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The earliest stages of adaptation in an experimental plant population: strong selection on QTLS for seed dormancy

XUEQING HUANG,* JOHANNA SCHMITT,† LISA DORN,‡ CONVERSE GRIFFITH,§ SIGI EFFGEN,* SHAUN TAKAO,¶ MAARTEN KOORNNEEF*,** and KATHLEEN DONOHUE¶

**Max Planck Institute for Plant Breeding Research, Carl-von-Linne Weg 10, 50829 Cologne, Germany, †Department of Ecology and Evolutionary Biology, Brown University, Box G, Providence, RI, 02912, USA, ‡Department of Biology and Microbiology, University of Wisconsin, Osh-Kosh, Oshkosh, WI, 54901 USA, §Kisatchie National Forest, United States Department of Agriculture Forest Service, 9912 Highway 28 West, Boyce, Louisiana 71409, USA, ¶Department of Biology, Duke University, Box 90338, Durham, NC 27708, USA, **Laboratory of Genetics, Wageningen University, Arboretumlaan 4, 6703 BD, Wageningen, The Netherlands*

Abstract

Colonizing species may often encounter strong selection during the initial stages of adaptation to novel environments. Such selection is particularly likely to act on traits expressed early in development since early survival is necessary for the expression of adaptive phenotypes later in life. Genetic studies of fitness under field conditions, however, seldom include the earliest developmental stages. Using a new set of recombinant inbred lines, we present a study of the genetic basis of fitness variation in *Arabidopsis thaliana* in which genotypes, environments, and geographic location were manipulated to study total lifetime fitness, beginning with the seed stage. Large-effect quantitative trait loci (QTLs) for fitness changed allele frequency and closely approached 90% in some treatments within a single generation. These QTLs colocated with QTLs for germination phenology when seeds were dispersed following a schedule of a typical winter annual, and they were detected in two geographic locations at different latitudes. Epistatically interacting loci affected both fitness and germination in many cases. QTLs for field germination phenology colocated with known QTLs for primary dormancy induction as assessed in laboratory tests, including the candidate genes *DOG1* and *DOG6*. Therefore fitness, germination phenology, and primary dormancy are genetically associated at the level of specific chromosomal regions and candidate loci. Genes associated with the ability to arrest development at early life stages and assess environmental conditions are thereby likely targets of intense natural selection early in the colonization process.

Keywords: adaptation, colonizing species, *DOG1*, *DOG6*, dormancy, germination, life history, phenology

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Introduction

In this century of rapid climate change, species range shifts, and widespread human introductions of exotic species, organisms often encounter novel selection pressures in new locations and environments. To understand the process of adaptation to novel environments, it is critical to know which traits adapt first, or which favourable alleles are fixed first. It has been shown theoretically that the sequence of allelic substitutions can constrain future evolutionary pathways, especially

Correspondence: Kathleen Donohue, Fax: (919) 660-7293; E-mail: K.donohue@duke.edu

when loci interact epistatically to influence fitness (Weinreich *et al.* 2005). Likewise at the organismal level, the prior adaptation of some traits can influence the adaptive value of other traits and consequently their evolutionary trajectories. Therefore, identifying loci that are under strong selection at early stages of adaptation and assessing their phenotypic effects is of great relevance for characterizing the process of adaptation to new locations.

Traits expressed early in development may be under especially strong selection at the early stages of adaptation, since organisms first need to express adaptive, or at least viable, phenotypes in order to survive to express adaptive phenotypes later. Traits that are associated with habitat selection may also be important for colonizing new locations; by selecting a habitat to which an organism is already adapted, the requirements for further adaptive evolution to changed conditions are diminished (Templeton & Rothman 1982). In plants, habitat selection occurs through dispersal (of seeds, vegetative propagules, or the growth form of ramets or other morphological structures, for example) and phenology (Bazzaz 1991). The timing of developmental transitions can be an especially effective mechanism of seasonal habitat selection in plants, since the timing of one transition determines the seasonal environment experienced by all subsequent developmental stages (Donohue 2005). As such, phenology may play an important role in early adaptation to new locations or changed climate (Walther 2004; Willis *et al.* 2008). Here we test whether variation at loci associated with early developmental stages, and especially those associated with phenology, is subject to natural selection at the earliest stages of adaptation in an experimental population of the colonizing species *Arabidopsis thaliana*.

Natural populations *in situ*—even if introduced in historic time—will have been exposed to natural selection; inviable genotypes or those that fail to reproduce effectively will have already been purged from the population. One approach to investigating the very earliest stages of adaptation and establishment involves using a naïve sample of genotypes that have never been exposed to natural selection and examining their performance in locations in which the species has successfully established in its region of introduction. In this manner, traits that are critical for initial establishment of a population can be identified. Here we present results of such an experiment in which an experimental population of *A. thaliana*, which is genetically and phenotypically diverse in many life-history traits (Donohue *et al.* 2005a,b), was studied in old-field sites—the most abundant habitat in which *A. thaliana* has established in its introduced range in North America.

The experimental population used in this study was a new set of mapped recombinant inbred lines derived from two natural populations. Transgressive segregants in recombinant populations extend the range of phenotypic and genetic variation beyond that present in the parental populations but which is still within plausible bounds of extant natural variation. In fact, recent population-genetic studies of *A. thaliana* conducted at small spatial scales have found evidence for natural recombinant inbred lines in natural populations, suggesting that occasional outcrossing in a highly selfing species can create the sort of recombinant genetic variation found in experimentally produced recombinant inbred lines (J. Borevitz & K. Bomblies, personal communication). Using environmental and geographic manipulations, we conducted a quantitative trait loci (QTL) analysis of fitness in the field to identify key traits and loci associated with the earliest stages of adaptation to different geographic locations within an introduced range. Unlike other QTL analyses of fitness-related traits, we included the earliest developmental stage of seeds in our study specifically to address the possibility that early stages are subject to especially strong selection during colonization of new locations.

Materials and methods

Recombinant inbred lines and map

Recombinant inbred lines were derived from two accessions of *A. thaliana*: one from Calver, England (Cal), and the other from Tacoma, Washington (Tac), with Tac as the maternal parent. These lines exhibit abundant genetic variation in germination behaviour and germination plasticity to seed-maturation conditions (Munir *et al.* 2001). The Tac parental line is more dormant than the Cal line, and it also displayed greater sensitivity of germination to the photoperiod of seed maturation. Seeds from Calver were acquired through the Arabidopsis Biological Resource Center at Ohio State University (stock CS1062). Seeds from Tacoma were collected by T. Mitchell-Olds. One hundred and fifteen recombinant lines were derived from F_2 crosses and maintained for seven generations by single seed descent. More details on the lines and their maintenance are presented in Donohue *et al.* (2005a).

Genotyping of single F9 plants was performed. Total genomic DNA was isolated from fresh flower buds in greenhouse-grown plants using DNeasy 96 Plant Kit (QIAGEN) and quantified using a spectrophotometer ND-1000 Nanodrop (PeQLab, Germany). A total of 115 F9 DNA samples were then genotyped with simple sequence repeat (SSR, or microsatellite) and SNP markers. Primer sequences for SSR markers have been

described in The Arabidopsis Information Resource (TAIR; http://www.arabidopsis.org) or in the MSAT database (http://www.inra.fr/internet/Produits/vast/msat.php). Table S1 (Supporting Information) provides detailed PCR protocols, conditions, and primers for the SSR markers. In addition, a set of 149 Framework SNPs among 384 Arabidopsis accessions developed by the Borevitz laboratory (http://borevitzlab.uchicago.edu/resources/molecular-resources/snp-markers) was used to genotype each RIL. The SNP genotyping was performed by the SEQUENOM company (USA; http://www.sequenom.com/), which used MassARRAY® iPLEX Gold for custom SNP genotyping. The primer design and the preparation of DNA sample were conducted according to the specifications of the company.

A set of 101 markers covering most of the *A. thaliana* genetic map at intervals of 1–11 cM was constructed with the JoinMap programme (version 4.0, http://www.kyazma.nl). This resulted in a genetic map similar in length to that of other *A. thaliana* populations (El-Lithy *et al.* 2006; O'Neill *et al.* 2008) and with segregation distortion at a limited number of positions (Fig. 1). The RILs are being submitted to the Arabidopsis Biological Resource Center with the map and marker data and will be available to the public.

Field experimental design

Conditions during seed maturation have pronounced effects on dormancy and germination (reviewed in Gutterman 1992; Donohue 2009), and the photoperiod of seed maturation has been shown to influence germination in this and other species (e.g. Hayes & Klein 1974; Gutterman 1994; Munir *et al.* 2001). Seeds of the 115 RILs were therefore matured under two photoperiods in Conviron E72 Growth chambers in order to replicate the photoperiod of seed maturation during June and November when plants have been observed to mature seeds in natural populations of *A. thaliana* in North America: 'Short days' comprised 10 h light/14 h dark at 22 °C, and 'Long days' comprised 14 h light/10 h dark at 22 °C. Seeds were grown in two batches so that fresh seeds could be dispersed in June and in November. Within each temporal batch, plantings were staggered so that seeds from all genotypes and from both photoperiods were harvested simultaneously. These seeds were used in the field experiment and in the laboratory studies of germination discussed below, with the exception of the quantification of primary dormancy ('$DSDS_{50}$').

Two field sites were used, one in Lexington, Kentucky (KY), USA and a more northerly site in Bristol, Rhode Island (RI), USA (Fig. S1, Supporting Information). Both sites experience a distinct winter season, but in the KY site, summer drought arrives in June and frequently remains through October, whereas in RI the summers and autumns are somewhat wetter. Autumn flowering from rapid-cycling autumn germinants has been observed in naturalized populations in RI and elsewhere in New England (Donohue 2009), and are commonly seen in the UK and elsewhere in Europe (e.g. the Netherlands) and Japan (Thompson 1994).

Seeds were dispersed only in June in KY, as natural seed dispersal was observed there only in June. Seeds were dispersed in June and in November in RI since natural populations near the RI field site have been observed to disperse seeds during both of those months. Field sites were established in blocks, with the RI site as a split-plot design, with different blocks for each dispersal season. Seeds were deposited into peat pots filled with Metromix 360 (Scotts Sierra, Marysville, OH, USA) in order to control for site-specific soil effects and enable a more controlled contrast of climatic differences. Twelve seeds of a given genotype were placed in a given pot, and each genotype had nine replicate pots for each treatment (two photoperiod treatments and three location-dispersal treatments: KY, June; RI-June; RI-November). The pot was the unit of analysis.

The germination timing of each seed was recorded through weekly monitoring, and the mean germination timing of all germinants within a pot was calculated. The total proportion of seeds that germinated during the course of the experiment in each pot was also recorded. A single random focal individual was followed in each pot, and all other germinants were plucked from the pot during each census. The focal individual was followed throughout its life, its adult traits were recorded (contingent on survival), and its total lifetime fitness was estimated as the total number of fruits it produced (zero if it died before reproducing). Other adult traits included the day of bolting (initiation of reproduction), the time interval between bolting and first flower, the number of rosette leaves at the time of bolting, the rosette diameter at the time of bolting, the final height of the plant, the number of basal and total branches. Plasticity to treatments was calculated for each genotype as the difference in the genotypic mean phenotype across treatments (plasticity to photoperiod = long-day - short-day; plasticity to site = RI - KY; plasticity to season = November - June). More details on the field design are provided in Donohue *et al.* (2005a).

Laboratory studies of dormancy and germination

Mature seeds dispersed in the field may be dormant or non-dormant, depending on the degree to which primary dormancy was induced during seed maturation.

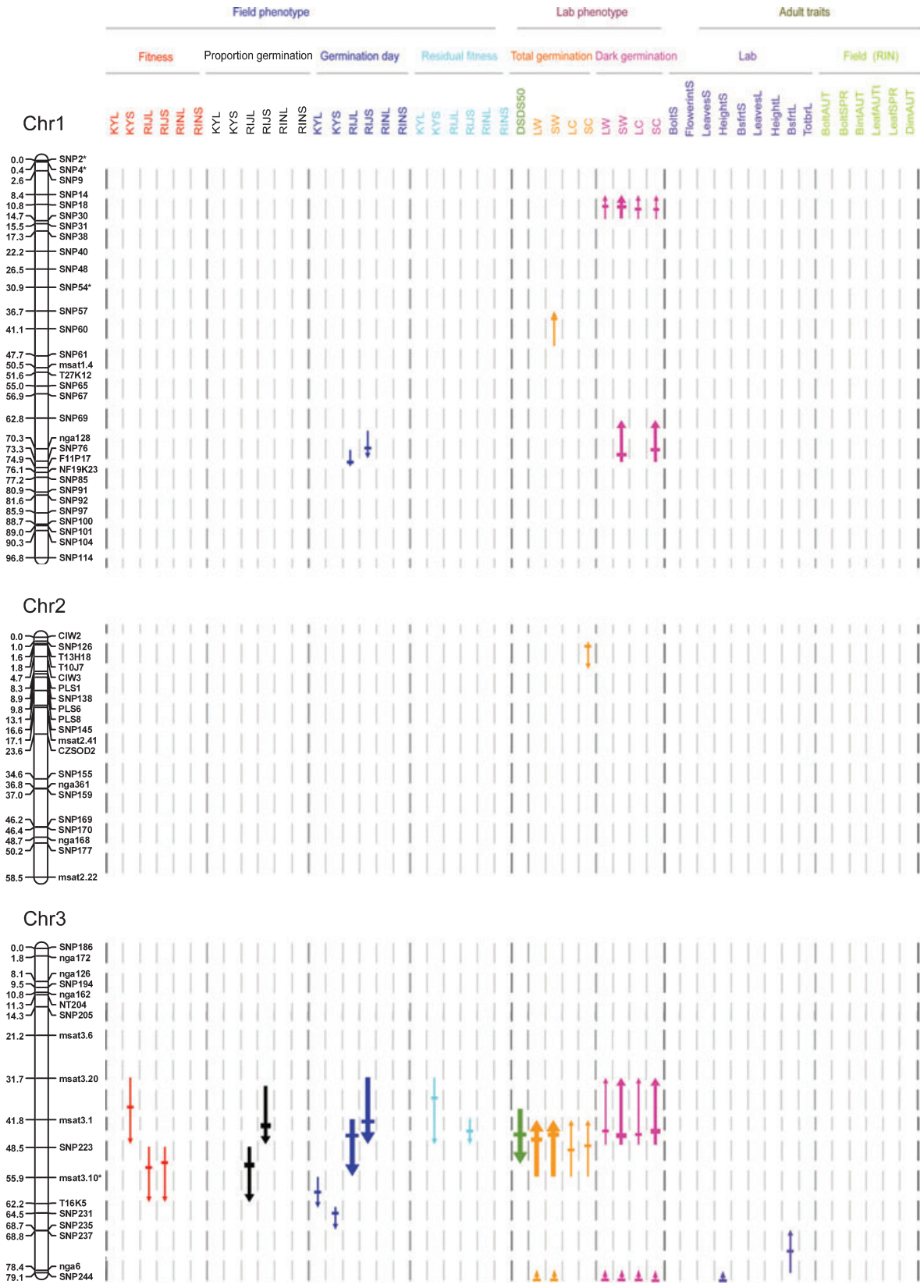

Fig. 1

Fig. 1 The Cal × Tac linkage map showing the locations of QTLs for the traits analysed. Regions of segregation distortion are marked by asterisks indicating significance level of the distortion (*$P < 0.05$, **$P < 0.01$). Tick marks on the arrows indicate the optimized location of the QTL based on MQM mapping in MapQTL. The length of arrows indicates the 2.4-LOD support intervals. The direction of the arrow indicates the allelic effects. Upward: the Tac allele is associated with an increase in the trait; downwards: the Tac allele is associated with a decrease in the trait. The thickness of the arrow refers to the LOD score value. Thin line indicates LOD score 2.4–5.0; Medium line indicates LOD score 5.0–10.0; Thick line indicates LOD score over 10.0. See text for explanation of the variables. Only traits with significant QTLs are shown. KYS = Kentucky, June, Short-day; KYL = Kentucky June, Long-day; RIJS = RI, June, Short-day; RIJL = RI, June, Long-day; RINS = RI, November, Short-day; RINL = RI, November, Long-day; LW = long-day seed maturation, warm imbibition: SW = short-day seed maturation, warm imbibition; LC = long-day seed maturation, warm followed by cold imbibition; SC = short-day seed maturation, warm followed by cold imbibition. For adult traits, suffix S = short days, suffix L = long days, suffix AUT = autumn germination cohort; suffix SPR = spring germination cohort; Bolt = Bolting day; Flowerint = time interval between bolting and flowering; Leaves/leaf = number of leaves at bolting; Height = plant height, Bsfrt = number of fruits on basal branches; Totbr = total number of branches; Bint = time interval between germination and bolting; Dim = Diameter at time of bolting. [Correction added after online publication 12 February 2010: the first of the two parts of Fig. 1 was replaced.]

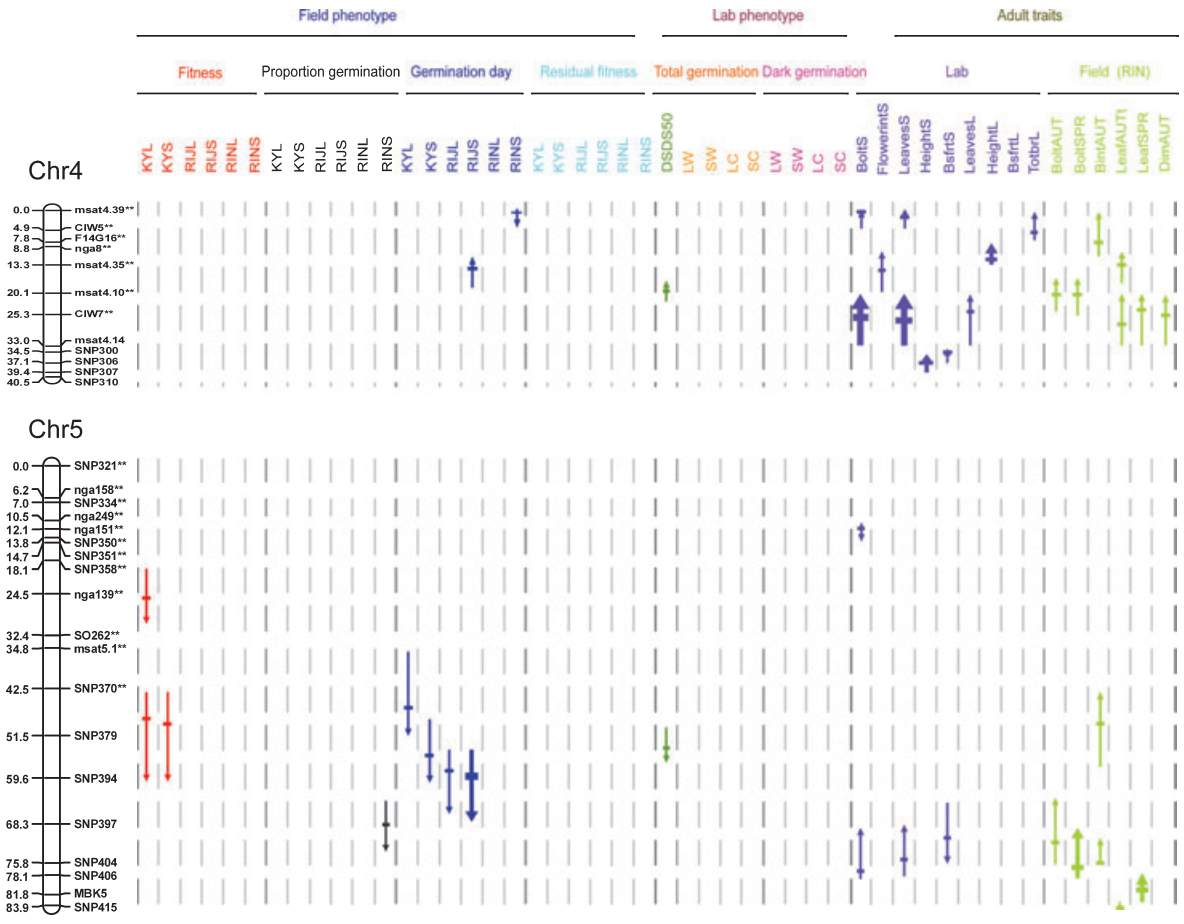

Fig. 1 (continued).

Primary dormancy was scored by assessing the germination of seeds that experienced different durations of dry storage (Alonso-Blanco *et al.* 2003; Bentsink *et al.* 2006). Seeds were matured in an air-conditioned greenhouse in Cologne, Germany with supplemental lighting, using a 16-h light 20 °C day and 8h dark 18 °C dark night. Three plants of a given genotype were grown in a pot in a randomized block design with two pots per genotype, and seeds were harvested by individual plant. Seeds were stored dry at room temperature for 1, 4, 8 and 13 weeks. Between 95% and 100% of the seeds were completely non-dormant after 13 weeks in most genotypes. Between 50 and 100 seeds from each of four plants per genotype (two from each of the two pots) were evenly sown on filter paper soaked with 0.7 mL demineralized water in a 6-cm Petri dish. Petri dishes

were placed in moisture chambers consisting of plastic trays containing a filter paper saturated with tap water and closed with transparent lids. Moisture chambers were stored in a climate chamber (25 °C, 16-h light period). The total number of viable seeds and the number of germinating seeds were scored, and the percentage of germinating seeds was calculated after 1 week. Curves of germination percentage converted into probit units as a function of the time of storage provided the kinetics of seed dormancy of each genotype. Measurements of germination proportions at all time points were used for probit regression on a logarithm timescale (SPSS, version 13.0). The dormancy of a genotype was estimated in a single parameter as the number of *d*ays of *s*eed *d*ry *s*torage ('after ripening') required to reach 50% germination ($DSDS_{50}$).

When seeds are dispersed during the typical spring or summer dispersal season, fresh seeds experience a warm summer period during which they may be induced into secondary dormancy (if they were dispersed as non-dormant). When cooler autumn conditions arrive, seeds experience fluctuating cool temperatures that, in many species including *A. thaliana*, break dormancy (Baskin & Baskin 1983, 1998). We therefore measured germination proportions after a warm imbibition period, and germination proportions after a warm period followed by a cold period. Secondary dormancy induction by warm temperature was measured using fully after-ripened seeds of all genotypes (100% germination of all genotypes assessed 8 months after harvest) of the same batch of seeds that were matured under short and long days for the field experiment. Fifteen seeds of a given genotype were placed on 0.5% agar Petri plates, with 12 plates per genotype. Plates were the unit of analysis. These seeds were then imbibed in the dark at 30 °C for 7 days, and then placed in the light at 22 °C. The proportion of viable seeds that germinated was counted after 14 days. The proportion of viable seeds that germinated was also determined for seeds that experienced first a warm (7 days at 30 °C) then a cold (5 days at 4 °C), dark imbibition period before being placed at the germination-permissive temperature of 22 °C in the light. Plasticity to treatments was calculated for each genotype as the difference in the genotypic mean phenotype across treatments (plasticity to photoperiod = long-day - short-day; plasticity to temperature = cold - warm).

QTL analysis

Genotypic means for each treatment were used in all QTL analyses. MapQTL (version 5.0, http://www.kyazma.nl) was used to identify and locate QTLs linked to molecular markers, using both interval mapping and multiple-QTL model mapping (MQM) methods, as described in the MapQTL reference manual. A LOD threshold of 2.6 was applied to declare the presence of a QTL. We verified this threshold for interval mapping by applying the permutation test to each data set (1000 repetitions) and found a $P < 0.05$ LOD varying between 2.4 and 2.6 (Doerge & Churchill 1996). The estimated additive effect and the percentage of variance explained by each QTL, as well as the total variance explained by all of the QTLs affecting a trait, were obtained in the final MQM model. For this, different cofactor markers were tested around a putative QTL position (Van Ooijen & Maliepaard 1996) and those maximizing the LOD score were selected as the final cofactors. Some traits were not normally distributed and could not be transformed to normality (Fig. S2, Supporting Information). In such cases, nonparametric Kruskal–Wallis test was used to verify significance levels. It should be noted however that such tests are conservative, do not control for obscuring cofactors, and are analogous to single-marker analysis.

To test for significant differences in QTL effects across environments (QTL–environment interactions) and colocation of QTL for different traits, we conducted multiple-trait composite interval mapping (CIM) analysis ('multiple-traits analysis') (Jiang & Zeng 1995) using Windows QTL Cartographer (version 2.5, Basten *et al.* 2004; Wang *et al.* 2005). We used 'hypothesis four' ('Test GxE' in QTL Cartographer) that tests for QTL × environment (or trait) interaction. We also verified QTLs identified with MapQTL using multiple interval mapping (MIM) in QTL Cartographer, using forward regression marker selection and default parameters.

To test for epistasis, MIM was used in QTL Cartographer, and the model was refined by searching for QTL interactions. This method tests for epistatic interactions among QTLs that exhibit main effects. To test for epistasis involving loci without main effects, a genome-wide scan for epistasis was conducted using EPISTAT (Chase *et al.* 1997). Regions with significant epistasis were identified according to LOD scores, and only those with LOD scores greater than 50 were investigated further. The markers with the most significant interaction within a significant region were identified using ANOVAs of marker pairs. In addition QTL × QTL × environment interactions were further verified with ANOVAs in the SAS statistical package (SAS 9.1.3).

Results

QTLs for fitness in the field

Fitness in KY was lower than in RI, with many seedlings dying from drought before reproducing, and the survivors also had lower fruit production (Donohue

et al. 2005b). Most seeds that were dispersed in November as opposed to June survived to germinate and reproduce, as they did not encounter summer drought conditions between germination and flowering, although being smaller, they had less fruit production. Donohue *et al.* (2005b) gives more details of fitness and germination differences across treatments.

Considering lifetime fitness of germinants, a small number of QTLs with large effects was detected for seeds dispersed during June, the typical dispersal time for a winter annual (Fig. 1; Table S2, Supporting Information). This measure of fitness is the total number of fruits produced throughout the lifetime of the plant, from germination through senescence. In the southern location, KY, one QTL on chromosome 3 and two QTLs on chromosome 5 were identified that, when their main effects were combined, explained up to 26% of the variance in fitness. In the northern location, RI, when seeds were dispersed in June as they were in KY, a single QTL on chromosome 3 explained up to 20% of the variance in fitness. For both loci, the Cal alleles increased fitness. When seeds were dispersed later in the season in the northern site, neither of the previous QTLs was detected. Multiple-trait QTL analysis that tested for genotype × environment interactions identified the QTL on chromosome 3 as having significantly stronger effects on fitness in June-dispersed seeds than in November-dispersed seeds (Table 1). No significant QTL × site interaction was detected, despite the fact that the QTLs on chromosome 5 were significant only in KY. Moreover, the QTL on chromosome 3 detected in KY overlapped with the QTL detected in RI, indicating it may be associated with the same locus. The photoperiod of seed maturation had no effect on the detection of QTLs for fitness in any treatment. Thus upon first introduction to old-field habitats, a small number of loci are subject to intense natural selection in both northern and southern locations.

Significant epistasis for lifetime fitness was detected (Fig. 2; Tables 2 and S3, Supporting Information). The main-effect QTLs on chromosome 3 and chromosome 5 interacted to influence fitness in the southern location (significant in short-day seeds), and the two QTLs on chromosome 5 interacted in both sites (significant in long-day seeds). A genome-wide scan for epistasis identified several interactions between QTLs without main effects on fitness (Fig. 2; Tables 2 and S3, Supporting Information). In all field treatments, the majority of interactions were such that native combinations of alleles had higher fitness than recombinants, and the Cal–Cal combination had higher fitness than Tac–Tac in all such cases. However, approximately one-third of the interactions were such that recombinant genotypes had the highest fitness. Therefore, the Cal parental population harbours adaptive combinations of alleles, but recombination nevertheless can create novel combinations of alleles that are more highly adapted to these habitats of new introduction.

Table 1 QTL-environment interactions. Likelihood ratios for tests that QTL effects differ across environments. The likelihood ratio (columns 3 and 4) compares the model with the observed effect of the QTL to the model constrained to be the same in the two environments. Only QTLs with significant interactions are shown. Boldface indicates a significant interaction. Significant LR threshold = 11.96. Upper: QTL-environment interactions for field phenotypes. KYS = Kentucky, June, Short-day; RIJS = RI, June, short-day; RIJL = RI, June, long-day. Lower: QTL-environment interactions in the laboratory. WL = Warm, long-day; WS = Warm, short-day. 'Diff from $DSDS_{50}$?' gives the LR that tests whether the effect size of the QTLs differ significantly from those for $DSDS_{50}$

QTL	Chr	Position (cM)	QTL × Site	QTL × season
Fitness				
RIJL	3	0.5587	7.62	**30.50**
RIJS	3	0.5586	6.54	**15.36**
Proportion of seeds germinated				
RIJL	3	0.5447	**21.54**	3.05
RIJS	3	0.4385	**18.50**	6.5
Germination day				
RIJL	1	0.7331	7.15	**12.91**
RIJL	3	0.4585	**15.59**	**50.73**
RIJS	3	0.4285	**16.10**	**39.17**
Residual fitness				
RIJS	3	0.4585	1.53	**12.69**

QTL	Chr	Position (cM)	QTL × photoperiod	QTL × temperature	Diff. from $DSDS_{50}$?
Germination in light in the laboratory					
WL	3	0.3975	0.27	**35.47**	**15.45**
WL	3	0.7579	0.01	**29.64**	8.66
WS	3	0.4585	0.08	**44.99**	**19.05**
WS	3	0.7843	0.00	**16.12**	2.20

Several pairs of interacting alleles had consistent effects on lifetime fitness across all environmental treatments, as indicated by the lack of QTL × QTL × environment interactions (Table S4, Supporting Information). This indicates that some gene combinations can improve fitness across a broad range of latitude and in populations with fundamentally different life histories, such as seed dispersal in autumn as opposed to summer. However, some interactions were detected only in one of the seasons of seed dispersal or photoperiods of seed maturation, indicating that flowering phenology can influence epistasis for fitness through its effects on seed maturation conditions and the season of seed dispersal.

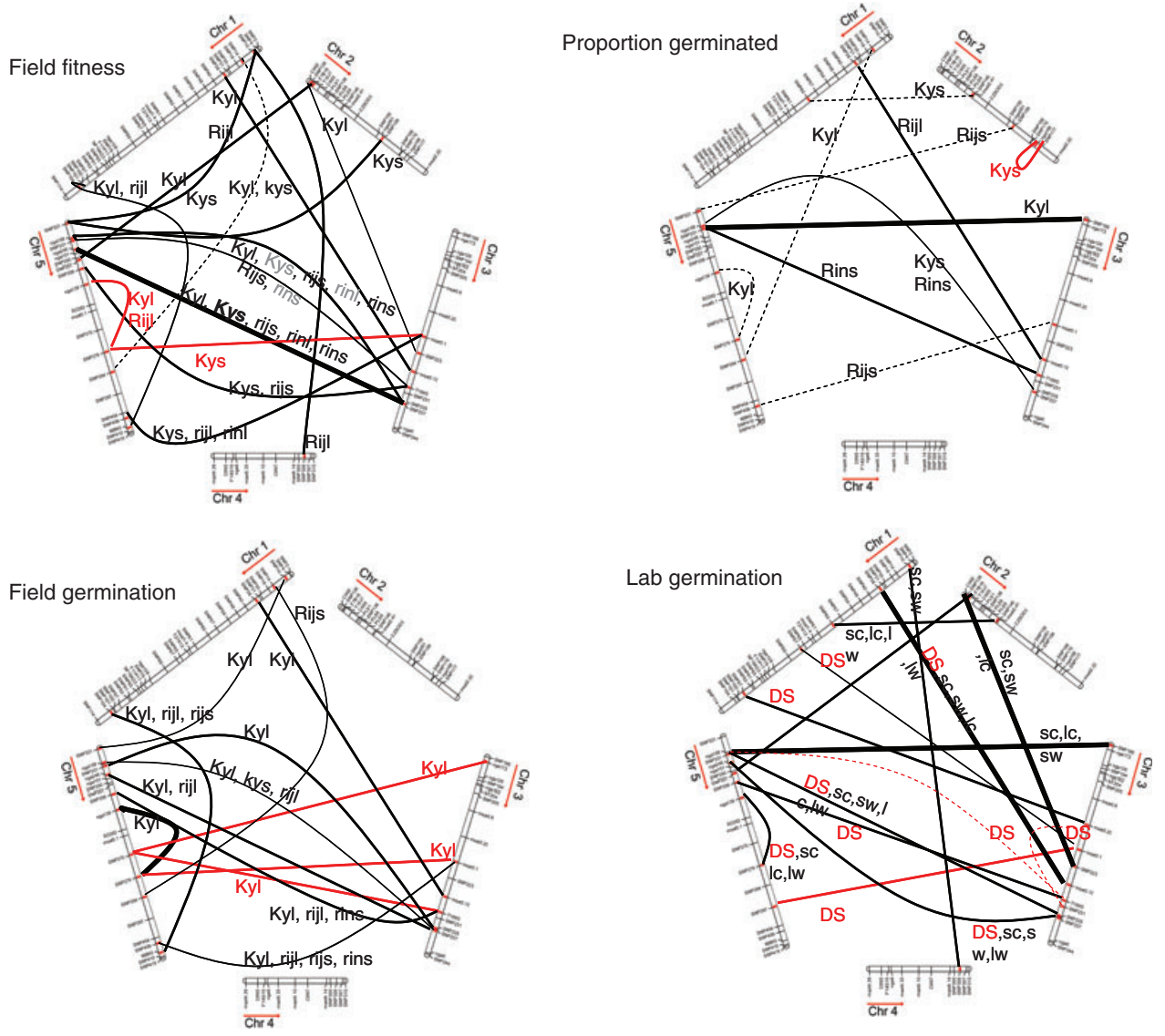

Fig. 2 Epistatic interactions associated with variance in field phenotypes and germination in the laboratory. Red lines indicate significant interactions between main-effect QTL, identified in QTL Cartographer MIM. The level of significance is indicated by the width of the line, based on the trait that showed the highest significance level (shown in bold), as indicated in Table S2 (Supporting Information). Dotted line = $P < 0.1$; Thin line = $P < 0.05$: Medium line = $P < 0.01$; Thick line = $P < 0.001$. The treatments that show significant epistatic influences are indicated near one of the markers. KYS = Kentucky, June, Short-day; KYL = Kentucky June, Long-day; RIJS = RI, June, Short-day; RIJL = RI, June, Long-day; RINS = RI, November, Short-day; RINL = RI, November, Long-day; WL = warm imbibition, long-day seed maturation: WS = warm imbibition, short-day seed maturation; CL = warm followed by cold imbibition, long-day seed maturation; CS = warm followed by cold imbibition, short-day seed maturation.

An even earlier component of fitness is whether seeds germinate or not. For seeds dispersed in RI during June, one QTL was detected on chromosome 3 that was significantly associated with the proportion of seeds that germinated over the course of the whole experiment (Fig. 1; Table S2, Supporting Information). These QTLs were associated with 29% (long-day seeds) to 26% (short-day seeds) of the variance in germination proportion, and their effects were significantly stronger in RI than in KY (Table 1). Photoperiod had no significant effect on QTL effects in any treatment, as indicated by the lack of significant QTL × photoperiod interaction (Table 1). The QTL on chromosome 3 overlapped with a QTL for lifetime fitness. A different QTL, on chromosome 5, was detected for germination proportion when seeds were dispersed in November, and like the QTL detected in June-dispersed seeds, its effect did not differ significantly across dispersal seasons. This QTL was

Table 2 Summary of epistatic interactions. '#Interactions' indicates the number of epistatic interactions detected between markers. 'Conditional/Interactive' indicates the number of epistatic interactions that are conditional/the number of epistatic interactions that are interactive. Conditional indicates that the magnitude of the difference between alleles depends on the allele at the other locus. Interactive indicates that the rank order of alleles of one locus changes depending on the alleles at the other locus. 'Native/Recombinant' indicates the number of interactions in which the native genotype combination had the highest phenotypic value/the number of interactions in which a recombinant genotype combination had the highest phenotypic value. ''Highest native genotype'' indicates which native genotype combination had the highest value. Values are given for each treatment. 'Q × Q × E' gives the number of significant QTL × QTL × Environment interactions; 'Photo' gives the number of interactions for KY-June, RI-June, and RI-November, respectively; 'Site' gives the number of interactions based on a comparison of KY-June and RI-June; 'Season' gives the number of interactions based on a comparison of RI-June and RI-Nov. CC = Cal–Cal, TT = Tac–Tac

	KYJune, Long	KYJune, Short	RI June, Long	RI June, Short	RI Nov, Long	RI Nov, Short	Q × Q × E
Fitness							
#Interactions	9	5	5	5	3	4	Photo:1, 3, 8
Conditional/interactive	8/1	4/1	3/2	5	2/1	3/1	Site: 0
Native/recombinant	6/3	4/1	3/2	4/1	3/0	0/4	Season: 6
Highest native genotype	CC	CC	CC	CC	CC	NA	
Germination day							
#Interactions	9	2	6	2	0	2	Photo:0, 2, 0
Conditional/Interactive	4/5	1/1	2/4	0/2	0	2/0	Site: 3
Native/Recombinant	8/1	2/0	5/1	1/1	0	2/0	Season: 5
Highest native genotype	CC	CC	CC	CC	NA	CC	
Proportion germinated							
#Interactions	3	2	1	2	0	1	Photo:1, 0, 0
Conditional/Interactive	0/3	1/1	0/1	1/1	0	0/1	Site: 8
Native/Recombinant	0/3	1/1	1/0	1/1	0	0/1	Season: 7
Highest native genotype	NA	CC	CC	CC	NA	NA	
	$DSDS_{50}$	Long, Warm	Long, Cold	Short, Warm	Short, Cold	Q × Q × photo	Q × Q × temperature
Germination in light							
#Interactions	7	4	7	7	8	1	0
Conditional/Interactive	7/0	2/2	5/2	4/3	6/2		
Native/Recombinant	7/0	2/2	4/3	4/3	5/3		
Highest native genotype		TT	TT	TT	TT		
Germination in dark							
#Interactions	NA	1	2	5	3	0	0
Conditional/Interactive	NA	1/0	1/1	2/3	1/2		
Native/Recombinant	NA	0/1	1/1	2/3	2/1		
Highest native genotype		NA	TT	TT/CC	TT		

associated with 11% of the variance in germination proportion. In all cases, the Cal allele had the greater percentage of seed germination. Some epistatic interactions also significantly influenced germination proportion (Tables 2 and S3, Supporting Information). The majority of these interactions showed a change in the rank order of the phenotypes of the alleles, depending on the allele at the other locus. The majority of interactions also resulted in recombinant genotypes having higher germination proportions than native genotypes. In cases in which the native genotype did have a higher germination proportion, the Cal–Cal genotype had the highest.

Those main-effect QTLs that were significantly associated with fitness also showed large changes in the allele frequency of their associated markers within one generation (Fig. 3; Table 3). The Cal alleles started below 50% in all cases. In June-dispersed seeds in RI, the Cal alleles of the markers on chromosome 3 (Msat 3.1 and Msat 3.10) increased in frequency after some lines failed to germinate (first episode of selection). After germination of June-dispersed seeds, those Cal alleles further increased their frequency in both locations, based on the total lifetime fitness estimate that assumes that fruit production is proportional to seed production, exceeding 80% (and usually closely approaching 90%) in one generation. The Cal alleles associated with the QTLs on chromosome 5 (nga139 and SNP379) likewise attained frequencies from less than 50% to up to 88% in one

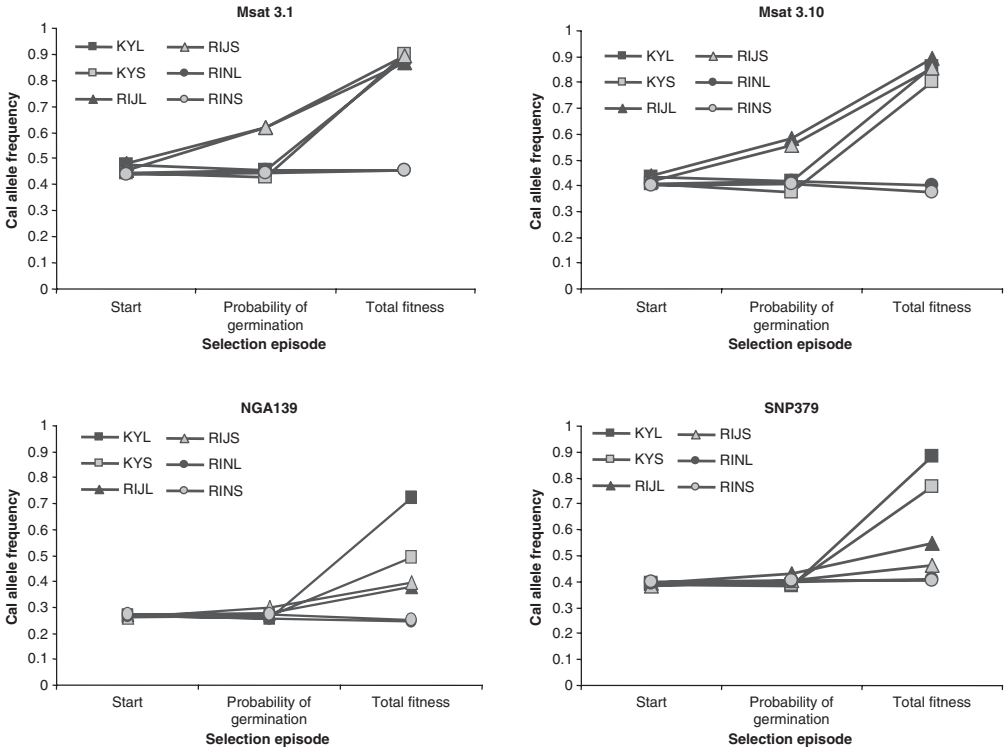

Fig. 3 Change in marker frequencies within one generation. 'Start' = the starting allele frequency, when seeds were first put in the field. 'Probability of germination' = the allele frequency of all successful germinants. 'Total fitness' = the estimated allele frequency in the progeny generation, based on the numbers of fruits produced. The differential ability to germinate under field conditions changed allele frequencies first. Differential survival to reproduction and differences in reproductive output changed allele frequencies further. Msat 3.1 and Msat 3.10 are located on chromosome 3. NGA139 and SNP379 are located on chromosome 5. See Fig. 1 for positions. See Table 3 for tests of significance of the changes in allele frequencies.

generation in June-dispersed seeds. For the markers on chromosome 5, differential failure of germination did not contribute to the increase allele frequency, but the Cal alleles rose when germinants carrying Tac alleles differentially failed to reproduce or produced fewer fruits.

QTLs for germination phenology colocate with fitness QTLs

In the southern location, KY, many seeds germinated within a few days after being dispersed in the field (Fig. S3, Supporting Information). Some germination continued throughout summer, and a second pulse of germination occurred in late September. When seeds were dispersed in June in the more northern location in RI, germination proceeded at low levels throughout the summer, and a peak of germination occurred in mid October. When seeds were dispersed in November in RI, the majority of seeds germinated within 2 weeks of being dispersed. Thus variance in germination timing was minimal in November-dispersed seeds, most likely because the cold temperatures broke any dormancy immediately after dispersal. See Donohue *et al.* (2005a) for more details on the germination timing and its variance.

The QTL analysis identified two QTLs for germination phenology in KY: one on chromosome 3 and one on chromosome 5 for both short-day and long-day seeds (Fig. 1; Table S2, Supporting Information). The main effects of these QTLs combined were associated with 27% of the variance in germination timing. When seeds were dispersed during June in RI, four QTLs were identified: one on chromosome 1, one on chromosome 3, one on chromosome 4 (short-day seeds only), and one on chromosome 5. The QTL on chromosome 3 was associated with 32% (short-day seeds) to 45% (long-day seeds) of the variance in germination timing, and the main effects of all QTLs combined explained 60.5% (short-day seeds) to 62.7% (long-day seeds) of the variance in germination phenology. For all but the QTL on chromosome 4, the Tac allele was associated with accelerated germination. The QTL on chromosome 1 was significantly stronger in RI than in KY (Table 1). The QTL on chromosome 3 detected in RI was significantly stronger in RI than KY, but the effect of the QTL

Table 3 Change in allele frequencies. F-ratios from analysis of variance to test for significant changes in allele frequencies of markers for two time intervals: 'Start to Germination' = from the start of the experiment until after seeds germinated; indicating the change in allele frequency due to differences in germination proportions. 'Germination to Total fitness' = after germination through seed set; total fitness is estimated as total fruit production, and the ending allele frequency is the estimated allele frequency of the seeds of the next generation, assuming fruit production is proportional to seed production. This indicates the change in allele frequency due to postgermination survival and fruit production. Blocks were used as the unit of analysis. For KY, $N = 3$ blocks. For RI, $N = 9$ blocks. See Fig. 3 for magnitudes of changes in allele frequencies

Treatment	Marker	Start to germination	Germination to total fitness
KYL	Msat3.1	0.51	162.68***
KYS	Msat3.1	0.17	80.05***
RIJL	Msat3.1	7.96**	18.12***
RIJS	Msat3.1	24.69***	76.47***
RINL	Msat3.1	0.07	5.19*
RINS	Msat3.1	0.09	0.35
KYL	Msat3.10	0.04	17.60**
KYS	Msat3.10	0.25	29.55**
RIJL	Msat3.10	51.73***	254.34***
RIJS	Msat3.10	15.21***	76.32***
RINL	Msat3.10	0.13	2.14
RINS	Msat3.10	0.05	1.83
KYL	NGA139	0.01	9.75*
KYS	NGA139	0.15	182.92***
RIJL	NGA139	0.01	1.49
RIJS	NGA139	0.88	8.66**
RINL	NGA139	0.15	0.55
RINS	NGA139	0.00	0.15
KYL	SNP379	0.00	28.56**
KYS	SNP379	0.02	41.00***
RIJL	SNP379	0.27	1.39
RIJS	SNP379	0.20	4.25^{+}
RINL	SNP379	0.07	1.46
RINS	SNP379	0.02	0.21

$^{+}P < 0.1$.
$^{*}P < 0.05$.
$^{**}P < 0.01$.
$^{***}P < 0.001$.

on chromosome 3 detected in KY did not differ significantly from its effect in RI, suggesting that these QTLs on chromosome 3 overlap (Tables 1 and S2, Supporting Information). The QTLs on chromosomes 1 and 3 in RI were also significantly stronger for June-dispersed seeds than November-dispersed seeds. Only one QTL for germination phenology was detected when seeds were dispersed in November. It was located on chromosome 4, and the Tac allele was associated with accelerated germination. In this treatment, germination was too synchronous to reveal many differences among genotypes. The photoperiod of seed maturation did not significantly influence detection of QTLs for germination in any treatment.

Significant epistasis was detected for germination timing (Fig. 2; Tables 2 and S3, Supporting Information). As with fitness QTLs, the majority of epistatic interactions were conditional, such that differences between alleles at one locus were detectable only in combination with one of the alleles at the other locus. Likewise, most epistatic interactions were such that the Cal–Cal genotype had the most delayed germination. Five interactions differed significantly, or marginally so, across dispersal seasons, with the interactions apparent primarily in June-dispersed seeds (Table S4, Supporting Information). Three interactions differed across sites, with one being stronger in KY and the others slightly stronger in RI (Table S2 and S4, Supporting Information). Thus epistatic variance in germination phenology is contingent both on geographic location and dispersal phenology.

Plasticity of germination to maternal photoperiod, geographic location, and season of seed dispersal was analysed. Plasticity always mapped to a QTL that was also a QTL for germination in at least one of the treatments used to calculate the plasticity (Table S5, Supporting Information). Therefore, we found no evidence for genes that regulate the plasticity of germination independently of the germination phenotype itself.

QTLs for germination timing colocated with QTLs for lifetime fitness of germinants (Fig. 1; Tables 4 and S2, Supporting Information). In nearly every case, the relative effect of QTLs for fitness and germination did not differ significantly. The exception is that the QTLs located on chromosome 5 for fitness of long-day, KY seeds had significantly stronger effects on fitness than on germination, as indicated by the multiple trait analysis, although the location of the second QTL was similar and the percent variation explained was comparable. While the location of the QTLs for germination phenology in KY differed slightly from the location of QTLs for fitness, the effect of the fitness QTLs did not differ significantly when compared to the effect size at that same position for germination, and vice versa. In nearly all cases, the Tac alleles were associated with decreased fitness and with faster germination. In addition, 34% of the epistatic interactions for fitness were also significant interactions for germination timing. Therefore, both main-effect and epistatic QTLs for fitness colocate with QTLs for germination timing under field conditions.

The QTL for germination phenology on chromosome 3 also colocated with the QTL for germination proportion, suggesting that seasonal requirements for germination that affect germination timing can also affect the probability of being able to germinate at all. Thus

Table 4 Upper: Tests that QTLs for germination in the laboratory and field have equivalent effects on field fitness. The likelihood ratio compares the model with the observed effect of the QTL for fitness to the model constrained have equivalent effects for fitness and field germination. It tests whether field or laboratory germination QTLs are equivalent to fitness QTLs. Lower: Tests that QTLs for fitness and germination in the laboratory have equivalent effects on field germination. The likelihood ratio compares the model with the observed effect of the QTL for field germination to the model constrained have equivalent effects for field germination and fitness or equivalent effects for field germination and laboratory germination ($DSDS_{50}$, germination in warm, germination in cold). It tests whether laboratory germination QTLs or fitness QTLs are equivalent to field QTLs for germination. Boldface indicates a significant departure from equality of QTL effects in the two traits. Significant LR threshold = 11.96. NB: Positions differ slightly between CIM and MQM. Positions presented here are based on CIM

Fitness QTL

QTL	Chr	Position (cM)	Field germination	$DSDS_{50}$	Total Warm	Total Cold
KYL	5	34.43	**12.51**	2.35	**14.51**	**14.51**
KYL	5	43.55	**13.51**	5.37	**16.25**	**16.23**
KYS	3	45.85	10.48	0.23	**16.15**	**17.38**
KYS	5	47.55	5.76	2.04	**14.31**	**12.98**
RIJL	3	55.87	4.04	**16.97**	**15.09**	**26.75**
RIJS	3	41.94	0.5625	8.64	**16.22**	**19.90**

Field germination QTL

QTL	Chr	Position	Fitness	$DSDS_{50}$	Warm	Cold
KYL	3	60.87	2.67	0.01	**14.99**	**14.11**
KYL	5	40.84	9.79	0.00	**20.43**	**20.50**
KYS	3	65.50	0.03	0.25	**13.44**	11.36
KYS	5	52.46	0.11	0.01	**17.04**	**16.00**
RIJL	1	73.31	0.14	3.08	**14.93**	**14.84**
RIJL	3	45.85	0.09	**20.96**	**50.89**	**50.73**
RIJL	5	47. 55	0.00	1.57	8.34	8.35
RIJS	1	70.29	0.02	1.93	10.03	10.72
RIJS	3	42.85	−0.11	**13.35**	**49.28**	**46.26**
RIJS	4	13.29	0.97	5.03	5.64	6.94
RIJS	5	59.57	1.22	4.28	7.28	6.20
RINS	4	0.00	0.02	0.00	4.57	5.07

germination QTLs colocate with QTLs for both fitness components measured in this study: germination success and fruit production after germination.

When germination timing was controlled for, and the residual variation in lifetime fitness was analysed, one QTL was identified on chromosome 3 (Fig. 1; Table S2, Supporting Information). It was detected in short-day seeds dispersed during June in KY (16% of the variance) and in short-day seeds dispersed during June in RI (11% of the variance). Its effect differed significantly across dispersal seasons (Table 1). This QTL also colocated with a QTL for germination phenology and the proportion of seeds that germinated in RI, indicating that this QTL has fitness effects not only through its effects on germination, but through other mechanisms as well.

No colocation of QTLs for adult life-history traits and QTLs for lifetime fitness in the field

In the field, a QTL analysis of adult life-history traits was compromised by the strong selection on germination when seeds were dispersed in June such that many genotypes did not survive to express adult life-history traits. We did find QTLs for adult life-history traits when plants were grown under controlled conditions, however (Fig. 1; Table S2, Supporting Information). Effect sizes of QTLs detected when plants were grown in long days (14 h) were somewhat weaker than those detected when plants were grown under short days (10 h), and QTLs for reproductive timing and leaf number at the time of bolting were significantly stronger in short-day plants. The proportion of variance accounted for ranged from 7% to almost 25% in short-day plants and 12–23% in long-day plants. QTLs for reproductive timing ('bolting time') in short days colocated with those for leaf number at the time of bolting in plants grown in short and long days. Importantly, none of these QTLs colocated with QTLs for field fitness in June-dispersed seeds, or with residual fitness, indicating that selection on these QTLs was weak in the field.

In seeds dispersed in RI during November, most genotypes did survive to express adult traits, so a QTL analysis was conducted on these field-expressed traits. Most genotypes also had some autumn and spring germinants, so we compared QTLs for adult traits separately in each cohort. Some modest sized QTLs were detected for reproductive timing and size at reproduction in both cohorts (Fig. 1; Table S2, Supporting Information). QTLs for bolting time were near each other or indistinguishable between the two cohorts, except the effect size of the QTL on chromosome 5 was significantly stronger for spring germinants. Interestingly, an additional QTL for the interval between germination and bolting was detected in the autumn cohort and had a significantly larger effect in that cohort when based on a joint analysis of the trait in both cohorts. In the autumn cohort, QTLs for leaf number and rosette diameter colocated, and the effect sizes of those QTLs did not differ strongly among cohorts, except the effect size of the QTL in the middle of chromosome 4 was somewhat stronger in autumn germinants. A second QTL for leaf number was detected in both cohorts, and an additional QTL was detected in the autumn cohort, but its effect size did not differ significantly across cohorts.

Several of these QTLs colocated with QTLs for the traits detected under laboratory conditions. In short, QTLs were detected for adult traits, and germination timing significantly altered effect sizes of some of these QTLs. None of these QTLs colocated with the one QTL for field lifetime fitness or with QTLs for residual fitness in the November-dispersed seeds, suggesting that they are not under strong selection in the field.

QTLs for germination in the field and laboratory

Variation in germination phenology in the field, and even the total proportion of seeds that were able to germinate during the course of the experiment, could be the result of primary dormancy induction, secondary dormancy induction, or breakage of secondary dormancy. A large QTL for primary dormancy ($DSDS_{50}$), tested in seeds matured under glasshouse conditions in Cologne, was detected on chromosome 3 and was associated with 31% of the variance in that trait (Fig. 1; Table S2, Supporting Information). It colocated with a QTL for field germination phenology and with the QTL for field fitness in seeds that were dispersed in June in both KY and RI, although its effect on primary dormancy was significantly larger than its effect on field germination and fitness in RI (Table 3), as indicated by the multitrait analysis. A smaller QTL for primary dormancy, on chromosome 5 (6.6% of the variance), also colocated with field germination phenology in seeds dispersed in June in both locations, and it colocated with a QTL for fitness in KY (Table 3). A nearly significant, suggestive QTL on chromosome 4 was associated with 6.3% of the variance in primary dormancy and colocated with a QTL for field germination of seeds dispersed during June in RI. Variation in primary dormancy therefore apparently translates to variation in field germination phenology and fitness.

Regarding secondary dormancy induction by warm temperature, a QTL on chromosome 3 colocated with the major QTL for primary dormancy and also with germination timing of June-dispersed seeds (Fig. 1, Tables 4 and S2, Supporting Information), but its effect on secondary dormancy was weaker than its effect on field germination phenology. A second QTL, also on chromosome 3, and a third QTL, on chromosome 1, did not colocate with field germination or with primary dormancy. The effect sizes of all fitness QTLs differed significantly from effect sizes for QTLs for secondary dormancy induction, and many of the effect sizes of QTLs for field germination phenology also differed significantly from those for secondary dormancy induction, as indicated by the multitrait analysis. Thus the unique QTLs for secondary dormancy were not as closely associated with field phenology or fitness as were those for primary dormancy.

Regarding secondary dormancy breakage by cold treatment, a QTL located on chromosome 3 colocated with a QTL for primary dormancy and germination timing in the field, although its effect was larger for field germination in RI (Table 4). An additional QTL located on chromosome 2 was unique to the cold-treated seeds and accounted for 12.8% of the variance in germination of short-day seeds after a cold treatment. This unique QTL did not colocate with QTLs for either field phenology or fitness QTLs.

QTLs for germination in the dark resembled those for total germination, unsurprisingly, except that two QTLs on chromosome 1 were detected that were not detected for total germination. Interestingly, the QTL near the centre of chromosome 1 was detected for short-day seeds, and it colocated with field germination phenology of seeds dispersed during June in RI, suggesting that there could have been some slight burial of seeds in RI and that light sensitivity influenced their field germination behaviour. The QTL on the top of chromosome 1, however, was unique to dark germination and did not colocate with QTLs for field phenology or fitness.

Generally, effects of QTLs for field germination and lifetime-fitness resemble those for primary dormancy more than those for secondary dormancy induction and breakage. QTLs for primary dormancy colocate with QTLs for field germination and with field fitness when seeds are dispersed in June. Thus genes associated with primary dormancy are likely the targets of intense natural selection early in the colonization process because of their effects on germination timing.

Discussion

Single QTLs can have surprisingly large effects on fitness when naïve genotypes are exposed to natural selection and when all life stages, including the earliest, are considered. Under one of our experimental conditions, a single QTL explained 13.6% of the variance in one fitness component (ability to germinate) and 28.9% of the variance in another fitness component (lifetime fitness of germinants). Allele frequencies of fitness QTLs changed substantially during the course of a single generation in some treatments, and in some cases closely approached 90%. Apparently, chromosomal regions with large additive effects on fitness segregate among natural populations, enabling only a subset of genotypes to successfully colonize and establish in such old-field sites as studied here. It is somewhat surprising to find such large QTLs for fitness, since fitness is thought to be an ecologically and genetically complex trait.

Previous studies that examined QTLs for fitness in *A. thaliana* under natural conditions found somewhat smaller QTL effects (Weinig *et al.* 2003; Malmberg *et al.* 2005). This study differed from those by including a very early life stage: the seed.

Major QTLs for fitness colocated with, and are likely to be, QTLs for germination phenology, which is regulated in large part by primary dormancy induction. The ability to arrest development at a very early stage and assess seasonal conditions appears to be under extremely strong natural selection at the earliest stages of colonization. Germination phenology is likely to be one of the earliest stages of adaptation to new environments for two obvious reasons. First, germination is a form of habitat selection; the timing of germination determines the seasonal environment experienced by all subsequent life stages (Donohue 2005). By germinating only under a predictable subset of environmental conditions, plants do not need to adapt de novo in other traits expressed at later stages, provided those traits are adapted to the subset of environments determined by their germination phenology. As such, habitat selection in general, and germination cuing in particular, may facilitate colonization and range expansion by exposing plants to environmental conditions to which they are already adapted. Second, germination is expressed at one of the earliest life stages; an organism has to survive past the early stages before it can express any adaptive phenotype at later life stages. Therefore, all else being equal, early life stages may be among the first to exhibit adaptation to novel conditions, although this has seldom been tested observationally or experimentally.

The sequence of adaptation of traits can have important consequences for the evolution of entire life histories. Germination timing has been shown to influence the expression of later life history traits as well as selection on later traits (Weinig 2000; Korves *et al.* 2007; Wilczek *et al.* 2009). Thus epistasis for fitness can occur through the interaction of germination and subsequent traits under selection. Germination phenology is therefore likely to be of especial importance in the process of adaptation, not only because it itself is under strong selection but because it influences natural selection on traits expressed at later life stages as well.

Likewise at the genetic level, alleles that are fixed earlier can shape how alleles at other loci are selected subsequently (Weinreich *et al.* 2005). In this study, allele frequencies of markers for QTLs associated with germination phenology and fitness changed dramatically within a single generation. Despite some apparent segregation distortion, which caused low starting frequencies of Cal alleles, the Cal alleles of these markers approached 90% in many cases after a single growing season. Selection on germination timing likely contributed strongly to these allele frequency changes and could even perhaps contribute to eventual fixation at these loci. Such fixation could alter selection on other loci that interact with them epistatically. These markers did interact epistatically with each other and with other loci to influence fitness. Thus selection on germination timing has the potential to influence the evolutionary trajectories of these loci and of loci interacting epistatically with them throughout the genome.

The two largest and most consistent QTLs for germination phenology and primary dormancy are within the same region as QTLs for dormancy identified in other studies of primary dormancy (Bentsink & Koornneef 2008) that used RILs derived from a cross between the laboratory strain Landsberg and the Cape Verdi Island ecotype (Alonso-Blanco *et al.* 2003) and between Bay-0 and Shahdara (Meng *et al.* 2008). The QTL on chromosome 5 most likely represents *DOG1*, which has since been cloned and identified as a gene of unknown molecular function that is expressed most highly in seeds at the late stages of seed maturation (Bentsink *et al.* 2006). Preliminary studies also indicate that the Cal and Tac parents used in this study have a number of DNA sequence differences in both coding and regulatory regions of *DOG1* (J. de Meaux, I. Kronholm and M. Debieu, personal communication). The larger QTL on chromosome 3 is in the same region as *DOG6*, which appeared as the major dormancy QTL in the Ler × Sha RIL population (Clerkx *et al.* 2004) and in other crosses involving Ler and dormant accessions. This locus has not yet been cloned.

Despite strong selection favouring primary dormancy, natural variation exists in dormancy and dormancy QTLs (and in fitness QTLs) as shown by this and other studies (Alonso-Blanco *et al.* 2003; Clerkx *et al.* 2004; Schmuths *et al.* 2006). The observation that similar QTLs were detected in laboratory experiments as in field conditions indicates that primary dormancy loss as measured in laboratory conditions reflects well the dormancy differences expressed in the field. This is the case despite the fact that the environmental conditions during germination are very different in the laboratory and field. That the germination phenotype depends on the conditions during imbibition as well, however, is also shown in this report and by the fact that genotype × germination-environment QTLs have been detected in other studies of *A. thaliana* (Jansen *et al.* 1995; van der Schaars *et al.* 1997; Laserna *et al.* 2008; Meng *et al.* 2008).

In this study, the major QTLs for fitness and germination were detected in both field sites, and the main effects of those QTLs were in the same direction in both sites. This indicates that the same alleles of these major QTLs were favoured in both sites. In both sites, some

seed dormancy was essential for avoiding summer conditions, which were typically fatal in KY and stressful in RI (Donohue *et al.* 2005b). While dormancy was strongly favoured in both sites when seeds were dispersed in June, stabilizing selection resulted in a slightly earlier (2 weeks) optimum germination timing in the autumn in RI as compared to KY.

Given this pattern of selection on germination timing, what maintains genetic variation for dormancy and fitness QTLs? First, not all QTLs were detected in all environmental conditions, so such variation may be neutral or nearly so under some conditions. In particular, selection against loss-of-dormancy alleles would not occur under conditions, such as dispersal in cold temperatures, in which primary dormancy is broken almost immediately after dispersal, as occurred in the November-dispersed cohort.

Second, balancing selection may contribute to the maintenance of variation dormancy alleles. Under conditions of seed dispersal in November, natural selection actually favoured accelerated germination (Donohue *et al.* 2005b), although this effect was much weaker than the stabilizing selection on germination timing detected in June-dispersed seeds. Nevertheless, this suggests that selection against primary dormancy may exist in rapid-cycling populations with autumn-flowering cohorts.

Third, stabilizing selection may contribute to the maintenance of variation in dormancy alleles. Interestingly, the additive effects of QTLs for germination timing indicated that alleles that delay germination are present in the Cal ecotype, but that one Cal locus (on chromosome 4) carries an allele that accelerates germination. Given the strong stabilizing selection on germination observed in both geographic locations when seeds were dispersed during the most typical dispersal season, this result is consistent with the hypothesis that an optimal intermediate phenotype may be caused in this parental line by a balance of alleles with opposing effects. This same general phenomenon may explain why QTLs associated with dormancy loss are maintained in natural populations, even when some degree of dormancy is advantageous.

Finally, epistasis may contribute to dormancy and fitness variation. In this study, the observed epistasis was primarily conditional epistasis, such that allelic effects were only apparent in combination with particular alleles at other loci. In this manner, epistasis acts similarly to environment-dependent expression of QTL effects; only in some genetic backgrounds are QTLs exposed to natural selection while such alleles are masked from selection in other backgrounds, remaining neutral or nearly so. In a smaller number of cases, the direction of the effect of the QTLs depended on alleles at other loci, which would lead to background-dependent selection on the QTLs.

Epistasis significantly influenced both fitness and germination, as was found in another study in *A. thaliana* (Malmberg *et al.* 2005). While the majority of epistatic interactions were such that native allelic combinations had higher fitness, an appreciable proportion of interactions showed that novel combinations of alleles had higher fitness than native combinations. This suggests an interesting potential role of recombination for adaptation to new environments (Rieseberg *et al.* 2003a,b). Moreover some native and recombinant combinations gave fitness advantages across both sites and life-histories. Recombination and epistasis therefore have the potential to create high-fitness colonizing genotypes that retain a fitness advantage over a wide range of ecological conditions. Such epistasis may even contribute to the ability of some introduced genotypes, and thereby species, to become widely distributed (Ellstrand & Schierenbeck 2000).

To understand the earliest stages of adaptation to new locations or environments, it is important to consider the earliest life-history stages. When the earliest life stages were exposed to natural selection, single QTLs had surprisingly large effects on fitness. Seed dormancy appears to be a critical attribute early in the process of adaptation—simply arresting development and assessing environmental conditions was under intense natural selection in this experimental population, and it likely contributed to impressive changes in allele frequencies within a single generation. The importance of dormancy goes beyond direct selection on germination itself, however, since the seasonal timing of germination early in life determines the seasonal environment, and the environment of natural selection, of all subsequent life stages. As such, early phenological traits are likely to be particularly important for the early stages of adaptation to new locations.

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References

Alonso-Blanco C, Bentsink L, Hanhart CJ, Blankestijn-de Vries H, Koornneef M (2003) Analysis of natural allelic variation at

seed dormancy loci of *Arabidopsis thaliana*. *Genetics*, **164**, 711–729.

Baskin CC, Baskin JM (1998) *Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination*. Academic Press, San Diego.

Baskin JM, Baskin CC (1983) Seasonal changes in the germination responses of buried seeds of *Arabidopsis thaliana* and ecological interpretation. *Botanical Gazette*, **144**, 540–543.

Basten CJ, Weir BS, Zeng Z-B (2004) *QTL Cartographer, Version 1.17*. Department of Statistics, North Carolina State University, Raleigh, NC.

Bazzaz FA (1991) Habitat selection in plants. *The American Naturalist*, **137**, S116–S130.

Bentsink L, Koornneef M (2008) Seed dormancy and germination. *The Arabidopsis Book*. American Society of Plant Biologists: 1–18.

Bentsink L, Jowett J, Hanhart CJ, Koornneef M (2006) Cloning of *DOG1*, a quantitative trait locus controlling seed dormancy in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA*, **105**, 17042–17047.

Chase K, Adler FR, Lark KG (1997) Epistat: a computer program for identifying and testing interactions between pairs of quantitative trait loci. *Theoretical and Applied Genetics*, **94**, 724–730.

Clerkx EJM, Ei-Lithy ME, Vierling E, Ruys GJBlankestijn-De Vries H, Groot SPC, Vreugdenhili O, Koornneef M (2004) Analysis of natural allelic variation of *Arabidopsis* seed germination and seed longevity traits between the accessions Landsberg *erecta* and Shakdara, using a new recombinant inbred line population. *Plant Physiology*, **135**, 432–443.

Doerge RW, Churchill GA (1996) Permutation tests for multiple loci affecting a quantitative character. *Genetics*, **142**, 285–294.

Donohue K (2005) Niche construction through phenological plasticity: life history dynamics and ecological consequences. *New Phytologist*, **166**, 83–92.

Donohue K (2009) Completing the cycle: maternal effects as the missing link in plant life cycles. *Philosophical Transactions of the Royal Society of London B, Biological Sciences*, **364**, 1059–1074.

Donohue K, Dorn LA, Griffith C, *et al.* (2005a) Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. *Evolution*, **59**, 740–757.

Donohue K, Dorn LA, Griffith C, *et al.* (2005b) The evolutionary ecology of seed germination of *Arabidopsis thaliana*: variable natural selection on germination timing. *Evolution*, **59**, 758–770.

El-Lithy ME, Bentsink L, Hanhart CJ, *et al.* (2006) New Arabidopsis recombinant inbred line populations genotyped using SNPWave and their use for mapping flowering-time quantitative trait loci. *Genetics*, **172**, 1867–1876.

Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences, USA*, **97**, 7043–7050.

Gutterman Y (1992) Maternal effects on seeds during development. In:*Seeds: The Ecology of Regeneration in Plant Communitie* (ed Fenner M), C. A. B. International, Wallingford, UK.

Gutterman Y (1994) Effect of day length during plant development and caryopsis maturation on flowering and germination, in addition to temperature during dry storage and light during wetting, of *Schismus arabicus* (Poaceae) in the Negev desert, Israel. *Journal of Arid Environments*, **33**, 439–448.

Hayes RG, Klein WH (1974) Spectral quality influence of light during development of *Arabidopsis thaliana* plants in regulating seed germination. *Plant & Cell Physiology*, **15**, 643–653.

Jansen RC, Van Ooijen JW, Stam P, Lister C, Dean C (1995) Genotype-by-environment interaction in genetic mapping of multiple quantitative trait loci. *Theoretical and Applied Genetics*, **91**, 33–37.

Jiang C, Zeng Z-B (1995) Multiple trait analysis of genetic mapping for quantitative trait loci. *Genetics*, **140**, 1111–1127.

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. *The American Naturalist*, **169**, E141–E157.

Laserna MP, Sanchez RA, Botto JF (2008) Light-related loci controlling seed germination in Ler × Cvi and Bay-0 × Sha recombinant inbred-line populations of *Arabidopsis thaliana*. *Annals of Botany*, **102**, 631–642.

Malmberg RL, Held S, Waits A, Mauricio R (2005) Epistasis for fitness-related quantitative traits in *Arabidopsis thaliana* grown in the field and in the greenhouse. *Genetics*, **171**, 2013–2027.

Meng P-H, Macqueta A, Loudet O, Marion-Poll A, Northa HM (2008) Analysis of natural allelic variation controlling *Arabidopsis thaliana* seed germinability in response to cold and dark: identification of three major Quantitative Trait Loci. *Molecular Plant*, **1**, 145–154.

Munir J, Dorn L, Donohue K, Schmitt J (2001) The influence of maternal photoperiod on germination requirements in *Arabidopsis thaliana*. *American Journal of Botany*, **88**, 1240–1249.

O'Neill CM, Morgan C, Kirby J, *et al.* (2008) Six new recombinant inbred populations for the study of quantitative traits in Arabidopsis thaliana. *Theoretical and Applied Genetics*, **116**, 1183–1185.

Rieseberg LH, Raymond O, Rosenthal DM, *et al.* (2003a) Major ecological transitions in wild sunflowers facilitated by hybridization. *Science*, **301**, 1211–1216.

Rieseberg LH, Widmer A, Arntz AM, Burke JM (2003b) The genetic architecture necessary for transgressive segregation is common in both natural and domesticated populations. *Philosophical Transactions of the Royal Society of London B*, **358**, 1141–1147.

Schmuths H, Bachmann K, Weber WE, Horres R, Hoffmann MH (2006) Effects of preconditioning and temperature during germination of 73 natural accessions of *Arabidopsis thaliana*. *Annals of Botany*, **97**, 623–634.

Templeton AR, Rothman ED (1982) Evolution in fine-grained environments. 2. Habitat selection as a homeostatic mechanism. *Theoretical Population Biology*, **19**, 326–340.

Thompson L (1994) The spatiotemporal effects of nitrogen and litter on the population dynamics of *Arabidopsis thaliana*. *Journal of Ecology*, **82**, 63–68.

Van Der Schaars W, Alonso-Blanco C, Leon-Kloosterziel KM, *et al.* (1997) QTL analysis of seed dormancy in Arabidopsis using recombinant inbred lines and MQM mapping. *Heredity*, **79**, 190–200.

Van Ooijen JW, Maliepaard C (1996) *MapQTL Version 3.0: Software for the Calculation of QTL Positions on Genetic Maps. Plant Genome IV Abstracts*. World Wide Web site: http://www.intl-pag.org.

Walther G-R (2004) Plants in a warmer world. *Perspectives in Plant Ecology Evolution and Systematics*, **6**, 169–185.

Wang S, Basten CJ, Zeng Z-B (2005) *Windows QTL Cartographer 2.5 User Manual*. N.C. State University, Bioinformatics Research Center.

Weinig C, Stinchcombe JR, Schmidtt J (2003) QTL architecture of resistance and tolerance traits in *Arabidopsis thaliana* in natural environments. *Molecular Ecology*, **12**, 1153–1163.

Weinig C (2000) Differing selection in alternative competitive environments: shade-avoidance responses and germination timing. *Evolution*, **54**, 124–136.

Weinreich DM, Watson RA, Chao L (2005) Perspective: Sign epistasis and genetic constrain on evolutionary trajectories. *Evolution*, **59**, 1165–1174.

Wilczek AM, Roe JL, Knapp MC, *et al.* (2009) Effects of genetic perturbation on seasonal life history plasticity. *Science*, **323**, 930–934.

Willis CGR, Ruhfel B, Primack RB, Miller-Rushing AJ, Davis CC (2008) Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 17029–17033.

The authors' research programs address the evolutionary ecology and genetics of adaptation, and the quantitative-genetic and molecular-genetic basis of adaptively significant life-history traits in plants. For authors at the Max Planck Institute for Plant Breeding, the research focus is on QTL mapping and the identification and molecular characterization of genes associated with natural variation in plant traits, including dormancy and flowering time. For authors at Duke University, the research focus is on the evolutionary ecology of adaptation, phenotypic plasticity, and niche construction, with a present focus on germination timing under natural conditions. The authors at Brown University conduct research on the ecological genetics of adaptation and plasticity, with a present focus on flowering time adaptation in *Arabidopsis thaliana*. Lisa Dorn studies natural genetic variation in germination and flowering in *A. thaliana*, and Converse Griffith studies plant communities, land management, and conservation.

Supporting Information

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

Table S1 Primer conditions for microsatellite markers used in the construction of the Cal × Tac map.

Table S2 QTLs for life-history and fitness traits in the field and lab.

Table S3 Epistatic interactions. Genotypic means and standard errors of the phenotypes of genotypes of interacting markers.

Table S4 Tests for QTL × QTL × environment interactions. 'Interaction' refers to the marker pair that had a significant interaction.

Table S5 QTLs for plasticity of germination in the field and lab.

Fig. S1 Experimental design. Seeds were matured in growth chambers.

Fig. S2 Frequency distributions for all traits analyzed.

Fig. S3 Germination schedule. *X*-axis shows the date (in Julian days).

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