some survival QTL, such as QTL5.95 in Norwich, collocated with QTL for other traits, but others did not, suggesting that viability selection may have acted on QTL underlying traits not directly measured in our study, such as pathogen resistance or drought tolerance.

Differential mortality across sites implied sample size variation, which can affect the power to detect QTL for other traits. To test this hypothesis, we simulated data sets of equal size as the empirical observation from the field for varying levels of heritability and partitioning of the genetic variance between genotype and family (Data S2 and Figs. S7 to S9). We concluded that given the structure of our observed sample, the estimated heritability should systematically be biased in the same direction, with no difference across sites. This ascertained that differential mortality alone could not explain the difference in the level of genetic variance across sites that would eventually decrease our power to detect QTL for specific sites.

Candidate genes for life history variation

Three bolting time QTL detected in multiple environments were further investigated to identify the specific locus underlying the variation using near isogenic lines (NILs). QTL1.04 cosegregated with the *CRY2* polymorphism reported by El-Assal *et al.* (2001), who showed functionally that the *CRY2-Cvi* allele accelerates flowering in short days. Similarly, we observed earlier flowering in RILs carrying the *Cvi* allele of this QTL under short day conditions in Cologne and Halle. Given the cosegregation of our QTL with this gene and the coherence of

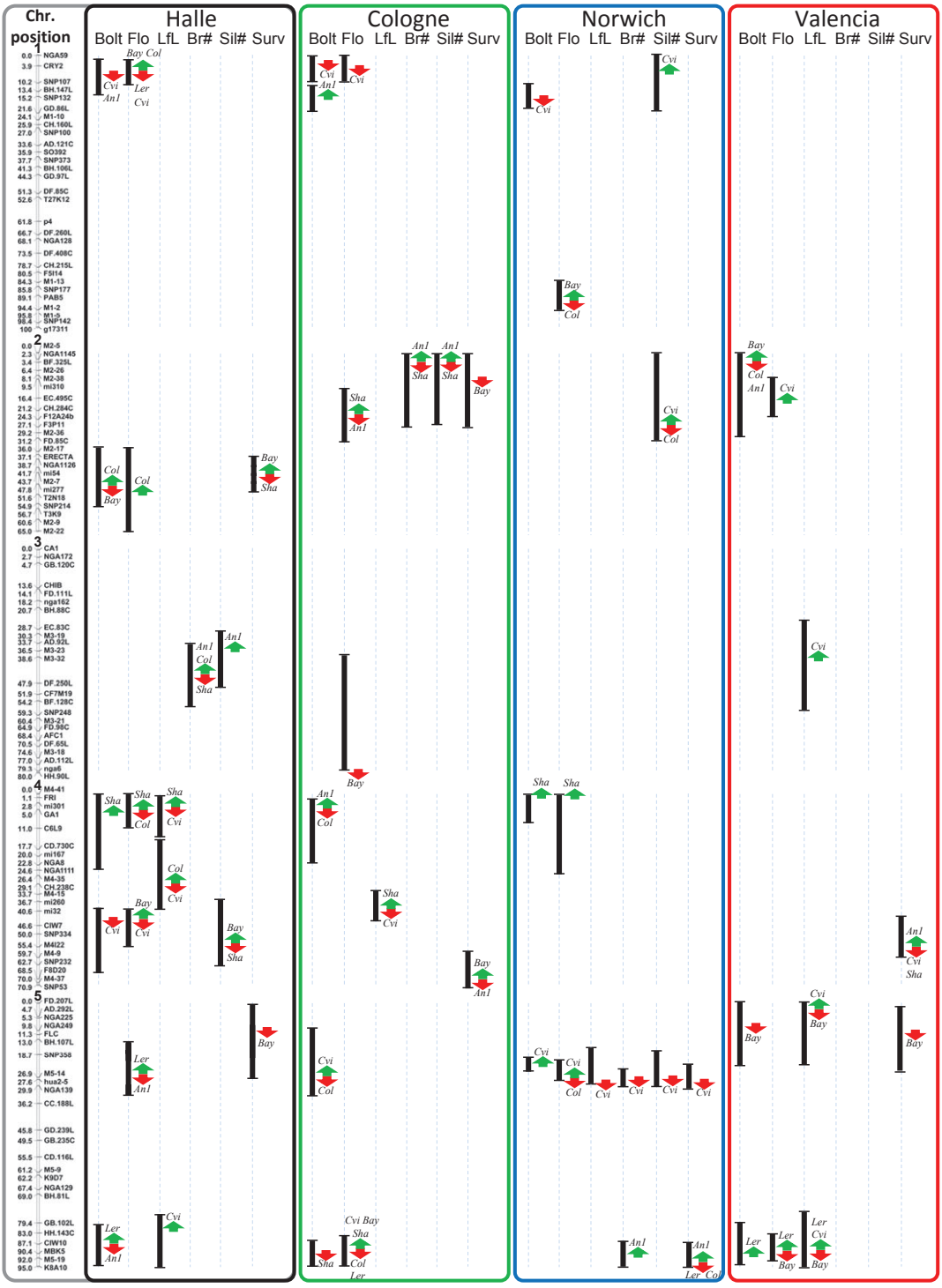

Fig. 1 Quantitative trait loci (QTL) map detected in the four environments through multi-cross analysis using the iterative QTL approach (see Materials and methods). Bold interval represents the LOD-2 confidence interval, arrow and parent names are reported for the parental alleles of significant effect (*z*-test, *P-val*<0.05, green/up arrow when positive, red/down when negative). The values of the QTL additive effects are reported in Table S3.

the genetic effect, *CRY2* is likely to underlie this QTL. We tested NILs where the *CRY2* allele from *Cvi* was introgressed in the *Ler* background and these NILs flowered significantly earlier than the *Ler* control in all locations but Valencia (Fig. S10). We will hereafter refer to QTL1.04 as 'CRY2'.

QTL4.01, on the top of chromosome 4, cosegregated with the *FRI* gene marker, (Johanson *et al.* 2000). This QTL was due to the delaying effect of the *Sha* allele on flowering in Halle and Norwich. *Sha* was the only parent carrying a functional *FRI* allele which was shown to delay flowering compared with the *Ler* allele (El-Lithy *et al.* 2004) or to the *Bay* allele (Loudet *et al.* 2002). In contrast, neither the *Ler*x*Col*, nor *Ler*x*Cvi* crosses showed an effect associated with the *FRI* locus (Ungerer *et al.* 2002). We also planted *Col* NILs carrying the functional *Sf2* allele of the *FRI* gene, known to delay flowering in the laboratory similarly to the *Sha* allele (Clerkx *et al.* 2004). Unfortunately, in Norwich, all 45 *FRI*- functional NIL plants died before producing any siliques. However, a field experiment with these lines in the same sites the previous year demonstrated large effects of FRI functionality in Norwich (Wilczek *et al.* 2009). *FRI* is therefore the likely candidate for the QTL4.01 in Halle and Norwich, and we will consequently refer to QTL4.01 as 'FRI'.

QTL5.95 colocated with the *MAF2-5* genes cluster. This QTL was due to the effect of the *Ler* allele delaying flowering. This QTL was previously reported (El-Lithy *et al.* 2004; Li *et al.* 2006; Huang *et al.* 2011; Salomé *et al.* 2011). Functional assays using the *Ler* allele of *MAF2* have shown a similar effect to what we found and *Sha* is known to harbour a major insertion in *MAF2* and deletion of *MAF3* (Rosloski *et al.* 2010). We tested a NIL in which a 550 Kbp genomic segment from *Sha* that included the *MAF2-5* genes introgressed into the *Ler* background. This NIL flowered significantly earlier than the *Ler* control in all locations (Fig. S10). We will therefore refer to QTL5.95 as '*MAF2-5*'.

Structural equation models of fitness across field sites

The final objective of the study was to join together the results obtained from QTL detection for different traits into a single model explaining how traits are interconnected in each environment. The SEMs in each environment fit the data fairly well with CFI and NFI greater than 0.9 but with high RMSEA values between 0.08 and 0.12 (Table S4). Bolting time and leaf length covaried positively in all sites except Cologne, although this relationship was weaker in Halle (Fig. 2). However, the standardized path coefficients between traits differed across planting sites, particularly the influence of bolting time on branch number, ranging from strongly negative in Norwich to non-significant in Halle. When summing direct and indirect paths, changes in structure of the paths leading to silique number in different sites resulted in a change in the overall selection coefficient for bolting time and leaf length at bolting (Table 2). In the oceanic climates (Cologne and Norwich), earlier bolting plants produced more branches and had higher fitness. In contrast, bolting time was not associated with fitness in Valencia, and in Halle, we observed weak selection for later bolting. The influence of leaf length on branch number, and thus indirectly on silique number, was low in Halle and Norwich, intermediate in Cologne and fairly strong in Valencia (Table S1). Selection for larger plants was substantial in Valencia and Cologne, but not in Halle or Norwich. On the contrary, in Norwich, we observed slight indirect selection for smaller plants due to strong selection for early bolting.

The three QTL for which potential candidate genes were identified had opposite effects on fitness in different environments (Table 2). '*CRY2*' was the one for which the selection gradient was the strongest. The *Cvi* allele of '*CRY2*' was predicted to be the target of selection, either positively in Cologne or negatively in Norwich (direct selection on silique number) and to a lesser extent in Halle (indirect selection through bolting time). For '*FRI*', the *Sha* allele was predicted to be the main target of selection through its delaying effect on bolting time, positively in Halle and negatively in Norwich. Finally, the '*MAF2-5*' showed a more complex pattern. With this QTL being the result of multiple allelic contrasts, selection favoured the *Sha* allele in Cologne and the *Ler* and *Cvi* allele in Valencia while purging the *Bay* allele.

We finally summed all the standardized coefficients leading to each trait in each model to understand the partitioning of the causal variation between QTL and correlated traits effects and the residual variance (microenvironmental). This showed that for each environment, genetic variation had a greater influence on early traits (bolting time and leaf length) than on later traits, as a consequence of the increased influence of correlated traits and the accumulation of environmental microvariation throughout plant life history (Fig. 3, GLM, P-value = 0.00323).

Discussion

When dissecting selection on QTL for life history variation driving fitness in multiple natural environments across the native range of *Arabidopsis thaliana*, we found that mostly different loci contributed to life history variation. Nonetheless, some major QTL were shared across environments, with their effect size varying significantly. We observed differences in the correlations between life history and fitness traits across environments and expression of tradeoffs. As a consequence, the direction of selection on timing of reproduction and therefore on life history QTL, changed across field sites. In particular, three QTL, putatively corresponding to known functional variation at the *CRY2*, *FRI* and

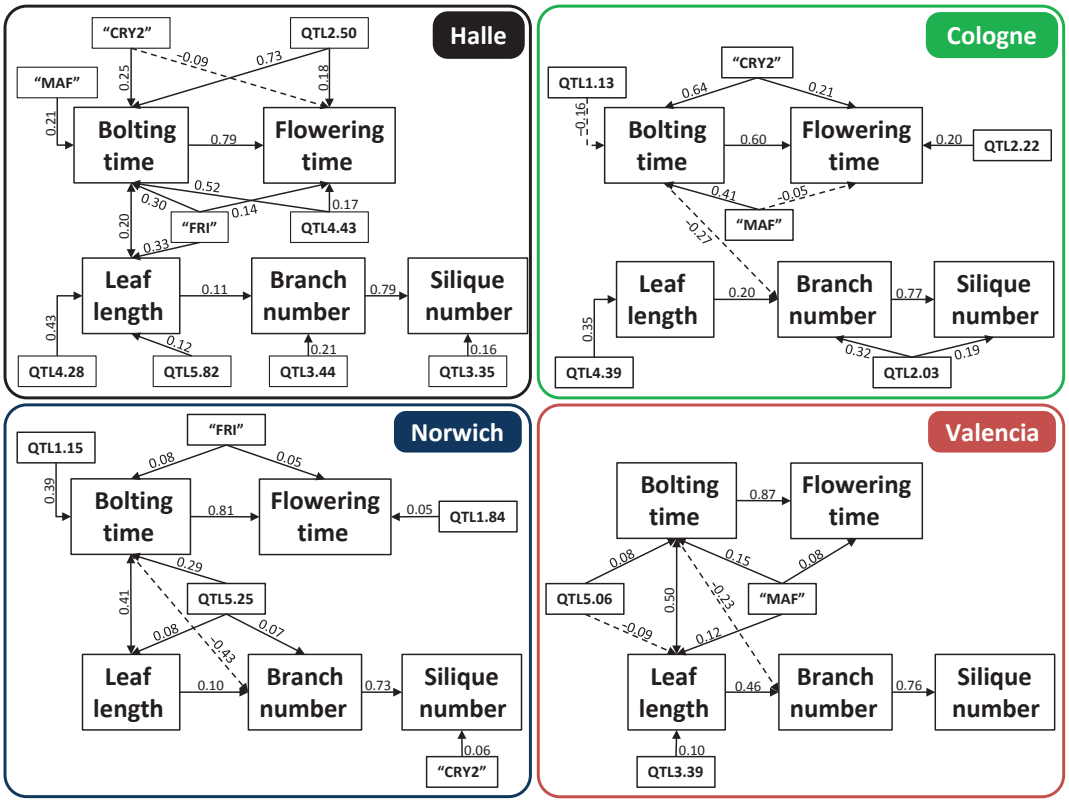

Fig. 2 Structural equation models presenting the influence of bolting time, flowering time, leaf length and branch number on fruit number together with their associated quantitative trait loci (QTL) effects. All the paths presented are significant with *P-val*<0.01 for paths between QTL and phenotype (fixed effects) and *P-val*<0.05 for path between phenotypes (random effects). Negative pathway coefficients are reported as dashed lines, positive ones are reported as solid lines. The summary statistics for the models in the different planting sites are reported in Table S4.

Table 2 Total selection coefficients estimated from the models presented in Fig. 2

		Halle		Cologne		Norwich		Valencia	
		Selection Coef.	Allele (selection)	Selection Coef.	Allele (selection)	Selection Coef.	Allele (selection)	Selection Coef.	Allele (selection)
Traits	Bolting Time	0.017		−0.208		−0.284		0	
	Leaf Length at Bolting	0.087		0.154		−0.056		0.262	
QTL	"CRY2"	0.004	*Cvi* (−)	−0.133	*Cvi* (+)	0.060	*Cvi* (−)	*no QTL*	
	"FRI"	0.034	*Sha* (+) *Cvi* (−)	*no QTL*		−0.023	*Sha* (−)	*no QTL*	
	"MAF"	*no QTL*		−0.085	*Sha* (+)	*no QTL*		0.031	*Ler*, *Cvi* (+) *Bay* (−)

(+), positive selection; (−), negative selection; QTL, quantitative trait loci.

MAF2-5 loci showed evidence of differential selection across environments.

The Environment determines the effect of QTL

Major functional variants and null alleles at several flowering time loci are common in *A. thaliana* (Toomajian *et al.* 2006; Caicedo *et al.* 2009; Rosloski *et al.* 2010), raising the question of how such variation is maintained in the wild. To test for environment-dependent selection at specific loci, our sample included allelic variants at a set of 9, a priori flowering time candidate loci, including *CRY2*, *FRI*, *FLC*, *FRL1*, and *MAF2-3*. However, the position and magnitude of the genetic effects were computed *de novo* through multicross QTL mapping, which detected additional QTL not considered a priori in our

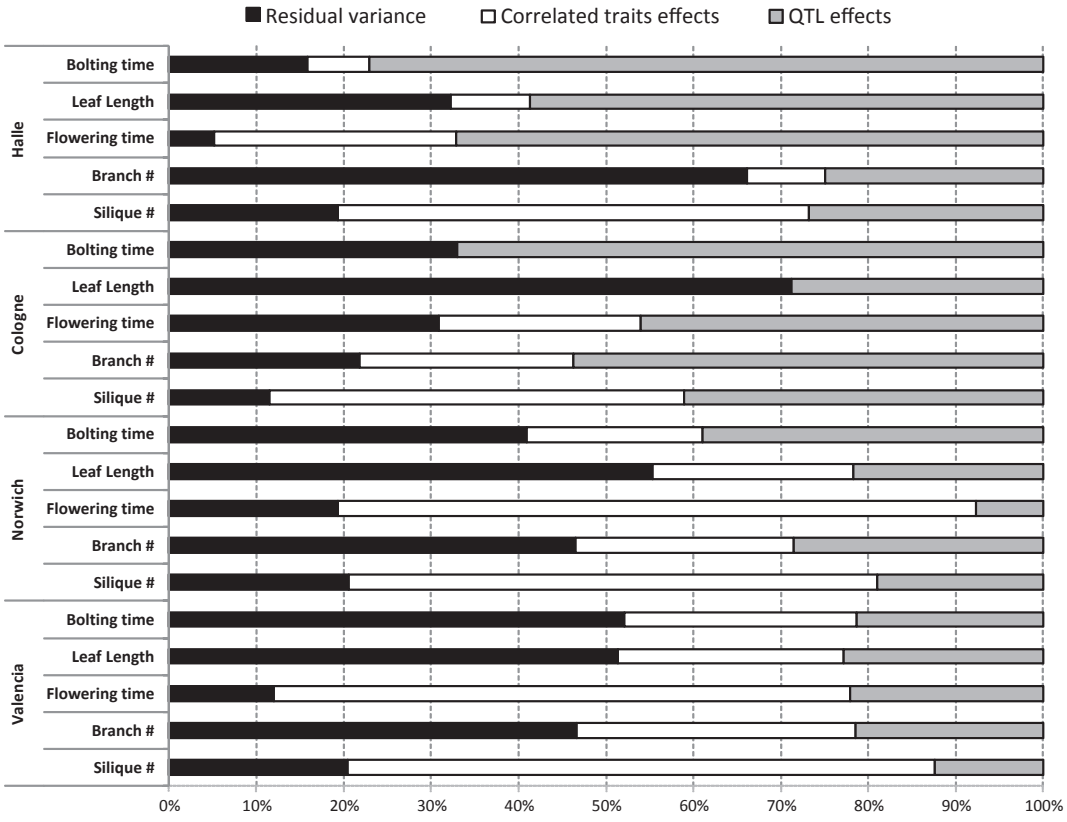

Fig. 3 Proportion of variation explained by the quantitative trait loci (QTL) effects, the influence of correlated traits and the residual microenvirenmental variance for each trait in the four environments, respectively. The proportion of variation due to QTL effects was calculated as the sum of all standardized coefficients from all QTL to a specific trait along all possible paths in Fig. 2 (direct and indirect) divided by the total variance for this trait in the structural equation model (SEM). The proportion of variation due to correlated trait was calculated in the same way but starting from all traits instead of QTL. The residual variance is the variance of the trait not explained by either QTL or correlated trait effects in the SEM.

sampling design. For example, QTL 4.43 spans the candidate gene *TSF*, already identified as a candidate QTL in a winter annual experimental planting in France (Brachi *et al.* 2010). Many of the QTL detected overlapped with those identified in controlled conditions using the same RILs (Jansen *et al.* 1995; El-Assal *et al.* 2001; Loudet *et al.* 2002; Ungerer *et al.* 2002; El-Lithy *et al.* 2004, 2006). Nonetheless, novel loci were also detected: out of the 38 QTL identified for life history and leaf length variation (30 if bolting and flowering time QTL that overlapped were considered identical), eight were not reported in prior studies; five of these novel QTL were specifically due to *Cvi* alleles, the parent having induced the most QTL.

Quantitative trait loci effects reported here were often attributable to a single allele from one parent contrasting with the ones of the five other parents (*CRY2* and *FRI*, Fig. 1 and Table S3). These allelic effects were consistent with known functional polymorphisms and consistent in direction across environments. For instance, the *Cvi* allele of *CRY2* accelerated bolting and the *Sha* allele of *FRI* delayed bolting in multiple environments. Many QTL effects were environment-specific, and we found no evidence of direct allelic effects reversing direction across environments suggesting conditional neutrality. However, conditional neutrality is statistically easier to detect than antagonistic pleiotropy, requiring only one significant test instead of two (Anderson *et al.* 2012). In addition, some QTL like *MAF2-5* were due to multiple contrasting alleles whose effects were more variable across traits and environments. This may be the consequence of the presence of allelic series at one locus or of multiple polymorphic genes in cluster (Salomé *et al.* 2011) with environment-specific expression. Finally, we found no effect of the previously reported weak *Ler* allele of the major flowering time gene *FLC* (Ungerer *et al.* 2002; Weinig *et al.* 2002; El-Lithy *et al.* 2004). Thus, the importance of major bolting/flowering time QTL detected under controlled conditions vary widely across natural environments, and some loci may be detected only under field conditions (Weinig *et al.* 2003; Brachi *et al.* 2010; Anderson *et al.* 2011).

A previous study identified loci associated with fitness in multiple environments in *A. thaliana*. Fournier-Level *et al.* (2011) performed a genome-wide association study (GWAS) of fitness on a panel of ecotypes grown in common gardens in four sites (including the Norwich, Halle and Valencia sites). Nearly all SNPs associated with fitness in that study exhibited conditional neutrality, although there was some evidence of weak genome-wide antagonistic pleiotropy between allelic effects measured in Finland vs. more southern field sites. Interestingly, there was minimal overlap between the fitness-associated loci identified by Fournier-Level *et al.* (2011) and by our experiment. This discrepancy may be due to differences in the samples used in the two studies; RIL families necessarily contained less genetic variation than the geographically diverse collection of ecotypes used by Fournier-Level *et al.* (2011). In addition, the GWAS methodology relies heavily on the pattern of linkage disequilibrium and is sensitive to allelic heterogeneity (Brachi *et al.* 2011), rare variants or loci with complex evolutionary history are thus likely to remain undetected. Our RIL sample segregated geographically differentiated InDel alleles of functional variants such as *FRI* (Korves *et al.* 2007) and *MAF2-3* (Rosloski *et al.* 2010), as well as a rare variant (*CRY2*) that would have been difficult to detect by GWAS. GWAS and QTL mapping are thus complementary approaches to identifying the loci underlying important life history traits in natural environments (Brachi *et al.* 2010).

The Environment determines the direction of selection on life history traits

Direction of selection on life history traits differed across environments. High mortality in Norwich and Cologne favoured early bolting, while later bolting was favoured in Halle and no net selection on bolting time was observed in Valencia. Longer leaves at bolting were advantageous in Valencia, Cologne, and Halle, but disadvantageous in Norwich. These environmental differences in selection were caused by differences in correlations between traits among sites, probably resulting from differences in plant growth rate and the timing and source of leaf damage and mortality. In the oceanic climates of Norwich and Cologne, we observed severe leaf damage and early mortality caused by *Albugo spp.*, a pathogenic oomycete usually present in wet fall and spring whose presence extends to winter in oceanic conditions (Kemen *et al.* 2011). In these sites, early-bolting plants were more likely to escape infection, whereas later bolting plants experienced greater pathogen-induced damage to leaves and were unable to produce branches and siliques before death. This trade-off between later bolting and branch production resulted in selection for early reproduction. In Norwich, this trade-off was so strong that it also caused net indirect selection for small leaves at bolting. In Valencia, we observed minimal pathogen damage to leaves, but substantial prereproductive mortality possibly due to water stress. Here, selection strongly favoured longer leaves at bolting, but the trade-off between bolting time and branch production resulted in no net selection on reproductive timing. In contrast, in Halle, where there was minimal pathogen damage and low pre-reproductive mortality, we observed no trade-off between bolting date and branch production. Consequently, selection acted through the weak correlation of later bolting with larger size, indirectly resulting in more branches and greater fecundity. Thus, environmental differences among sites resulted in differences in the architecture of life history tradeoffs and in the direction of selection on reproductive timing, as predicted by life history theory. This geographic variation in selection indirectly favoured alternate alleles at *CRY2* and *FRI* in different sites. Early *FRI* alleles were advantageous in Norwich, and early *CRY2* alleles were advantageous in Cologne, but indirect selection favoured late *FRI* and *CRY2* alleles in Halle. In addition, we are using the fruit number as a proxy for fitness and there might therefore be other unmeasured factors determining fitness, for instance seed viability, that can mitigate trade-offs between life history and fitness.

Our results show that both conditional neutrality in the allelic effects of life history QTL and indirect antagonistic pleiotropy due to environmental differences in life history tradeoffs can contribute to environmental differences in selection at candidate life history loci. Several other studies have reported conditional neutrality from field experiments with *A. thaliana* (Huang *et al.* 2010; Fournier-Level *et al.* 2011) and other species (Anderson *et al.* 2012). Evidence for antagonistic pleiotropy is rarer. Weinig *et al.* (2002) observed a trade-off for fitness at a QTL conferring high fecundity in a spring planting in Rhode Island, but low survival in a fall planting in North Carolina. Todesco *et al.* (2010) showed that the *ACD6* locus promoting resistance is also associated with slow growth and small size under laboratory conditions, suggesting a mechanism for the maintenance of intermediate frequency polymorphisms within populations. Anderson *et al.* (2012) found antagonistic pleiotropy contributing to local adaptation across sites in 2.8% of the genome in a field experiment with *Boechera stricta*, including a major flowering time QTL which contributed to local adaptation; in the same study 8% of the genome displayed conditional neutrality. Our SEM results suggest that antagonistic pleiotropy at a locus may depend upon the direct and indirect selection acting on loci underlying life history

traits. In some cases, an underlying life history trade-off (such as between bolting time and branch number) may produce multiple indirect paths to fitness that can cancel one another out, resulting in no net selection in certain environments (as with bolting time in Valencia).

Environmental factors determining the effect of 'genetic variation

In the low-mortality environments (Halle and Cologne), a larger proportion of variance was due to genetic effects compared to the high-mortality environments of Valencia and Norwich (possibly due to water stress and pathogen pressure, respectively), leading to greater heritability and influence of QTL in the less stressful environments. Such a pattern may be a statistical bias as differential mortality leads to an uneven numbers of observations across environment. We assessed this possible issue through simulation (Data S2 and Figs S7 to S9) and demonstrated that in our data, the level of differential mortality alone was not enough to explain the decrease of heritability following increased environmental stress. However, similar patterns were observed in a meta-analysis of animal wild populations (Charmantier & Garant 2005), with lower heritability in stressful environment. An explanation for such a pattern is that stressful conditions are typically different from the ones where an organism has evolved, and therefore, this organism may lack the appropriate genetic variation to respond to new environmental cues (Uller *et al.* 2002). Interestingly, the consequence of reducing heritability in stressful environment is a reduced selection efficiency which maintains more genetic variation and favours a higher level of phenotypic stasis (Wilson *et al.* 2006). This may indeed be an advantage when the populations are facing harsh environmental conditions, as it avoids genetic erosion.

Early traits such as bolting time or leaf length were highly heritable, whereas traits expressed later, such as branch number, were more affected by microenvironmental variation (Fig. 3). Thus QTL influencing the early stages of plant development are more likely to show strong response to selection (Huang *et al.* 2010). This may be a consequence of microenvironmental noise accumulating over the lifetime of the plant (Mitchell-Olds & Bergelson 1990), while genetic variation is fixed within one generation of the population considered. It may also result from past selection events which depleted the initial genetic variation at loci directly related to fitness (Mousseau & Roff 1987).

Overall, this study suggests that selection on loci controlling life history traits is crucial in the response to specific environmental variation. Only few loci seem to affect fitness directly and genetic selection mostly acts indirectly through life history and growth traits that determine fitness. Even if the direction of the genetic effects underlying these traits remains unchanged across environment, environmental variation mediates a major shift in the direction and intensity of the correlation between traits. Consequently, major functional allelic variants with stable direct effect on life history, may have opposite indirect effects on fitness in different environments, or be sheltered from selection in some environments. Such geographic variation in the direction or intensity of selection justifies therefore the persistence of functional genetic variation in life history trait across the species range.

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J.S., M.K., S.M.W. and A.M.W. conceived the study; J.S., S.M.W. and A.M.W. designed the experiment; B.P. and M.R. developed experimental materials; M.D.C., J.L.R., J.A., D.E., B.T.M., R.H.P., R.N.S. and A.M.W. performed the experiments; A.F.L., D.L.R., and J.S. designed the analyses; A.F.L. performed the analyses; A.F.L. and J.S. wrote the manuscript, revised by all authors.

Data accessibility

Input files and R analysis scripts for QTL mapping and SEM analysis are available at the Dryad data repository (http://datadryad.org/resource/doi:10.5061/dryad.1pg3n).

Supporting information

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

Data S1 Power to detect QTLs over the entire genome given the selection of specific lines.

Data S2 Effect of differential mortality on the estimation of heritability across environment.

Table S1 Description of the location, climate and soil characteristics of the four planting environments.

Table S2 Individual survival for each family in each planting site.

Table S3 Location and summary statistics of the detected QTL.

Table S4 Summary statistics for the SEMs.

Fig. S1 Power to detect a QTL for each locus (*k*-ratio, Zhang *et al*. 2010) given the various level of segregation distorsion in the di_erent RIL families.

Fig. S2 Density distribution of bolting time expressed in days and in photothermal units.

Fig. S3 Accumulation of photothermal units over time from the sowing date in each of the four planting sites.

Fig. S4 Density distribution of leaf length at bolting and photothermal units to flowering.

Fig. S5 Density distribution of branch and silique number.

Fig. S6 Pairwise correlation between the allelic e_ect of QTL colocating within 10 cM (Pearson's p, p-val<0.05).

Fig. S7 Estimation of the observed heritability for simulated datasets fro different level of initial heritability.

Fig. S8 Estimation of the observed heritability for simulated datasets fro different level of initial heritability.

Fig. S9 Estimation of the observed heritability for simulated datasets fro different level of initial heritability.

Fig. S10 Validation of candidate genes using NILs.