THE EARLIEST STAGES OF ADAPTATION IN AN EXPERIMENTAL PLANT POPULATION: STRONG SELECTION ON QTLS FOR SEED DORMANCY

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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 QTL for fitness changed allele frequency and closely approached 90% in some treatments within a single generation. These QTL co-located with QTL 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. QTL for field germination phenology co-located with known QTL 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.

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 favorable alleles are fixed first. It has been shown theoretically that the sequence of allelic substitutions can constrain future evolutionary pathways, especially when loci interact epistatically to influence fitness (Weinreich et al 2006). 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 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, Donohue 2005). 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. As such, phenology may play an important role in early adaptation to new locations or changed climate. 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 (Calver, England and Tacoma, Washington USA). 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 studies of local population genetics of *A. thaliana* have found evidence for 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 experimental recombinant inbred lines (J. Borevitz, K. Bomblies, pers. com.). Using environmental and geographic manipulations, we conducted a 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. 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 fifteen recombinant lines were maintained for nine generations by single seed descent. See Donohue et al. (2005a) for more details on the lines and their maintenance.

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, Germany) 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). PCR reactions were run in 10 µl total volume with 50 ng template DNA, 1µl10×PCR buffer (QIAGEN, Germany) 0.25 µM of the

forward and reverse primers, and an optimal concentration of $MgCl_2$ (1.5 , 2.25, 3 or 4 mM depending on the primer), 200 µM each dNTP, and 0.5 units of *Taq* polymerase(QIAGEN, Germany). All PCRs were run in 96-well format on MJ Research (Watertown, USA) DNA Engine TETRAD 2 Peltier thermal cycler. The PCR cycling conditions were as follows: 94°C for 5 min, 40 cycles with 94°C for 30 s, Tm for 30 s, and 72°C for 30 s, followed by 72°C for 10 min. The concentration of $MgCl_2$ and annealing temperature (Tm) was individually optimized for each primer pair, and this information is available upon request. PCR products were resolved on a 2–4% agarose gel, and bands were visualized by staining with ethidium bromide. The majority of these primers produced easily interpretable, single-locus, codominant banding patterns. The remainder produced more complex banding patterns, often with multiple unlinked dominant loci. Primers that produced easily scored polymorphisms (length variation and codominant inheritance) were selected as genetic markers.

A set of 149 Framework SNPs among 384 Arabidopsis accessions developed by the Borevitz lab (http://borevitzlab.uchicago.edu/resources/molecular-resources/snp-markers) was used to genotype all individual RILs. 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 100 markers covering most of the *A. thaliana* genetic map at intervals of 1 to 11 cM was constructed with the JoinMap program (version 4.0, 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 2007) and with segregation distortion at a limited number of positions. 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 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 and 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 seeds have been observed to mature seed in natural populations of *A. thaliana* in North America: "Short days" comprised 10h light/14h dark at 22ºC, and "Long days" comprised 14h light/10h dark at 22ºC. Seeds were grown in two batches so that fresh seeds could be dispersed in June and in November. Two field sites were used, one in Lexington, Kentucky (KY), USA and a more northerly site in Bristol, Rhode Island (RI), USA. 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 350 in order to control for site-specific soil effects and enable a more controlled contrast of climatic differences. 12 seeds of a given genotype were placed in a given pot, and each genotype had 10 replicate pots for each treatment (2 photoperiod treatments, and three location x dispersal treatments). The pot was the unit of analysis.

The germination timing of each seed was recorded through weekly monitoring, and pot means were used for germination estimates. A single random focal individual was followed in each pot, and all other germinants were plucked from the pot. 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). Adult traits included the day of bolting (start 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. See Donohue et al. (2005a) for more details on the field design.

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. 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 one week. Curves of germination percentage converted into probit units as a function of the time of storage provided the kinetics of seed dormancy of a genotype. Measurements of germination proportions at all timepoints were used for probit regression on a logarithm time scale (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 and 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 afterripenend seeds of all genotypes (100% germination of all genotypes assessed eight months after harvest) of the same batch of seeds that were matured under short and long days for the field experiment. 15 seeds of a given genotype were placed on 0.5% agar pertri plates, with 12 plates per genotype. Plates were the unit of analysis. These plates were then imbibed in the dark at 30ºC for 7 days, and then placed in the light at 22ºC. 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 30ºC) then a cold (5 days at 4ºC),

dark imbibition period before being placed at the germination-permissive temperature of 22ºC in the light.

QTL analysis:

Genotypic means for each treatment were used in all QTL analyses. QTL analysis was conducted using MapQTL (version 5.0, www.kyazma.nl) to identify and locate QTL 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 (1,000 repetitions) and found a $P < 0.05$ LOD varying between 2.4 and 2.6 (Doerge and 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 with MapQTL in the final MQM model. For this, different cofactor markers were tested around a putative QTL position (Van Ooijen and Maliepaard 1996) selecting as the final cofactors the closest marker to each QTL, i.e. those maximizing the LOD score. Some traits were not normally distributed and could not be transformed to normality. In such cases, non-parametric Kruskal-Wallis tests were used to verify significance levels. It should be noted however, that such tests are conservative, do not control for obscuring co-factors, and are analogous to single-marker analysis. We conducted multiple-trait composite interval mapping (CIM) analysis to test for QTL-environment interaction and trait co-location (Jiang and Zeng 1995) using Windows QTL Cartographer (Version 2.5; Basten et al. 1994, Wang et al. 2005). We used hypothesis four ("Test GxE"). We also verified QTL 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 QTL 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 x QTL x environment interactions were further verified with ANOVA in the SAS statistical package (SAS 9.1.3).

RESULTS

QTLs for fitness in the field:

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 S1). 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, 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 x 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 x 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 somewhat with the QTL detected in RI, suggesting 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, although it affected the epistasis that could be detected (see below). 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, S2). Some main-effect QTLs exhibited significant epistasis based on MIM. 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, Table S2). 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 combinations had the highest fitness. Therefore, the Cal parental population harbors adaptive combinations of alleles, but recombination nevertheless can create novel combinations of alleles that are more highly adapted to these habitats of new introduction.

Several pairs of interacting alleles had consistent effects on lifetime fitness across all environmental treatments (Table S3). 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. Hybridization between different introduced lines may therefore contribute to adaptation of introduced species across a wide geographic and ecological range. 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.

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 S1). While the location of the QTL was slightly different for seeds matured under long and short days, it was not significantly different (Table 1). 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). 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 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, S3). 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). The Cal alleles started below 50% in all cases. The Cal alleles of the markers on chromosome 3 increased in frequency in June-dispersed seeds in RI after some lines failed to germinate (first episode of selection). After germination, the Cal alleles further increased in June-dispersed seeds in both locations, based on the total lifetime fitness estimate that assumes that fruit production is proportional to seed production. In June-dispersed seeds, the Cal alleles associated with the QTLs on chromosome 5 attained frequencies from 50% to 88% in one generation. The Cal alleles of the QTLs on chromosome 3 exceeded 80% (and usually closely approached 90%) in one generation in both locations in June-dispersed seeds.

QTLs for germination phenology co-locate with fitness QTLs:

The life-history trait that was expressed at the earliest stage that we could record in the field was the timing of germination. In the southern location in KY, many seeds germinated within a few days after being dispersed in the field. Some germination continued throughout summer, and a second pulse of germination occurred in late September. Very low levels of germination continued throughout the winter and into spring. 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. Low levels of germination continued throughout the winter and into spring. When seeds were dispersed in November in

RI, the majority of seeds germinated within two 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 schedules.

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 S1). 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 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. 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 QTL on chromosome 1 detected in RI was significantly stronger in RI than in the southern site, KY (Table 2). The QTL on chromosome 3 was significantly stronger for seeds dispersed in June in RI than for the other treatments, but the effect of the QTL on chromosome 3 detected in KY did not differ significantly from its effect in RI, suggesting that these QTLs may overlap, even though the effect size was greater in RI. 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, S3). As with fitness QTLs, the majority of epistatic interactions were conditional, with differences between alleles at one locus being 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 interaction apparent primarily in June-dispersed seeds (Table S4). Three interactions differed across sites, with one being stronger in KY and the others slightly stronger in RI. Thus epistatic variance in germination phenology is contingent both on geographic location and dispersal phenology.

When plasticity of germination to maternal photoperiod, geographic location, or season of seed dispersal was analyzed, in no case did plasticity map to a QTL that was not also a QTL for germination in at least one of the treatments (Table S4). Therefore, we found no evidence for genes that regulate the plasticity of germination independently of the germination phenotype itself.

QTLs for germination timing co-located with QTLs for lifetime fitness of germinants (Fig. 1, Tables S1, 2). 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. This is consistent with natural selection for delayed germination when seeds are dispersed in June, just before summer drought conditions (Donohue et al 2005b). In addition, 34% of the epistatic interactions for fitness were also significant interactions for germination timing. Therefore, both additive and epistatic QTLs for fitness co-locate with QTLs for germination timing under field conditions.

The QTL for germination phenology on chromosome 3 also co-located 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 germination QTLs co-locate 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 analyzed, one QTL was identified on chromosome 3. 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. This QTL also co-located 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.

In short, fitness and germination phenology are genetically associated at the level of specific chromosomal regions. Genes associated with natural variation in germination phenology are therefore likely targets of intense natural selection early in the colonization process. However, such genes do not contribute much to fitness variation under conditions in which seeds are induced to germinate quickly and synchronously, as was the case in the November-dispersed cohort. Moreover, once adaptation in germination has occurred, other genes are associated with

residual variation in fitness. However, germination QTLs may even be associated with fitness through mechanisms other than germination under some conditions, through pleiotropic effects.

No co-location 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; because of inappropriate germination timing, many genotypes did not survive to express adult life-history traits. We did find QTLs for adult life-history traits when plants were grown in under controlled conditions, however (Fig. 1, Table S1). Effect sizes of QTLs detected when plants were grown in long days (14h) were somewhat weaker than those detected when plants were grown under short days (10h), and QTLs for reproductive timing and leaf number at the time of bolting were significantly stronger in short-day plants. The proportion variance accounted for ranged from 7% to almost 25% in short-day plants and 12% to 23% in long-day plants. QTLs for reproductive timing ("bolting time") in short days co-located with those for leaf number at the time of bolting in plants grown in short and long days. Importantly, none of these QTLs co-located 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. Moreover, 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 S1). 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 co-located, 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 co-located with QTLs for the traits detected under laboratory conditions. However, none of these QTLs co-located 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 lab:

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, 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 S1). It co-located 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 multi-trait analysis. A smaller QTL for primary dormancy, on

chromosome 5 (6.6% of the variance), also co-located with field germination phenology in seeds dispersed in June in both locations, and it co-located with a QTL for fitness in KY (Table 3). A nearly significant, suggestive QTL on chromosome 4, associated with 6.3% of the variance in primary dormancy, co-located 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 co-located with the major QTL for primary dormancy and also with germination timing of June-dispersed seeds (Fig. 1, Tables 3, S1), but its effect on germination after a warm treatment 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 co-locate 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 multi-trait 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 co-located with a QTL for primary dormancy and germination timing in the field, although its effect was larger for field germination in RI. 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 co-locate 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 center of chromosome 1 was detected for short-day seeds, and it co-located 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 behavior. The QTL on the top of chromosome 1, however, was unique to dark germination and did not co-locate with QTLs for field phenology or fitness.

Generally speaking, effects of field germination QTLs and lifetime-fitness QTLs differed more from QTLs for secondary dormancy induction and breakage than they differed from QTLs for primary dormancy. Variation in field germination phenology is therefore explained to a large degree by variation in primary dormancy, and QTLs for primary dormancy co-locate with QTLs for field germination and with field fitness when seeds are dispersed in June. Genes associated with primary dormancy are therefore likely the targets of intense natural selection early in the colonization process.

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 (Malmberg et al 2005, Weinig et al. 2003). This study differed from those by including a very early life stage: the seed.

Major QTLs for fitness co-located 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. 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 are

expected to 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. 2006). 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 and Koornneef 2008) that used RILs derived from a cross between

the lab 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). It was shown in the Ler x Cvi RIL population that differences in expression of this gene between accessions are related to differences in their dormancy levels. The major difference in the *DOG1* alleles in that study was an insertion in the promoter region of the Landsberg allele, causing it to have greatly reduced expression of *DOG1*. The larger QTL on chromosome 3 is in the same region as *DOG6*, which appeared as the major dormancy QTL in the Ler x 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 favoring 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 after-ripening as measured in lab 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 lab 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 x germination environment QTLs have been detected in other studies of *A. thaliana* (Jansen et al 1995, van der Schaars et al. 1997, Meng et al. 2008, Laserna et al. 2008).

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 favored 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 (Reiseberg et al. 2003a and 2003b). 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 and 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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FIGURE LEGENDS

Figure 1. The Cal X Tac linkage map showing the locations of QTLs for the traits analyzed. 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.

Figure 2: Epistatic interactions associated with variance in field phenotypes and germination in the lab. 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 Supplementary Table 1. Dotted line = $P < 0.1$; Thin line = $P < 0.05$: Medium line = $P < 0.01$; Thick line = $P < 0.001$. The traits 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.

Figure 3: Change in marker frequencies within one generation. 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.

Table 1. QTL-environment interactions. Likelihood ratios for tests that QTL effects differ across environments. The likelihood ratio 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 lab. WL = Warm, long-day; WS = Warm, short-day. "Diff from DSDS50?" gives the LR that tests whether the effect size of the QTLs differ significantly from those for DSDS50.

QTL	Chr	Position	QTL x Site	QTL x Season
Fitness				
RIJL	3	0.5587	7.6158	**30.5029**
RIJS	3	0.5586	6.5420	**15.3646**
Proportion of seeds germinated				
RIJL	3	0.5447	**21.5390**	3.0498
RIJS	3	0.4385	**18.5027**	6.5337
Germination day				
RIJL	1	0.7331	7.1506	**12.9172**
RIJL	3	0.4585	**15.5943**	**50.7253**
RIJS	3	0.4285	**16.1048**	**39.1682**
Residual fitness				
RIJS	3	0.4585	1.5322	**12.6875**

QTL	Chr	Position	QTL x Photoperiod	QTL x Temperature	Diff. from DSDS50?
Germination in light in the lab					
WL	3	0.3975	0.2735	**35.4702**	**15.4485**
WL	3	0.7579	0.0057	**29.6414**	8.6646
WS	3	0.4585	0.0815	**44.9931**	**19.0509**
WS	3	0.7843	0.0039	**16.1151**	2.2005

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—such that the magnitude of the difference between alleles depends on the allele at the other locus / the number of epistatic interactions that are interactive—such 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; CC = Cal-Cal, TT = Tac-Tac.

	KYJune, Long	**KYJune, Short**	**RI June, Long**	**RI June, Short**	**RI Nov, Long**	**RI Nov, Short**	**QxQxE**
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	
	DSDS50	**Long, Warm**	**Long, Cold**	**Short, Warm**	**Short, Cold**	**QxQxPhoto**	**QxQxTemperature**
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		

Table 3. Upper: Tests that QTLs for germination in the lab 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 lab germination QTLs are equivalent to fitness QTLs. Lower: Tests that QTLs for fitness and germination in the lab 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 lab germination (DSDS50, germination in warm, germination in cold). It tests whether lab germination QTLs or fitness QTLs are equivalent to field QTLs. 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	Field germination	DSDS50	Total Warm	Total Cold
KYL	5	34.43	**12.5101**	2.3545	**14.5110**	**14.5096**
KYL	5	43.55	**13.5055**	5.3743	**16.2508**	**16.2253**
KYS	3	45.85	10.4824	0.2302	**16.1478**	**17.3805**
KYS	5	47.55	5.7560	2.0385	**14.3147**	**12.9797**
RIJL	3	55.87	4.0408	**16.9682**	**15.0880**	**26.7461**
RIJS	3	41.94	0.5625	8.6357	**16.2167**	**19.8980**

Field germination QTL

QTL	Chr	position	Fitness	DSDS50	Warm	Cold
KYL	3	60.87	2.6712	0.0120	**14.9942**	**14.1094**
KYL	5	40.84	9.7947	-0.0015	**20.4347**	**20.5034**
KYS	3	65.50	0.0258	0.2541	**13.4389**	11.3624
KYS	5	52.46	0.1145	0.0059	**17.0400**	**16.0013**
RIJL	1	73.31	0.1355	3.0847	**14.9289**	**14.8438**
RIJL	3	45.85	0.0862	**20.9556**	**50.8911**	**50.7348**
RIJL	5	47. 55	0.0032	1.5725	8.3397	8.3467
RIJS	1	70.29	0.0157	1.9257	10.0278	10.7208
RIJS	3	42.85	-0.1068	**13.3485**	**49.2792**	**46.2648**
RIJS	4	13.29	0.9710	5.0285	5.6446	6.9363
RIJS	5	59.57	1.2232	4.2787	7.2783	6.1954
RINS	4	0.00	0.0166	0.0006	4.5714	5.0669

Supplementary Table 1. QTLs for life-history and fitness traits in the field and lab. A positive additive effect indicates that the Tac allele was associated with an increase in the trait. KYS = Kentucky, June, Short-day; RIJS = RI, June, short-day; RIJL = RI, June, long-day. WL = Warm, long-day; WS = Warm, short-day. Only treatments and traits in which significant QTL were detected are shown. "Residual fitness" refers to the residual variation in lifetime fitness after factoring out the effect of germination timing on lifetime fitness. "Flowering interval" refers to the interval between bolting and flowering. "QTLxPhoto" tests for significant differences in QTL effects on adult traits of plants grown in two photoperiods in the lab. "QTL x Germ. season" tests for significant differences in QTL effects on adult traits of plants that germinated in autumn versus spring. "(J)" indicates a significant QTL x Germination season interaction in the joint analysis of the trait in both germination cohorts, but no significant difference when each cohort was analyzed separately. [a]Not significant in Kruskal-Wallis test.

Trait	Marker	Chromosome	Position of peak QTL	LOD score peak	% Variance explained	Additive allele effect
Lifetime Fitness						
KYL	SNP379	5	47.541	2.98	12.2	-17.57
	nga139	5	24.47	2.93	11.6	-17.66
KYS	msat3.1	3	38.741	3.87	15.9	-14.71
	SNP379	5	48.541	2.59	10.8	-12.53
RIJL	msat3.10	3	53.46	3.42	13.6	-33.26
RIJS	msat3.10	3	52.46	4.37	20.3	-31.58
Proportion germination						
RIJL	msat3.10	3	53.46	6.61	28.9	-0.06
RIJS	msat3.1	3	43.835	5.64	26.3	-0.06
RINS	SNP397	5	68.245	2.64	11.2	-0.03
Germination day						
KYL	T16K5	3	59.858	2.98	11.6	-4.00
	SNP370	5	45.541	4.03	15.7	-4.90
KYS	SNP231	3	64.487	2.52	10.6	-5.06
	SNP379	5	54.450	3.27	16.5	-6.59
RIJL	SNP76	1	73.281	3.7	9.6	-21.88
	SNP223	3	45.835	13.85	45.2	-32.58

	SNP394	5		57.45	2.89	7.9	-13.50
RIJS	nga128	1		70.281	2.51	6.3	-11.90
	msat3.1	3		42.835	10.15	32.0	-27.48
	msat4.35	4		13.279	3.15	8.0	19.7
	SNP394	5		59.555	5.26	14.2	-18.55
RINS	msat4.39	4		0.00	2.84	11.9	-1.64
Residual fitness							
KYS	msat3.1	3		36.741	3.65	16.1	-14.66
RIJS	msat3.1	3		44.835	2.27	11.4	-23.19
Germination in the lab							
DSDS50	SNP223	3		45.552	10.55	31.0	-104.30
	msat4.10	4		18.819	2.43	6.3	565.24
	SNP379	5		52.762	2.67	6.6	-492.54
Total germination							
LW	SNP223	3		46.835	5.53	14.2	0.18
	SNP244	3		79.061	4.84	10.7	0.11
SW	SNP60	1		37.718	3.09	6.9	0.09
	SNP223	3		45.835	11.58	32.2	0.21
	SNP244	3		79.061	4.13	9.5	0.11
LC	SNP223	3		49.46	3.45	14.2	0.05
SC	CIW3	2		1.826	3.77	12.8	-0.06
	SNP223	3		48.46	3.86	13.6	0.07
Germination in the dark							
LW	SNP18	1		10.831	4.72	12.9	0.11
	msat3.1	3		44.835	3.84	10.9	0.11
	SNP244	3		79.061	4.09	11	0.11
SW	SNP18	1		12.831	6.75	14.5	0.12
	nga128	1		70.281	5.19	10.8	0.11
	msat3.1	3		45.835	5.66	12.5	0.13
	SNP244	3		79.061	2.95	5.8	0.08
LC	SNP18	1		10.831	3.64	10.5	0.09
	msat3.1	3		45.835	4.61	14.2	0.09
	SNP244	3		79.061	3.14	8.9	0.09
SC	SNP18	1		12.831	6.46	14.6	0.11
	nga128	1		71.281	5.27	12.1	0.10
	msat3.1	3		44.835	6.65	15.4	0.12
	SNP244	3		79.061	2.77	5.7	0.08

Trait	**Marker**	**Chr.**	**Position of peak QTL**	**LOD score peak**	**% Variance explained**	**Additive allele effect**	
Adult traits in the lab							
Short-day							**QTLxPhoto**
Days to bolting	msat4.39	4	1.00	3.91	8	2.91	**12.17**
	CIW7	4	25.076	10.55	24.7	5.04	**48.87**

	SNP404	5	76.819	3.69	7.4	2.34	**12.65**
# Leaves at bolting	msat4.39	4	1.00	3.79	7.1	2.14	**12.90**
	CIW7	4	26.297	10.93	24.4	4.02	**50.04**
	SNP404	5	75.245	4.96	9.6	2.18	**20.84**
Flowering interval	msat4.35	4	14.279	4.08	15.6	2.02	7.70
Height	SNP244	3	79.061	4.15	11.5	1.59	1.74
	SNP306	4	36.491	7.32	22.4	2.29	3.14
Number of fruits on basal branches	SNP300	4	34.012	3.66	13.3	-3.74	1.61
	SNP397	5	70.245	3.6	13.3	-3.702	9.31 **/15.21(J)**
Long-day							
# Leaves at bolting	CIW7	4	24.076	4.56	18.2	0.97	6.53
Height	nga8	4	11.783	5.81	22.9	2.53	1.21
Number of fruits on basal branches	nga6	3	73.781	2.41	11	2.16767	-1.74
Total branches	CIW5	4	4.898	2.99	12.3	2.35	0.07
Adult life-history traits in the field for November-dispersed seeds							
Autumn germinants							**QTL x Germ. season**
Date of bolting	msat4.10	4	20.076	2.97	10.3	1.00	-0.02
	SNP397	5	71.245	3.26	11.7	0.92	0.32
Interval between germination and bolting	nga8	4	7.779	3.05	9.2	1.49	0.56
	SNP379	5	48.541	2.84	10.4	1.43	3.10 / **13.47 (J)**
	SNP404	5	75.245	2.54	8.9	1.27	0.51
# Leaves at bolting	msat4.35	4	12.783	2.62	5.8	0.78	1.22
	CIW7	4	27.297	4.75	11.5	0.92	**12.77**
	SNP415	5	83.897	3.9	8.6	0.59	0.51
Rosette diameter at bolting	CIW7	4	25.297	4.67	18.5	1.12	2.62

Spring germinants							
Date of bolting	msat4.10	4	20.076	3.29	10.1	1.94	4.77
	SNP404	5	75.819	5.1	16.3	2.05	**14.11**
# Leaves at bolting	CIW7	4	24.076	4.56	11.3	0.60	1.82
	MBK5	5	80.1	6.74	17.9	0.66	0.05

Supplementary Table 2. Epistatic interactions. Genotypic means and standard errors of the phenotypes of genotypes of interacting markers. "Markers" indicates two markers that were shown to have significant epistasis in either Epistat or QTL Cartographer MIM. Genotypes Cal and Tac indicate the parental allele at the first and second markers, respectively. "C/I" indicates whether the non-additive interaction is conditional—such that the magnitude of the difference between alleles depends on the allele at the other locus—or interactive—such that the rank order of alleles of one locus changes depending on the alleles at the other locus. " Extreme" indicates the genotype that has the most extreme phenotype. "F-residual" (given only for fitness traits) presents the F-ratio of the interaction between loci when germination day is used as a co-factor, and tests whether the observed epistasis for fitness can be accounted for by epistasis for germination day. F-ratio indicates the F-ratio of analysis of variance. All interactions except those marked with "[a]" had LR over 50 in Epistat. "[a]" indicates a significant LOD score for the interaction effect in QTL Cartographer MIM. Instead of the F-ratio, the LOD score is given. *P < 0.05, **P < 0.01, ***P < 0.001.

Markers		**Cal Cal**	**Cal Tac**	**Tac Cal**	**Tac Tac**	**F-ratio**	**C/I**	**F-residual**	**extreme**
t10j7 x snp351	freq	15	39	15	33				
	fitKYL	13.1 (5.9)	9.4 (5.0)	62.6 (29.7)	9.0 (3.5)	6.01*	C	6.89*	TC
	GermSC	0.99 (0.01)	0.96 (0.01)	0.80 (0.06)	0.89 (0.04)	3.08+	I		CC
	GermLC	0.98 (0.01)	0.96 (0.01)	0.79 (0.07)	0.94 (0.02)	9.96**	I		CC
snp223 x snp126	freq	21	20	34	30				
	fitKYL	14.4 (4.3)	51.5 (20.0)	7.8 (5.9)	4.2 (3.3)	4.89*	C	1.05	CT
	GermSC	0.96 (0.01)	0.67 (0.07)	0.96 (0.01)	0.97 (0.01)	35.02***	C		TT
	GermSW	0.53 (0.06)	0.22 (0.05)	0.82 (0.05)	0.86 (0.03)	12.07***	I		TT
	GermLC	0.94 (0.02)	0.79 (0.06)	0.97 (0.01)	0.97 (0.01)	9.11**	C		TT/TC
msat3_10x snp38	freq	20	22	28	35				
	fitKYL	49.7 (18.0)	10.4 (3.9)	0.3 (0.2)	5.8 (3.3)	7.09**	C	0.69	CC

	fitRINS	21.5 (1.5)	24.4 (2.4)	30.8 (3.8)	22.9 (1.5)	4.2*	C	4.83*	TC
	gdayKYL	171.8 (3.3)	163.2 (2.0)	158.1 (0.4)	159.2 (1.0)	7.02**	C		CC
	perRIJL	0.29 (0.02)	0.22 (0.02)	0.12 (0.02)	0.16 (0.01)	7.14**	minor I		CC
	DSDS50	28.6 (3.7)	20.1 (6.0)	1.1 (0.4)	6.7 (4.1)	2.94+	I		CC
	GermSC	0.73 (0.06)	0.95 (0.01)	0.98 (0.01)	0.95 (0.02)	19.25***	I		TC
	GermSW	0.24 (0.05)	0.560 (0.06)	0.85 (0.03)	0.84 (0.05)	13.53***	C		TT/TC
	GermLC	0.82 (0.05)	0.92 (0.02)	0.97 (0.01)	0.97 (0.01)	4.62*	C		TC/TT
	GermLW	0.31 (0.07)	0.55 (0.06)	0.79 (0.04)	0.84 (0.04)	3.32+	I		TT
t16k5 x snp358		18	29	16	41				
	fitKYL	67.2 (21.5)	8.8 (2.8)	0.3 (0.3)	4.6 (2.6)	12.37***	C	2.49	CC
	fitKYS	45.0 (11.6)	12.7 (4.3)	0.1 (0.1)	10.7 (5.8)	8.81**	C	9.44**	CC
	fitRIJS	92.6 (26.8)	53.5 (11.0)	4.6 (3.1)	22.2 (6.1)	4.22*	C	4.8*	CC
	fitRINL	25.5 (2.6)	23.2 (1.9)	18.4 (1.7)	24.8 (2.2)	3.17+	C	2.57	CC
	gdayKYL	175.1 (4.6)	163.3 (4.6)	157.5 (0.2)	159.2 (0.9)	9.73**	C		CC
	gdayRIJL	275.7 (8.6)	261.1 (7.0)	200.7 (8.2)	223.2 (7.6)	4.6*	I		CC
	gdayRINS	348.8 (1.0)	344.2 (0.8)	345.2 (0.9)	344.3 (0.6)	4.59*	C		CC
	DSDS50	36.4 (9.6)	14.6 (2.6)	0.3 (0.3)	4.1 (1.5)	9.74**	I		CC
msat3_1 x snp406	freq	23	24	32	25				
	fitKYS	49.1 (12.0)	16.0 (6.7)	1.0 (0.9)	5.0 (2.1)	8.22**	C	8.64**	CC
		113.5				6.09*	C	3.68+	CC
	fitRIJL	(22.1)	59.0 (16.1)	12.5 (6.5)	33.1 (13.6)				
	fitRINL	28.2 (2.7)	19.4 (1.7)	22.7 (2.1)	23.9 (2.5)	4.76*	C	4.41*	CC
	gdayKYL	171.8 (3.5)	163.2 (2.2)	158.2 (0.4)	160.2 (1.6)	6.06*	C		CC
	gdayRIJL	282.3 (7.1)	254.3 (9.4)	215.9 (6.4)	218.0 (8.9)	3.60+	C		CC
	gdayRIJS	286.7 (5.6)	259.8 (7.7)	219.5 (9.0)	224.3 (9.4)	3.57+	C		CC
	gdayRINS	347.7 (0.8)	344.7 (0.8)	344.3 (0.8)	344.8 (0.9)	4.53*	C		CC
	perRIJS	0.26 (0.02)	0.22 (0.03)	0.11 (0.02)	0.16 (0.02)	4.01*	I		CC
snp139 x snp379	freq	13	15	28	50				
	fitKYL	93.1 (27.3)	1.4 (1.4)	10.6 (4.0)	3.7 (1.6)	25.42***	C	3.04+	CC
	fitRIJL	122.7 37.8)	24.8 (10.3)	41.5 (14.0)	47.9 (10.4)	8.36**	C	5.87*	CC
	gdayKYL	181.2 (5.6)	158.3 (0.8)	162.3 (1.7)	159.9 (0.8)	24.03***	C		CC
	perKYL	0.68 (0.04)	0.78 (0.02)	0.79 (0.02)	0.76 (0.02)	5.11*	C		TC
	perRIJL	0.25 (0.03)	0.15 (0.03)	0.19 (0.02)	0.19 (0.01)	3.26+	C		CC
	DSDS50	38.4 (13.2)	6.0 (2.2)	12.6 (3.5)	6.7 (1.5)	8.4**	C		CC
	GermSC	0.81 (0.07)	0.94 (0.02)	0.93 (0.03)	0.92 (0.02)	4.07*	C		CT
	GermSW	0.41 (0.10)	0.74 (0.07)	0.69 (0.07)	0.71 (0.05)	4.38*	C		CT

	GermLW	0.49 (0.10)	0.76 (0.07)	0.67 (0.06)	0.68 (0.05)	3.46+	C		CT
	darkSW	0.24 (0.09)	0.53 (0.07)	0.45 (0.07)	0.50 (0.04)	3.06+	C		CT
	darkLC	0.22 (0.06)	0.48 (0.06)	0.38 (0.06)	0.389 (0.04)	4.14*	C		CT
	darkLW	0.27 (0.07)	0.56 (0.07)	0.43 (0.06)	0.45 (0.04)	4.59*	C		CT
nga249 x snp237	freq	15	16	30	40				
	fitKYL	70.8 (26.1)	0 (0)	10.0 (3.2)	9.5 (5.0)	13.45***	C	4.64*	CC
	fitKYS	51.6 (15.2)	0.1 (0.1)	12.1 (4.0)	14.0 (5.9)	12.4***	C	12.85***	CC
	fitRIJS	114.0 36.3)	11.1 (5.8)	49.3 (10.2)	23.9 (5.5)	7.23**	C	6.85*	CC
	fitRINL	27.9 (3.3)	17.6 (1.6)	23.7 (1.7)	24.5 (2.1)	5.06*	C	4.54*	CC
	fitRINS	23.1 (2.7)	35.0 (6.3)	25.0 (1.6)	21.8 (1.3)	7.22**	C	6.11*	CT
	gdayKYL	174.2 (4.8)	157.4 (0.1)	163.7 (1.6)	161.0 (1.7)	8.42**	C		CC
	gdayRIJL	280.8 10.2)	202.6 (8.7)	258.3 (7.3)	229.9 (7.3)	7.24**	C		CC
	reproRIN								CC
	L	35.7 (5.1)	25.5 (2.4)	30.2 (1.7)	32.1 (2.6)	3.84+	C		
	reproRINS	34.3 (3.9)	50.1 (10.4)	32.4 (2.0)	27.7 (1.6)	5.96*	C		CT
	DSDS50	32.9 (9.2)	2.5 (1.4)	16.9 (4.7)	4.8 (1.7)	4.15*	C		CC
	GermSC	0.81 (0.07)	0.98 (0.01)	0.91 (0.02)	0.94 (0.02)	4.08*	C		CT
	GermSW	0.31 (0.08)	0.90 (0.04)	0.57 (0.06)	0.79 (0.05)	9.57**	C		CT
	GermLC	0.79 (0.07)	0.98 (0.01)	0.93 (0.02)	0.96 (0.01)	8.98**	C		CT
	GermLW	0.33 (0.07)	0.87 (0.03)	0.53 (0.06)	0.80 (0.04)	5.4*	C		CT
	darkSC	0.13 (0.05)	0.53 (0.06)	0.34 (0.06)	0.52 (0.05)	3.16+	C		CT/TT
	darkSW	0.15 (0.06)	0.60 (0.06)	0.42 (0.06)	0.55 (0.05)	6.25*	C		CT
	darkLW	0.17 (0.05)	0.57 (0.07)	0.35 (0.05)	0.55 (0.04)	2.92+	C		CT
snp104 x mbk5	freq	18	28	32	24				
	fitKYL	9.5 (4.7)	22.5 (12.2)	28.1 (10.7)	2.0 (2.0)	4.04*	I	0.29	TC
	fitRIJL	31.4 (11.9)	49.3 (13.7)	85.5 (19.8)	29.0 (12.7)	4.94*	I	2.32	TC
	gdayKYL	162.2 (1.7)	164.0 (2.3)	166.4 (2.9)	158.7 (0.8)	4.04*	C		TC
	gdayRIJL	239.9							TC
		(12.6)	254.2 (10.0)	262.4 (9.7)	219.5 (6.3)	3.16+	I		
	gdayRIJS	244.3							TC
		(10.4)	246.0 (10.2)	252.5 (9.1)	219.8 (7.7)	8.38**	C		
snp237 x nga158	freq	18	27	12	46				
	fitKYL	55.2 (21.1)	14.3 (7.6)	0 (0)	8.8 (4.7)	5.64*	C	0.43	CC
	fitKYS	35.0 (9.7)	19.2 (8.3)	0.2 (0.2)	13.0 (5.5)	3.00+	C	2.25	CC
	fitRIJS	104.7			23.7 (5.2)	5.04*	C	4.8*	CC
		(34.6)	52.0 (10.5)	9.4 (6.1)					

	fitRINL	27.4 (2.7)	23.6 (1.9)	18.1 (1.8)	23.7 (2.0)	3.36+	C	2.49	CC
	fitRINS	22.2 (2.2)	25.9 (1.8)	36.3 (8.4)	22.6 (1.3)	8.95**	C	7.94**	TC
	gdayKYL	172.5 (4.2)	163.9 (1.7)	157.3 (0.01)	160.7 (1.6)	5.67*	Minor I		CC
	gdayKYS	203.0 22.6)	166.9 (2.7)	160.3 (1.6)	162.2 (1.4)	4.77*	C		CC
	gdayRIJL	278.6 (9.3)	257.9 (7.7)	206.8 (9.8)	226.7 (7.1)	4.41*	I		CC
	perKYS	0.64 (0.06)	0.73 (0.02)	0.84 (0.02)	0.77 (0.02)	4.68*	I		TC
	perRINS	0.97 (0.02)	1.00 (0.02)	1.02 (0.02)	0.97 (0.01)	4.98*	minor I		TC
	reproRIN								CC
	L	35.2 (4.3)	29.9 (1.8)	25.5 (2.5)	31.5 (2.5)	3.14+	I		
	reproRINS	32.1 (3.0)	33.7 (2.4)	49.9 (13.4)	29.7 (2.0)	5.99*	C		CT
	DSDS50	30.7 (7.9)	16.6 (5.1)	2.1 (1.7)	4.7 (1.6)	3.21+	I		CC
	GermSC	0.82 (0.06)	0.91 (0.02)	0.98 (0.01)	0.24 (0.02)	3.55+	I		TC
	GermSW	0.35 (0.07)	0.57 (0.06)	0.94 (0.03)	0.79 (0.04)	7.98**	I		TC
	GermLC	0.80 (0.06)	0.94 (0.02)	0.98 (0.01)	0.97 (0.01)	7.93**	C		TC/TT
	GermLW	0.35 (0.07)	0.53 (0.06)	0.85 (0.04)	0.82 (0.04)	3.33+	C		TC
snp321 x snp159	freq	12	20	35	33				
	fitKYL	6.9 (4.3)	37.5 (15.4)	16.8 (9.5)	10.5 (6.1)	2.92+	Minor I	0.0	CT
	fitKYS	1.9 (1.7)	37.4 (12.0)	18.9 (7.4)	6.9 (2.7)	8.41**	Minor I	9.79**	CT
	gdayKYL	160.8 (1.7)	168.9 (3.9)	163.2 (1.7)	161.1 (2.0)	3.82+	I		CT
	gdayRIJS	232.1 14.5)	273.4 (10.0)	241.4 (9.1)	239.2 (8.6)	3.73+	I		CT
	perIJS	0.12 (0.04)	0.20 (0.03)	0.20 (0.02)	0.16 (0.02)	4.21*	I		CT/TC
	DSDS50	10.9 (5.0)	19.0 (4.0)	16.8 (5.7)	4.4 (1.3)	4.3*	I		CT
	GermSC	1.0 (0.01)	0.87 (0.05)	0.89 (0.04)	0.94 (0.02	5.33*	I		TT
	GermSW	0.74 (0.08)	0.49 (0.08)	0.69 (0.06)	0.72 (0.06)	3.62+	I		CC
	GermLC	0.98 (0.01)	0.87 (0.05)	0.94 (0.02)	0.94 (0.02)	3.41+	C		CC
	GermLW	0.68 (0.08)	0.50 (0.08)	0.65 (0.06)	0.74 (0.06)	3.45+	I		TT
	darkSC	0.47 (0.09)	0.24 (0.06)	0.41 (0.06)	0.48 (0.06)	4.98*	C		TT
	darkSW	0.55 (0.08)	0.25 (0.06)	0.47 (0.06)	0.52 (0.06)	6.68*	C		CC
	darkLC	0.41 (0.07)	0.21 (0.05)	0.37 (0.05)	0.46 (0.05)	6.08*	I		TT
	darkLW	0.45 (0.09)	0.29 (0.06)	0.45 (0.05)	0.51 (0.05)	2.93+	I		TT
snp394 x snp14	freq	27	21	31	24				
	fitKYL	50.0 (16.1)	10.9 (5.1)	4.2 (2.6)	1.3 (0.9)	4.07*	C	3.24+	CC
	fitKYS	36.8 (11.4)	13.2 (5.3)	4.1 (1.8)	12.3 (6.0)	4.86*	C	4.10*	CC
	fitRIJS	53.2 (18.3)	17.0 (4.7)	35.5 (8.8)	61.4 (20.4)	4.46*	C	2.24	CC
	gdayRIJS	273.7 10.6)	242.9 (11.0)	224.0 (8.1)	238.4 (8.0)	5.52*	I		CC
	perKYL	0.73 (0.03)	0.80 (0.02)	0.77 (0.02)	0.74 (0.04)	4.07*	minor I		CT

	GermSW	0.51 (0.07)	0.78 (0.07)	0.73 (0.06)	0.72 (0.07)	4.06*	C		CT
	darkSC	0.23 (0.06)	0.56 (0.07)	0.40 (0.05)	0.51 (0.07)	3.25+	C		CT
	darkSW	0.262 0.06)	0.61 (0.07)	0.46 (0.05)	0.57 (0.07)	3.88+	C		CT
snp10 x snp306	freq	39	19	22	20				
	fitRIJL	32.1 (8.3)	48.9 (12.3)	42.5 (18.1)	33.1 (7.5)	7.11**	I	7.24**	CT
	GermSC	0.93 (0.02)	0.80 (0.08)	0.93 (0.02)	0.98 (0.01)	7.25**	I		TT
	GermSW	0.72 (0.05)	0.45 (0.09)	0.73 (0.07)	0.75 (0.08)	4.09*	I		TT
	darkSC	0.39 (0.05)	0.21 (0.06)	0.47 (0.06)	0.56 (0.08)	4.57*	I		TT
	darkSW	0.45 (0.05)	0.26 (0.06)	0.53 (0.06)	0.61 (0.08)	4.32*	I		TT
snp321 x snp10	freq	20	13	40	30				
	fitRIJL	114.3 28.0)	7.7 (4.5)	44.0 (10.3)	42.3 (15.0)	9.98**	I	5.17*	CC
	gdayKYL	171.8 (3.8)	157.6 (0.4)	162.7 (1.9)	161.2 (1.5)	6.76*	C		CC
t16k5 x snp331	freq	17	30	12	44				
	fitKYL	51.7 (20.0)	19.4 (8.7)	0 (0)	4.2 (2.4)	3.05+		0.71	CC
	fitRIJS	103.0 32.1)	52.0 (9.3)	2.3 (1.8)	21.5 (5.7)	5.67*		6.78*	CC
	Fitrinl	27.3 (2.7)	22.2 (1.8)	17.8 (2.2)	24.4 (2.0)	5.20*		4.34*	CC
	FitRINS	23.0 (2.4)	24.4 (1.7)	35.56 (8.4)	23.5 (1.4)	5.22*		3.55+	TC
	perRINS	0.96 (0.02)	1.01 (0.02)	1.03 (0.02)	0.96 (0.01)	9.16**	minor I		TC
	reproRIN								CC
	L	354 (4.3)	28.8 (1.7)	25.1 (3.1)	32.2 (2.5)	4.48*	minor I		
	DSDS50	33.6 (8.0)	16.9 (4.7)	0.5 (0.5)	3.7 (1.4)	4.91*	C		CC
5-Nga139 x 5-snp379	freq	13	15	28	50				
	fitKYL[a]	93.1 (27.3)	1.4 (1.4)	10.6 (4.0)	3.7 (1.6)	4.95** (25.42***)	C	3.04+	CC
3-Msat3.1 x 5-snp379	freq	20	27	21	37				
	fitKYS[a]	56.8 (12.6)	12.4 (5.6)	4.41 (2.4)	2.0 (1.1)	2.56+ (11.38**)	C	11.69***	CC
	gdayKYS[a]	198.6 (17.4)	161.7 (0.9)	163.9 (3.2)	161.8 (2.9)	5.350**	C		CC
	DSDS50 [a]	39.9 (8.3)	13.3 (2.2)	2.6 (2.1)	1.1 (0.6)	2.6*	C		CC
SNP331 x SNP186	freq	13	17	40	33				
	perKYL	0.63 (0.04)	0.82 (0.02)	0.77 (0.01)	0.76 (0.03)	11.81***	C		CT
	GermSC	0.76 (0.06)	0.98 (0.02)	0.93 (0.01)	0.93 (0.02)	10.43**	C		CT

	GermSW	0.44 (0.06)	0.70 (0.09)	0.76 (0.04)	0.67 (0.01)	5.79*	I	TC
	GermLC	0.74 (0.08)	0.97 (0.01)	0.95 (0.03)	0.96 (0.02)	18.57***	C	CT
	darkSC	0.15 (0.08)	0.42 (0.01)	0.50 (0.01)	0.42 (0.01)	7.4**	I	TC
	darkSW	0.18 (0.10)	0.46 (0.06)	0.57 (0.05)	0.46 (0.05)	9.01**	I	TC
snp235 x snp331	freq	18	12	27	37			
	gdayKYL	172.5 (4.2)	157.3 (0.1)	163.9 (1.7)	160.6 (1.6)	5.65*	C	CC
	gdayKYS	203.0 22.6)	160.3 (1.6)	166.9 (2.7)	162.1 (1.3)	4.81*	C	CC
	gdayRIJL	278.6 (9.3)	206.8 (9.8)	257.9 (7.8)	225.5 (7.0)	4.13*	C	CC
	DSDS50	30.7 (7.9)	2.1 (1.7)	16.6 (5.1)	4.74 (1.5)	3.25+	C	CC
	GermSC	0.82 (0.6)	0.98 (0.01)	0.91 (0.02)	0.94 (0.02)	3.52+	C	CT
	GermSW	0.35 (0.07)	0.94 (0.03)	0.57 (0.06)	0.79 (0.04)	7.96**	C	CT
	GermLC	0.80 (0.06)	0.98 (0.01)	0.94 (0.02)	0.97 (0.01)	7.97**	C	CT
	GermLW	0.35 (0.07)	0.85 (0.04)	0.53 (0.06)	0.82 (0.04)	3.42+	C	CT
snp145 x snp60	freq	35	26	23	21			
	perKYS	0.79 (0.01)	0.69 (0.05)	0.72 (0.04)	0.78 (0.03)	5.11*	C	CC
	GermSC	0.97 (0.01)	0.92 (0.03)	0.83 (0.05)	0.93 (0.02)	5.44*	I	CC
	GermLC	0.97 (0.01)	0.91 (0.03)	0.87 (0.04)	0.96 (0.01)	8.32**	I	CC
	GermLW	0.67 (0.05)	0.67 (0.07)	0.54 (0.08)	0.79 (0.05)	3.69+	C	TT
msat3.1 x snp67	Freq	26	21	27	31			
	DSDS50	31.4 (6.8)	16.1 (3.5)	1.0 (0.4)	2.2 (1.5)	4.76*	C	CC
msat3.20 x snp97	Freq	22	30	23	27			
	DSDS50	35.3 (8.0)	11.9 (2.3)	2.9 (2.0)	1.6 (1.0)	8.19**	C	CC
mat3_20 x snp231	freq	32	20	15	36			
	DSDS50	31.0 (5.6)	7.8 (3.0)	5.1 (3.1)	1.0 (0.6)	5.50*	I	CC
3-Nga172 x 5-snp370	freq	15	37	22	28			
	gdayKYL[a]	179.9 (5.1)	159.9 (1.0)	163.7 (2.2)	158.7 (0.7)	3.825*	C	CC
3-T16k5 x 5-snp370	freq	22	24	15	31			
	gdayKYL[a]	175.9 (3.8)	161.4 (1.38)	160.9 (2.38)	157.9 (0.3)	2.8400*	C	CC

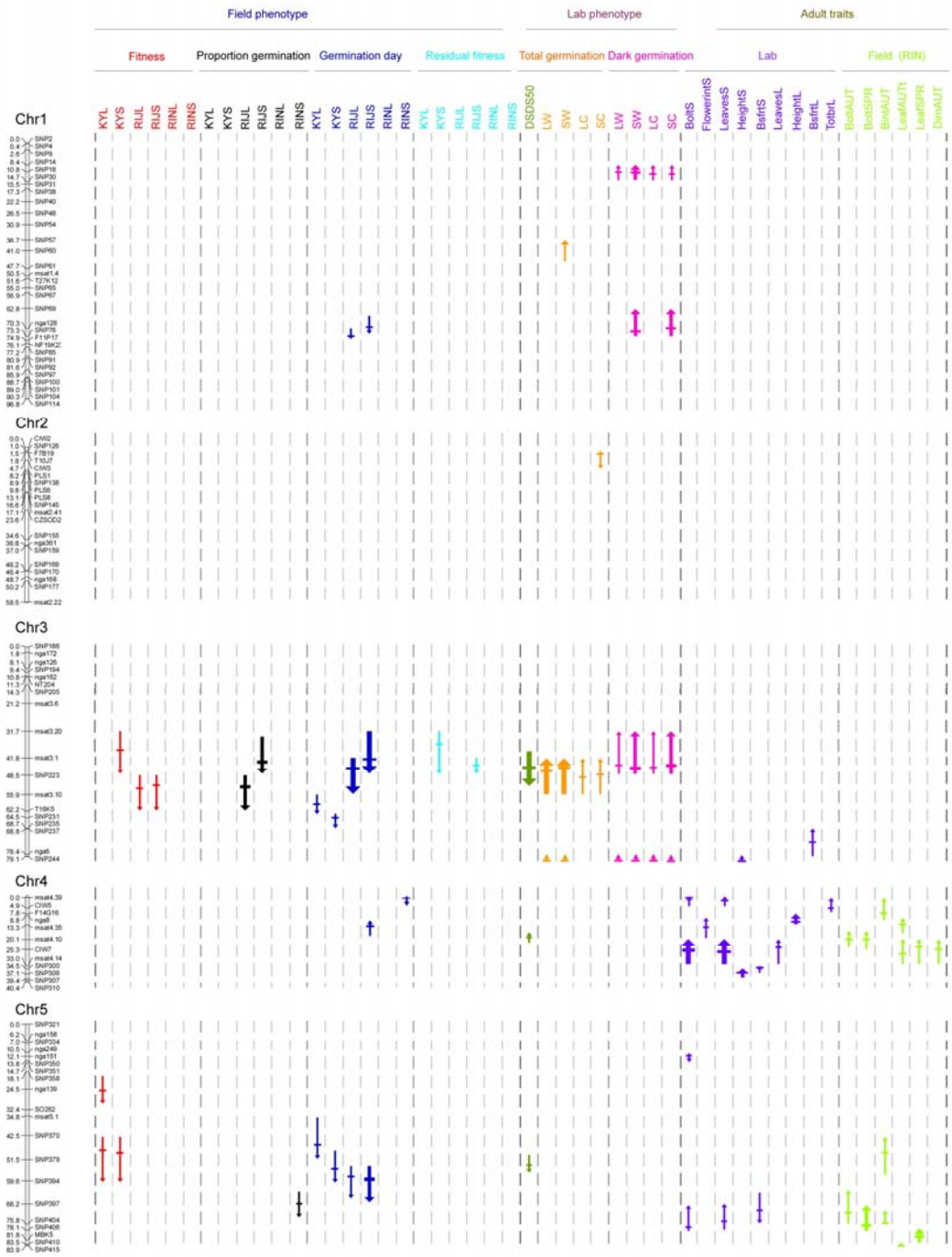
Supplementary Table 3: Tests for QTL x QTL x environment interactions. "Interaction" refers to the marker pair that had a significant interaction. F-ratios are given, based on ANOVA. In addition to these interactions based on field phenotypes, one pair of markers, SNP223 x SNP126, which was associated with variation in germination in the lab after a warm-then-cold imbibition treatment, demonstrated a QTLxQTLxPhotoperiod interaction (F = 4.90, P – 0.28). The treatment in which the significant effect was detected is indicated in parentheses: RIJS = RI, June, short-day; RIJL = RI, June, long-day. Lower: QTL-environment interactions in the lab. WL = Warm, long-day; WS = Warm, short-day. *P < 0.05, **P < 0.01, ***P < 0.001. [a] Direction of the interaction was consistent across treatments.

	Fitness			Germ day			Proportion germination		
Interaction	QxQx Photo	QxQxSite	QxQx Season	QxQx Photo	QxQx Site	QxQx Season	QxQx Photo	QxQx Site	QxQx Season
MBK5xSnp104	ns	0.87	6.67	ns	6.26*	[a]11.68***	ns	1.94	0.26
Sn186xSnp331	4.27*(RIN)	0.14	0.51	ns	1.80	1.59	ns	9.24**	2.16
Snp321xSnp159	ns	0.06	2.73	ns	3.01+	3.43+	ns	2.57	1.34
Snp306xSnp10	ns	2.43	9.83**	ns	0.70	3.56+	ns	0.17	4.17*
Snp321xSnp10	3.65+(RIN) 5.36*(RIJ)	1.05	5.59*	ns	[a] 7.43**	10.38**	ns	3.12+	0.00
Snp145xSnp60	ns	0.72	4.45*	3.10+(RIN)	0.11	2.69	6.52*(KY)	0.32	1.38
nga139xSnp379	9.21**(KY)	0.49	8.54**	ns	1.91	0.21	ns	8.32**	1.61
Snp394XSnp14	ns	0.15	4.35*	ns	2.36	8.96**	ns	4.44*	0.10
Snp331xT16K5	10.39**(RIN) 4.74*(RIJ)	0.10	0.87	4.81*(RIJ)	0.74	0.20	ns	0.95	7.05**
Msat3.10xsnp38	7.06**(RIN)	0.84	0.51	ns	0.05	0.51	ns	4.41*	4.31*
Nga158xSnp237	11.95***(RIN) 3.12+(RIJ)	0.27	1.74	ns	0.00	2.64	ns	4.35*	5.41*
Snp331xSnp235	11.65***(RIN) 3.16+(RIJ)	0.28	1.70	ns	0.01	2.34	ns	4.50*	5.11*
Nga249xSNp237	12.30***(RIN) 2.75+(RIJ)	0.78	3.70+	ns	2.39	4.94*	ns	5.47*	5.08*
T16K5xSnp358	5.78*(RIN)	0.27	3.97*	4.73* (RIJ)	0.19	0.22	ns	5.43*	10.52**
Msat3.1xSnp406	6.14*(RIN) 5.58*(RIJ)	0.03	2.08	ns	4.60*	[a] 7.13**	3.37+(RIN) 3.03+(KY)	2.10	0.61

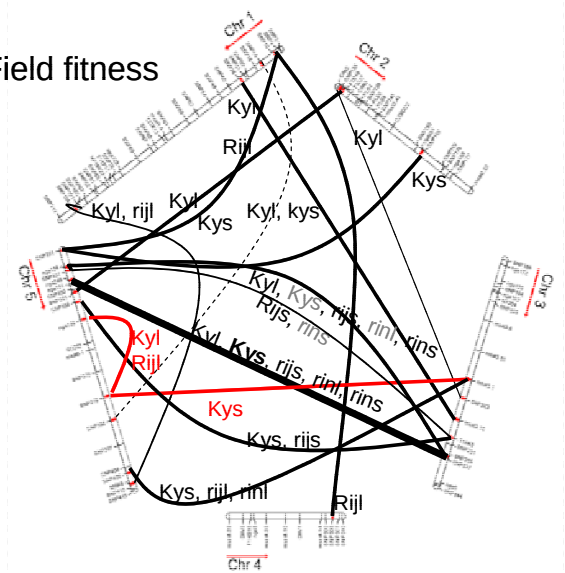
3-Nga172 x 5-snp370	ns	3.81+	ns	ns	ns	ns	ns	ns	ns
3-T16k5 x 5-snp370	ns	ns	3.88*	ns	ns	ns	ns	6.00*	4.81*
3-Msat3.1 x 5-snp379	ns	4.55*	ns	ns	ns	3.30+	ns	20.35***	3.38+
5-Nga139 x 5-snp379	9.21**(KY)	ns	8.54**	ns	ns	ns	ns	8.32**	ns

Supplementary Table 4. QTLs for plasticity of germination in the field and lab. A positive additive effect indicates that the Tac allele was associated with an increase in the trait. Only treatments and traits in which significant QTLs were detected are shown.

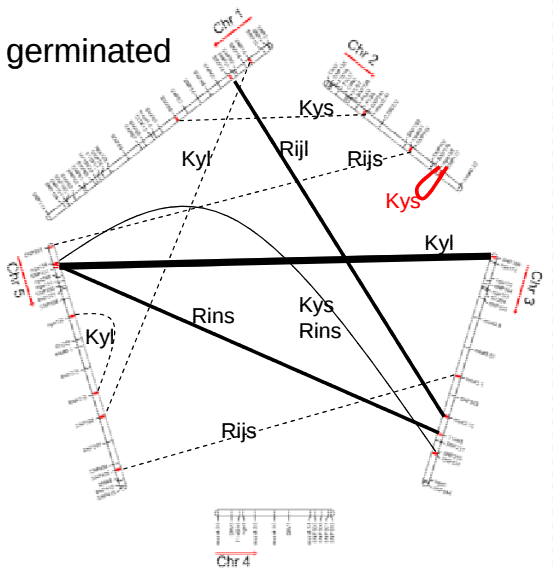
Trait	QTL	Chromosome	Position of peak QTL	LOD score peak	% of variance explained	Additive allele effect
Plasticity to site						
Germination day, Long-days	SNP223	3	45.835	9.02	38.7	-26.70
Germination day, Short-days	msat3.1	3	41.835	6.92	25.1	-21.41
	SNP394	5	59.555	2.87	9.4	-13.54
	msat4.35	4	13.279	2.76	9	18.64
Plasticity to season						
Germination day, Long days	SNP223	3	45.835	11.18	43.2	30.99
Germination day, short days	msat3.1	3	42.835	9.14	29.9	26.31
	SNP394	5	59.555	4.54	12.8	17.44
	msat4.35	4	13.279	3.01	8.1	-19.69
Plasticity to temperature in the lab						
Total germination, long days	SNP223	3	45.835	3.69	11.7	-0.15
	SNP244	3	79.061	3.17	8.3	-0.08
Total germination, short days	SNP223	3	45.835	8.7	27	-0.15
	SNP244	3	79.061	3.39	8.8	-0.09
	SNP54	1	30.89	3.27	8.5	-0.08
Dark germination, long days	nga6	3	78.42	3.13	13	-0.03


Field phenotype
Lab phenotype
Adult traits
Fitness
Proportion germination
Germination day
Residual fitness
Total germination
Dark germination
Lab
Field (RIN)
KYL KYS RJL RJS RINL RINS KYL KYS RJL RJS RINL RINS KYL KYS RJL RJS RINL RINS KYL KYS RJL RJS RINL RINS DSDS50 LW SW LC SC LW SW LC SC BoltS FloweringS LeavesS HeightS BsftrS LeavesL HeightL BsftrL TotbrL BoltAUT BoltSPR BrntAUT LeafAUTt LeafSPR DrnAUT
Chr1
Chr2
Chr3
Chr4
Chr5

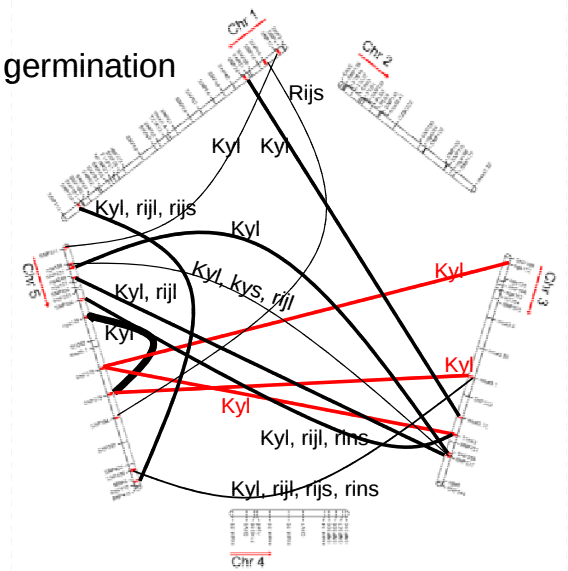
Field fitness



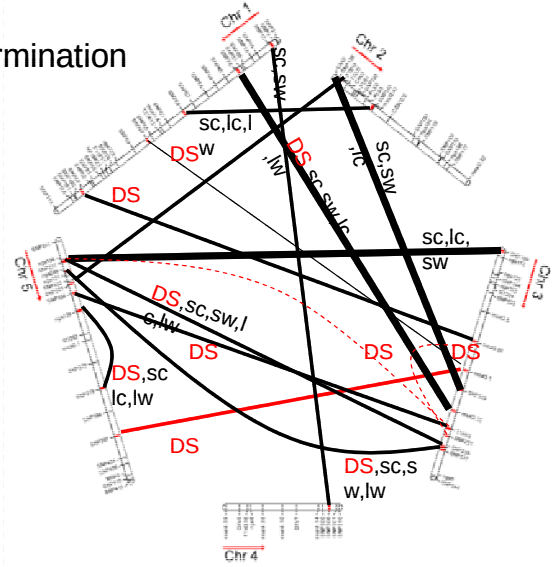
Proportion germinated

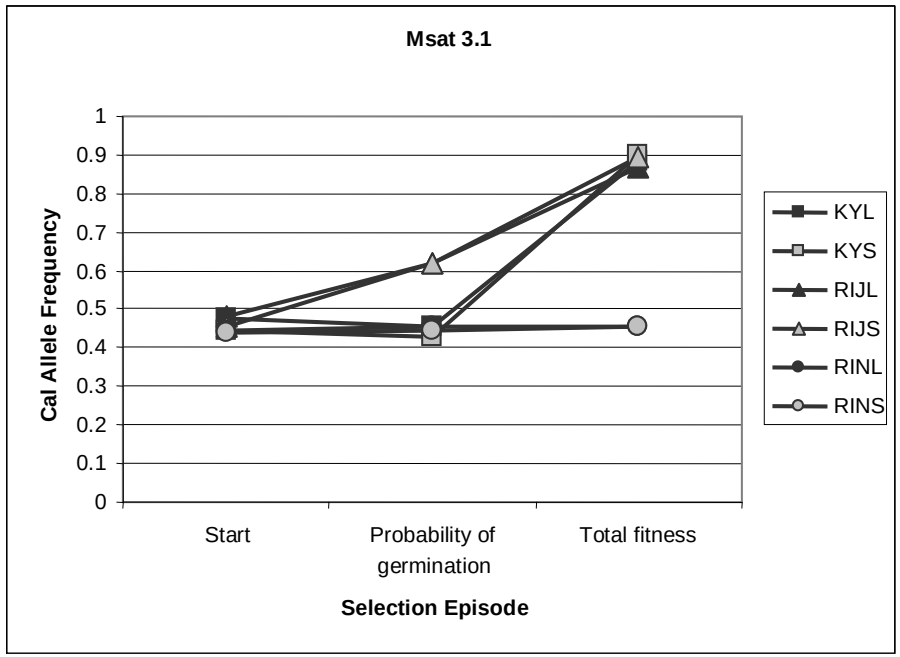


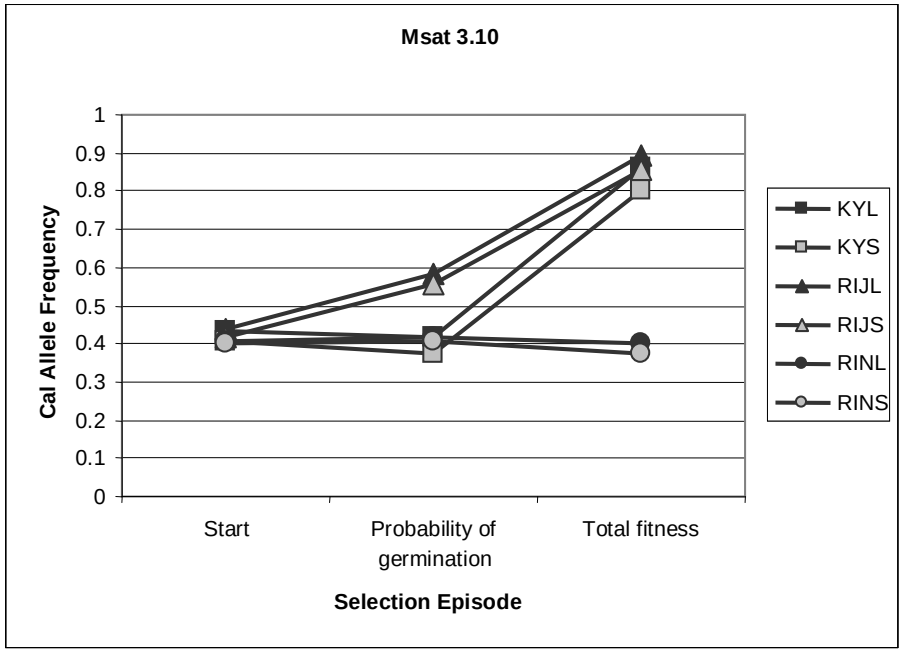
Field germination

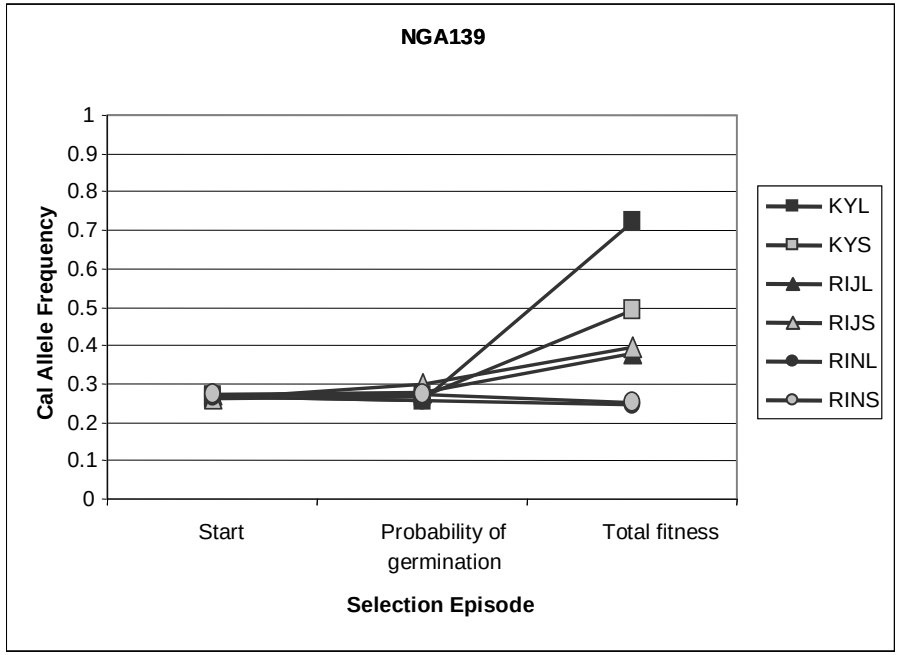


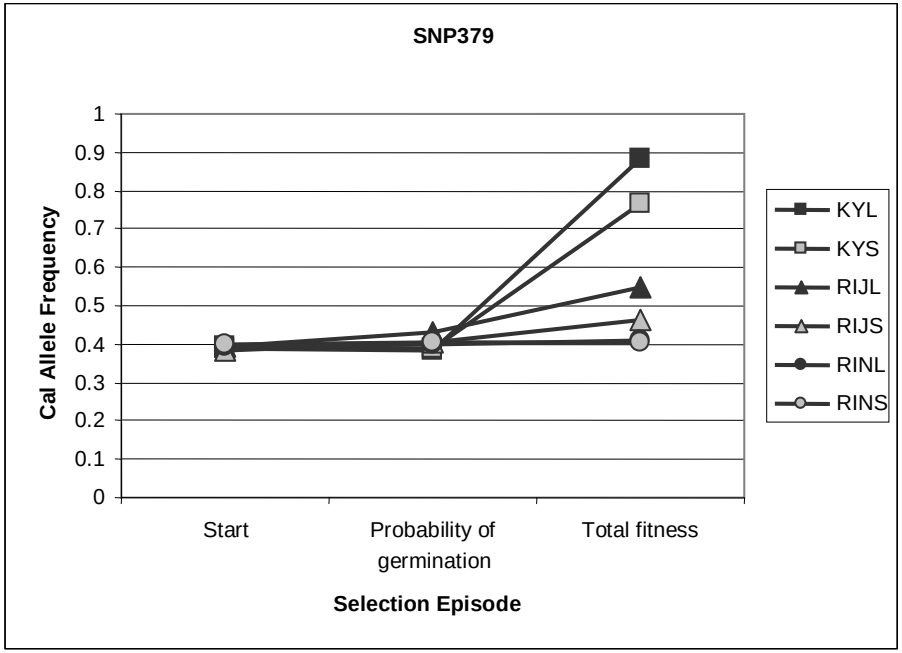
Lab germination




Msat 3.1
Cal Allele Frequency
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Start
Probability of germination
Total fitness
Selection Episode
KYL
KYS
RIJL
RIJS
RINL
RINS


Msat 3.10
Cal Allele Frequency
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Start
Probability of germination
Total fitness
Selection Episode
KYL
KYS
RIJL
RIJS
RINL
RINS


NGA139
Cal Allele Frequency
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Start
Probability of germination
Total fitness
Selection Episode
KYL
KYS
RIJL
RIJS
RINL
RINS


SNP379
Cal Allele Frequency
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Start
Probability of germination
Total fitness
Selection Episode
KYL
KYS
RIJL
RIJS
RINL
RINS