Genetics of microenvironmental canalization in *Arabidopsis thaliana*

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Canalization is a fundamental feature of many developmental systems, yet the genetic basis for this property remains elusive. We examine the genetic basis of microenvironmental canalization in the model plant *Arabidopsis thaliana*, focusing on differential developmental stability between genotypes in one fitness and four quantitative morphological traits. We measured developmental stability in genetically identical replicates of two populations of recombinant inbred (RI) lines and one population of geographically widespread accessions of *A. thaliana* grown in two different photoperiod-controlled environments. We were able to map quantitative trait loci associated with developmental stability. We also identified a candidate gene, *ERECTA*, that may contribute to microenvironmental canalization in rosette leaf number under long-day photoperiods, and analysis of mutant lines indicates that the *er*-105 allele results in increased canalization for this trait. *ERECTA*, which encodes a signaling protein, appears to act as an ecological amplifier by transducing developmental noise (e.g., microenvironmental variation) into phenotypic differentiation. We also measured genotypic selection on four plant architecture traits and find evidence for selection for both increased and decreased canalization at various traits.

developmental noise | developmental stability | *ERECTA* | phenotypic plasticity | quantitative trait locus mapping

Genetically identical organisms, even when raised in indistinguishable environments, rarely look exactly identical; even zygotic twins have different fingerprints. This phenomenon is, in part, due to the presence of developmental noise, which includes stochastic fluctuations in a developing system (e.g., minute, random differences between individuals in the timing of molecular interactions) or microenvironmental variation experienced by different individuals that otherwise inhabit the same macroenvironment (1). The extent to which developmental noise can perturb trait phenotypes is counterbalanced by the ability of organisms to buffer their developmental processes against environmental fluctuations through the process of environmental canalization (2–4). The concept of canalization rose to prominence over half a century ago when Waddington (5) and Schmalhausen (6) drew attention to developmental stability as a major feature of the ontogenetic process. Their pioneering studies have stimulated interest in the understanding of how developmental systems evolve and the roles of epistasis and cryptic genetic variation in the maintenance of genetic variation for phenotypes.

There has been intense interest in understanding the genetic basis for canalization and determining the evolutionary forces that may act to reduce phenotypic variance of traits (3, 7–9). Several measures of microenvironmental canalization, such as fluctuating asymmetry or developmental stability, have been shown to be heritable for quantitative traits (10), suggesting that selection can shape levels and patterns of environmental canalization. Moreover, population genetic models of canalization demonstrate that alleles selected for environmental canalization may also be responsible for genetic canalization, which buffers against mutational variation (7, 9).

Understanding the genetic architecture of canalization, particularly for quantitative traits, can clarify whether canalization for a particular trait evolves independently of the target phenotype, and can also facilitate the isolation of genes involved with canalization and help determine the molecular mechanisms that underlie developmental stability. Several studies suggest that Hsp90 is a candidate gene for genetic canalization in both *Drosophila* (11, 12) and *Arabidopsis* (13), in which this gene buffers qualitative variation in morphology. Hsp90 does not consistently account, however, for environmental canalization, nor for quantitative variation (12, 14–16), and it is likely that other genes contribute to the canalization of organismal development.

Studies of canalization have focused largely on animal systems, and relatively little is known about its role in plants (but see refs. 13 and 17–19 for examples on qualitative traits). Given the sessile nature of plants, their development and physiology are highly sensitive to environmental signals and may thus fundamentally differ from animals in the extent of microenvironmental canalization. We examine the genetics of microenvironmental canalization in the model plant *Arabidopsis thaliana*, focusing on differential developmental stability between genotypes in one fitness and four quantitative morphological traits. Plant systems, particularly selfing species such as *A. thaliana*, possess several intrinsic advantages in the study of canalization mechanisms, as their sedentary nature and the availability of inbred lines allow for genotypically replicated individuals to be grown in a randomized design in the same environment. Exploiting these characteristics of *A. thaliana*, we were able to dissect the genetic architecture of microenvironmental canalization and identify loci that may modulate phenotypic variability in various morphological and fitness-related traits. In addition, we were able to examine the nature of selection on canalization levels in two controlled photoperiods known to affect phenotypes.

Results and Discussion

Microenvironmental Sensitivity Varies Among Lines and Traits. To measure the degree of microenvironmental canalization, we estimated within-genotype variation on four quantitative plant morphological traits (rosette leaf number at time of bolting,

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Abbreviations: *ER*, *ERECTA*; *LS*, Levene's statistic; MCIM, multitrait composite interval mapping; QTL, quantitative trait locus; RI, recombinant inbred.

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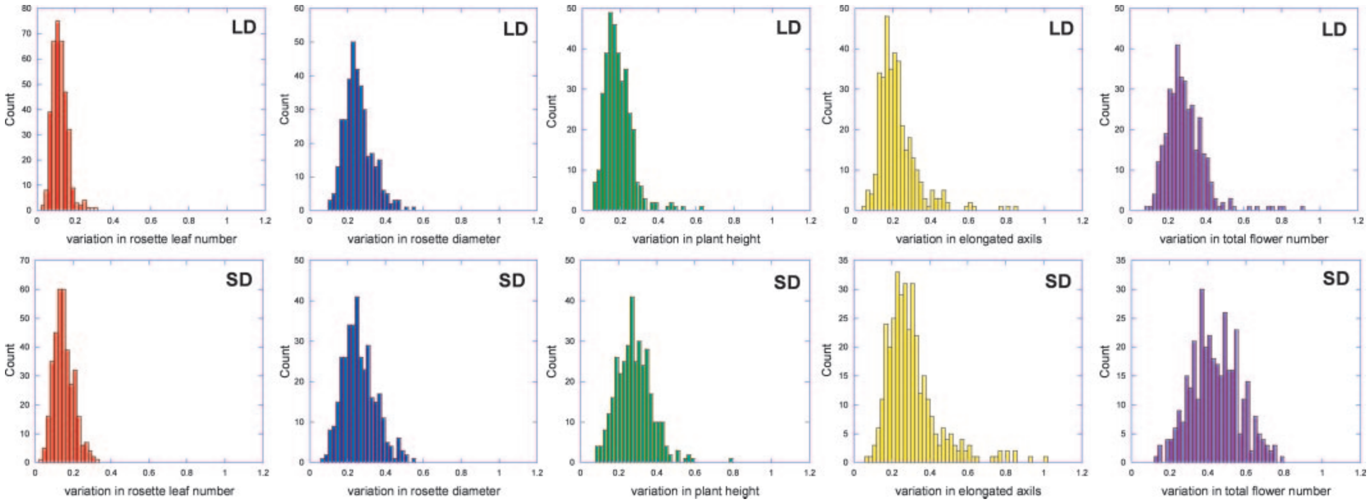

Fig. 1. Frequency distributions of variation (as measured by *LS*) in five traits measured in two environments, long days (LD) (*Upper*) and short days (SD) (*Lower*), for 360 *A. thaliana* accessions.

plant height, rosette diameter, number of elongated axillary branches) and one fitness trait (total flower number per plant) in two recombinant inbred (RI) mapping populations and one species-wide sample of accessions of *A. thaliana* (20, 21). Replicated *A. thaliana* lines were grown in environmentally controlled growth chambers, providing a common macroenvironment and ensuring that variation in phenotypes between replicates arises from developmental noise, most likely microenvironmental differentiation among replicated individuals. Given that the plants were grown in a spatially randomized experiment that included regular shuffling of growing locations within the chamber, we assume that each genotype in the experiments experiences a similar range of microenvironments and that differences in trait canalization between lines are thus genetic in origin. Many of the traits measured were highly correlated [supporting information (SI) Tables 3–5], although the relationship between line means and measured variation is weak (SI Fig. 3).

Large differences in the levels of microenvironmental canalization, as measured by Levene's statistic (*LS*), were observed among traits (Fig. 1), although the distributions did not vary substantially among the three populations examined. Broad-sense heritabilities (H^2) for canalization were estimated and ranged from 0–0.37 (Table 1), suggesting that the degree of variability for most trait phenotypes has a genetic basis. Consistent with previous reports on other organisms (10), however, these estimates are an order of magnitude lower than heritabilities for trait size (see Table 1).

For the sample of accessions, canalization of most traits showed significant environmental effects with respect to the photoperiod (ANOVA, $P < 0.0001$ for all traits except rosette diameter). For these significant traits, we observed that in the short-day conditions, the interindividual variation observed within in each genotype was greater, as is evident from the broader distributions in *LS* measures in short days relative to long days (Fig. 1).

Selection on Canalization. It is often assumed that canalization is selectively advantageous as it helps individuals reduce the risk of drift from the phenotypic optimum, although empirical support for this claim is weak (22). A negative relationship between measures of fitness and developmental *in*stability has been suggested (23), although this work has received strong criticism (22, 24). Other studies have found, at best, a weak relationship between developmental stability and fitness (25–29).

To account for trait correlations, we used a multivariate

Table 1. Broad-sense heritabilities (H^2) for canalization and trait means

Traits	Col × *Ler* (long days)	Col × *Ler* (short days)	Cvi × *Ler* (long days)	Cvi × *Ler* (short days)	Accessions (long days)	Accessions (short days)
RLN	**0.400**	**0.336**	**0.535**	**0.698**	**0.664**	**0.486**
RLN-*LS*	**0.068**	0.000	**0.035**	**0.073**	**0.058**	**0.062**
RD	**0.045**	**0.066**	**0.131**	**0.068**	**0.309**	**0.153**
RD-*LS*	0.010	**0.026**	**0.016**	0.012	**0.042**	**0.056**
PH	**0.529**	**0.577**	**0.563**	**0.493**	**0.454**	**0.281**
PH-*LS*	**0.036**	**0.053**	**0.030**	**0.033**	**0.101**	**0.087**
EA	**0.117**	**0.224**	**0.138**	**0.335**	**0.410**	**0.162**
EA-*LS*	**0.139**	**0.186**	0.000	**0.366**	**0.163**	**0.179**
TF	**0.040**	**0.133**	**0.238**	**0.118**	**0.293**	**0.076**
TF-*LS*	0.012	0.004	0.000	**0.145**	**0.081**	0.014

H^2 was estimated according to the formula $V_G/(V_G + V_E)$. For each trait, total phenotypic variance was partitioned into sources due to genotype (among-line variance, V_G) and residual (error) variance (V_E) by using restricted maximum likelihood (*REML*) with a generalized linear mixed model (*GLMM*). H^2 values were estimated in two sets of RI lines (Col × *Ler* and Cvi × *Ler* and in a set of 360 *A. thaliana* accessions. RLN, rosette leaf number; RD, rosette diameter; PH, plant height; EA, number of elongated axils; TF, total number of flowers produced. *LS* is a measure of variation for each trait. Values in bold indicate H^2 estimates that are significant at $P < 0.05$.

Table 2. Genotypic selection under long- and short-day environments

		Mean	Variation
Trait	N	β	β
RLN_LD	360	−0.0022	0.0017
RD_LD	360	0.26***	−0.21
PH_LD	360	0.020***	0.070
EA_LD	360	0.059***	−0.38**
RLN_SD	360	0.0027	−0.36
RD_SD	360	0.14***	0.64***
PH_SD	360	0.023***	0.34*
EA_SD	360	0.095***	−0.34***

Unstandardized multivariate genotypic selection gradients (β) on mean trait values and variation (as measured by *LS*) in 360 accessions of *A. thaliana* measured in both long-day (LD) and short-day (SD) environments. Each environment was treated separately, but all traits (means and variances) were included in a single multivariate analysis. RLN, rosette leaf number; RD, rosette diameter; PH, plant height; EA, number of elongated axils. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

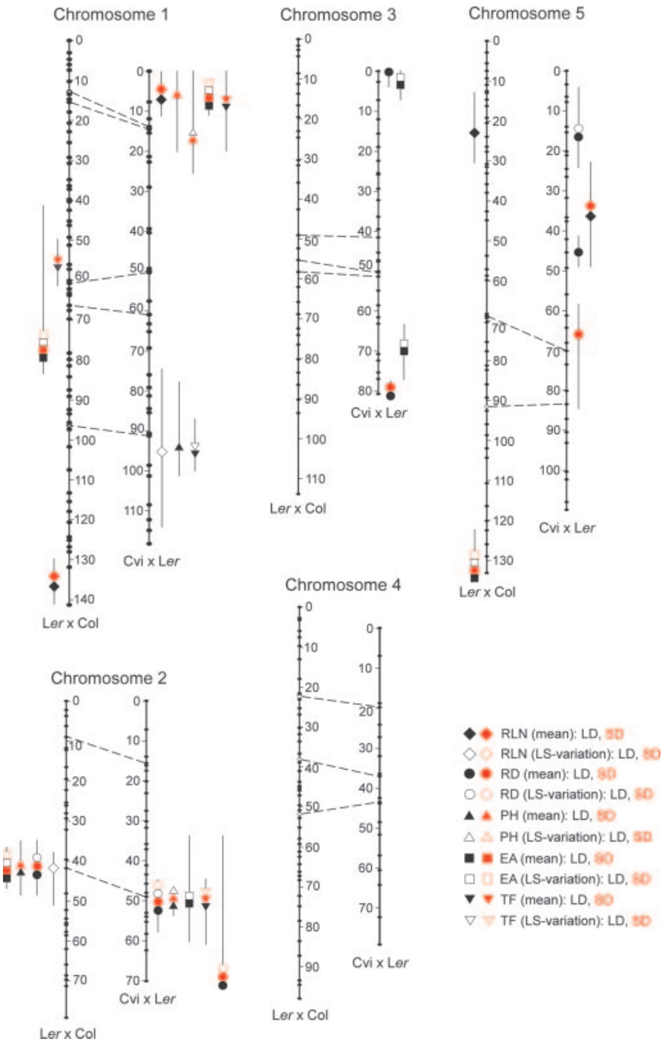

Fig. 2. *A. thaliana* genetic linkage maps constructed from the Col × Ler (left chromosomes) and Cvi × Ler (right chromosomes) RI lines [from Ungerer *et al.* (20)] with QTL positions. QTL positions were based on separate MCIM analyses performed on each of five traits: rosette leaf number (RLN), rosette diameter (RD), plant height (PH), total number of elongated axils (EA), and total flower number (TF). For each trait, a separate MCIM analysis was done that included measures of the means and variances (as measured by *LS*) from two environments, long days (LD) and short days (SD) for that particular trait. Filled symbols represent QTL individual effects of the mean; open symbols represent QTL effects on the variance. Black symbols denote measures taken in LD, whereas orange symbols represent measures taken under SD. Vertical lines associated with each QTL indicate 2-LOD support limits. Markers connected by dashed lines were mapped in both sets of RI lines. Map length units are in centimorgans.

genotypic selection analysis on our species-wide sample of accessions to examine whether there was evidence for selection on canalization for four morphological traits, using total flower number as a component of fitness. By using a genotypic rather than phenotypic selection approach, we have accounted for microenvironmental correlations (30) to provide a more direct measure of the relationship between traits and fitness. We observed significant selection on canalization for several traits under long- and short-day environments (Table 2). A significant relationship was detected between fitness and increased canalization for the number of elongated axillary branches in both environments (Table 2), although selection favors decreased canalization in both height and rosette diameter under short days. These results suggest trait-specific selection for both decreased and increased phenotypic variance. Selection for canalization could imply that robust developmental programming is associated with increased fitness, whereas the reverse may arise from selection for phenotypic plasticity to microenvironmental differences. Although we measured fitness and microenvironmental canalization in two controlled environments, it would be intriguing to pursue a similar experiment in the wild where microenvironmental differences are certainly magnified.

Mapping Genes for Trait Canalization. Because the amount of interindividual variation within a given genotype is heritable, we should be able to map the underlying genomic regions. We used a quantitative trait locus (QTL) mapping analysis of the variation on our five quantitative traits in two photoperiod environments in both the Col × L*er* and Cvi × L*er* RI mapping populations. A multivariate QTL analysis, using multitrait composite interval mapping (MCIM) (31), was run separately for each of the five traits, with both trait means and *LS* measured under long- and short-day conditions included in a single analysis. The multivariate method tests for the likelihood of a QTL affecting one or more of the four measures (mean and *LS* in long- and short-day environments) for a trait at a particular genomic position, by accounting for the correlational structure of the data. Because the means and variances for a single trait may be highly correlated and the same trait measured in two environments may not be independent, this multivariate approach allows us to tease apart affects due to each of these measures separately.

In total, we mapped 30 QTLs and 66 individual QTL effects for both the trait means and microenvironmental canalization. Three QTLs that were slightly below the experiment-wide threshold of significance when mapped jointly were also included, because each of these QTLs was highly significant in single-trait analyses. In addition, two regions contained overlapping QTLs for the same traits, which we accepted because they had distinct effects on the trait measures. Detailed map positions and additive effects for all QTLs are included in SI Tables 6 and 7.

There were 22 QTL individual effects controlling microenvironmental canalization among the five traits in both mapping populations (Fig. 2). Many of these QTLs mapped to the same position for a particular trait mean, indicating that the underlying loci affected both the main phenotype as well as its level of canalization. We also observe three QTLs that affect only the level of phenotypic variability and not the phenotypic mean of the trait (Fig. 2 and SI Tables 6 and 7), indicating that these genomic regions harbor genes that underlie canalization of a trait but not its main effect. Two of these QTLs affect canalization in rosette leaf number in long days: one on chromosome 2 in the Col × L*er* population and the other in chromosome 1 in the Cvi × L*er* population. A third QTL on chromosome 1 in the Cvi × L*er* mapping population affects canalization in plant height in long days. This finding suggests that a class of genes may function in part to modulate microenvironmental canalization in *A. thaliana* quantitative traits.

***erecta* Mutant Alleles Result in Greater Canalization.** Nearly half of the QTLs with individual effects for canalization (10 of 22) mapped to the same position, tightly linked to the *ERECTA* (*ER*) locus on chromosome 2. This region also contained one of the three QTLs that uniquely affected canalization, but not the phenotypic mean for rosette leaf number under long-day conditions. The L*er* line, which is one of the progenitors of both our mapping populations, is known to harbor a laboratory-created mutant allele of *ER* (32). Although L*er* is traditionally referred to as wild type and is commonly used in Arabidopsis genetic research due to its compact plant size, here we refer only to plants with functional *ER* alleles as wild type. We additionally tested four different *ER* mutants (*er*-2, *er*-111, *er*-116, and *er*-105) to see whether they also showed differences in the levels of microenvironmental canalization between mutant and wild-type alleles. The *er*-105 mutant was generated from fast-neutron irradiation of Col-0, which resulted in a ≈4-kb insertion near the start site of the *ER* gene, and a Northern blot analysis demonstrated no detectable RNA for this allele (33). The *er*-2 allele contains a frameshift mutation, *er*-111 contains a nonsense mutation, and *er*-116 allele has an in-frame deletion. The L*er* plant (*er*-1 allele, induced by irradiation of the Columbia accession) used in the RI line crosses contains a single nucleotide change that results in the replacement of an amino acid that is highly conserved among *ER*-homologous genes. Of the five *er* mutants used in this experiment, *er*-105 is the only clear null allele and it has a consistently strong phenotypic effect (33).

Using a multivariate ANOVA (MANOVA) to compare mutants from their respective congenics, two alleles (*er*-105: $F_{5,26} = 3.62$, $P < 0.05$; and *er*-2; $F_{5,23} = 2.32$, $P < 0.10$) showed some differences in the patterns of (co)variation from their wild types. Interestingly, these alleles only showed an effect in the long-day environment. With respect to the individual traits, only *LS* for rosette leaf number under long days was significantly different between mutant and control lines. Moreover, phenotypic variability in this trait was reduced solely in the *er-105* mutant relative to its isogenic wild-type line ($F_{1,30} = 16.52$, $P < 0.001$). Although this mutant had a strong effect on interindividual variation for rosette leaf number, there was no effect on mean rosette leaf number between mutant and control lines (data not shown), which agrees with the QTL mapping results. These results indicate that, at least for this trait, *ER* represents a gene that affects microenvironmental canalization of a trait but not the trait itself. Other mutant alleles, however, did not appear to affect canalization levels, which suggests that the ability to modulate developmental buffering is allele-specific and also possibly dependent on genetic background (see discussion below). Another possibility is that a gene linked to *ER*, but not *ER* itself, is responsible for microenvironmental canalization.

The Evolutionary and Quantitative Genetics of Canalization in *A. thaliana*. Plants may fundamentally differ from animal systems in the levels and patterns of canalization, because their sessile nature and continuous development make the former particularly prone to microenvironmental variation that can impact ontogenetic trajectories. In addition, homologous structures in a single plant may experience multiple microenvironments throughout its lifetime, which could shape the genetic or environmental control of canalization in distinct ways from animal systems.

In the model plant *A. thaliana*, we find variation in the levels of microenvironmental canalization in four morphological and one fitness trait among natural accessions. The heritabilities of trait canalization levels are predictably lower than those observed for the main trait phenotypes (10), although our results indicate clear genetic differentiation among genotypes in their ability to modulate the degree of developmental buffering.

It is typically assumed that increased canalization is evolutionarily advantageous and models tend to predict phenotypic buffering (1, 5, 7), although there is little direct evidence to support or refute this claim. It is possible that in some circumstances, particularly in plants, selection would favor reduced canalization and increased sensitivity to microenvironmental variation so that organisms maintain sufficient plasticity to respond to this variation. We observe differing patterns of selection among plant architecture traits, including both selection for either increased or decreased canalization among particular traits. One should be cautious, however, in overinterpreting these results, which were based on growth chamber conditions in which plants experienced differences in photoperiod. It is probable that patterns of selection will differ in the complex, fluctuating natural environments of *A. thaliana*.

One can use QTL mapping to identify genes that modulate the extent of canalization, and we find that in the majority of cases, there are QTLs that affect both the main trait phenotype as well as its canalization levels. There are three possible reasons for the correlations in QTLs between canalization levels and the main trait phenotypes: (*i*) there could be multiple genes within a single QTL region, some of which affect the main trait and others that affect the canalization levels; (*ii*) these QTLs may represent a statistical artifact, because calculation of *LS* includes the main trait phenotype; or (*iii*) these patterns confirm the widespread observation of increased phenotypic variance associated with mutant traits, which was the initial impetus for Waddington (5) to develop the concept of canalization. Although all of these explanations may underlie the observed QTL correlations, by using a multitrait mapping approach that incorporates the correlational structure of the data, the possibility of spurious statistical artifacts is greatly reduced. Indeed, we do find at least three cases of QTLs that affect canalization levels but not the main trait phenotypes. This suggests that genes exist that control the degree of developmental buffering without affecting the main phenotype of the specific trait.

Several of the QTLs, including one that solely affects levels of canalization for a trait (rosette leaf number under long days), map to a region that is known in our mapping lines to harbor a mutation at the *ER* gene. *ER* is a member of the leucine-rich repeat/receptor-like protein kinase (LRR-RLK) gene family (33) and plays a role in cell-cell signaling by phosphorylating serine/threonine residues (34). The gene is expressed at high levels in the shoot apical meristem of bolting plants and in organ primordia, at low levels in mature organs and leaves, and not at all in roots (35). *ER* is pleiotropic and has been implicated in several important functions in *Arabidopsis*, including leaf, flower, and fruit development (33, 36, 37), resistance to pathogens (38, 39), and regulation of plant transpiration (40).

Using *er* mutant lines, we observe that one strong allele (*er*-105) does affect the level of canalization of rosette leaf number, but not the total leaf number, as indicated by the QTL mapping analysis. Other *er* mutant alleles did not show any significant effect on canalization; this suggests that either an alternate gene linked to *ER* is responsible for the effect or the presence of allele specificity in the modulation of developmental buffering. Variation in allelic effects of *er* mutants, however, is common and highly dependent on the phenotype measured, although *er*-105 has a consistently strong phenotypic effect (33, 34, 39, 40). Moreover, the function of *ER* has also been shown to be dependent on genetic background (40). Our finding of differential effects of *er* mutant allele on canalization may also explain why the mapping of the long-day rosette leaf number canalization QTL to the *er* position is observed in the Col × L*er* but not the Cvi × L*er* population.

Interestingly, the effect of the *er-105* mutant allele, as well as L*er erecta* allele in the Col × L*er* population, is to increase canalization in rosette leaf number during long days. The consistency of our QTL and mutant allele analyses suggests that wild-type (functional) *ER* is a strong candidate for a gene that promotes (rather than buffers) phenotypic variance. This pattern is in contrast to most investigations, which have focused on genes such as Hsp90 that promote canalization and are described as evolutionary capacitors (11–13). Several other studies, however, have also demonstrated that mutants sometimes have increased environmental canalization relative to their wild-type counterparts (41, 42). One explanation is that the *ER* wild-type allele actually impedes canalization via a tradeoff with main trait expression of rosette leaf number. Another possibility is that *ER* may be an example of an ecological amplifier, by transducing microenvironmental variation into minute phenotypic differences among individuals. *ER* may serve as a signaling gene associated with microenvironmental sensitivity of rosette leaf number and that specific mutant *er* alleles increase environmental canalization by abolishing this signaling function.

Canalization is a fundamental feature of many developmental systems (5, 6), and there has been concerted effort to understand the genetic basis of developmental buffering as well as the evolutionary forces that shape the levels of phenotypic stability (1, 3, 7–9). Our study has established the variability in microenvironmental canalization levels in *A. thaliana*, shown differential selective forces that act on this trait and identified several QTLs that act on levels of developmental buffering. We now have an opportunity to continue to dissect the molecular genetic mechanisms that underlie microenvironmental canalization and to begin to understand how canalization evolves to modulate organismal developmental patterns.

Materials and Methods

Measuring Variation in *A. thaliana* Populations. Data were previously collected on five traits (rosette leaf number at time of bolting, plant height, rosette diameter, number of elongated axillary branches, total flower number per plant) in the Col × L*er* and Cvi × L*er* RI lines of *A. thaliana* (20). Fifteen replicates each of 96 Col × L*er* and 160 Cvi × L*er* RI lines were grown under both long-day (14 h light) and short-day (10 h light) conditions at the Southeastern Plant Environmental Laboratory (Raleigh, NC). In addition, 10 replicates each of 360 accessions from throughout the natural range of *A. thaliana* were grown in the same conditions in a separate experiment. Seeds were not vernalized, which could affect levels of microenvironmental canalization differentially among accessions. Detailed plant growth conditions as well as the randomized experimental design for phenotypic analysis, are discussed elsewhere (20, 21).

As a measure of microenvironmental canalization, we estimated within-genotype phenotypic variability by calculating two forms of the *LS* (42–44) for each individual line:

$$LS = \frac{|X_{ij} - \bar{X}_j|}{\bar{X}_j}$$

and

$$LS = |\log(x)ij - \text{median}(\log(Xj))|,$$

where *i* is each individual from line *j*. *LS* was averaged for each line as a standardized measure of variation for each trait, with the log-transformed *LS* based on the median being particularly robust to covariation with trait size (43–45). Because many individuals had zero values for number of elongated axils, we could not calculate the median form of *LS* for this trait. All analyses reported here use the mean form of *LS* to maintain consistency among traits. Measures in the two environments were treated separately. We estimated broad-sense heritabilities as described in ref. 20.

Estimating Genotypic Selection. Relative fitness was estimated separately for each environment by using the total flower number as the measured fitness component. Line means were used for each of the 360 *A. thaliana* accessions to estimate the effects of genotypic selection, thereby reducing biases due to environmental covariances between traits and fitness (30). Relative fitness was regressed on the unstandardized *LS* for all four quantitative traits simultaneously (30, 46) to estimate linear multivariate genotypic selection gradients, β, to account for correlations among traits.

QTL Mapping of Canalization Genes. We used the previously constructed *A. thaliana* linkage map (20) generated by Mapmaker/EXP 3.0 (47) as a framework for mapping canalization QTLs in each of the two RI populations. Each of the five traits (see above) was initially run separately for both means and *LS* by using composite interval mapping (CIM) (48, 49) as in Ungerer *et al.* (20). Because means and variances for a single trait were highly correlated, we also mapped QTLs by MCIM (31) using QTL Cartographer for Windows, version 2.5 (50, 51). In these analyses, both means and *LS* measured under long- and short-day conditions for each particular trait were combined in a single analysis. Experiment-wise significance levels ($\alpha = 0.05$) in all analyses were determined by permuting the phenotypes against the genotypes 1,000 times so that the correlations between traits were maintained (52).

To determine whether QTLs detected by MCIM had pleiotropic effects on the trait measures in each analysis, individual MCIM likelihood ratio test values were examined for each position where joint mapping indicated the presence of a QTL (31). Pleiotropy was indicated by the rejection of the null hypothesis of no more than one trait having a Likelihood ratio (LR) test value greater than a significance threshold value of 5.99 ($X^2_{0.05,2}$) at a particular QTL position as determined by the model parameters estimated jointly by MCIM.

Mutant Analyses. Fifteen replicates each of four *er* mutants (*er*-2, *er*-111, *er*-116, and *er*-105) and their wild-type progenitor accessions as control lines were planted in a completely randomized design in both long- and short-day phytotron conditions as described in ref. 20. For each plant, the same five traits were measured as previously described. *LS*'s were estimated for each individual and averaged per mutant or control line for all five traits in both environments. The effect of each mutation was compared with its otherwise identical congenic by using a multivariate ANOVA (MANOVA) under each environment (short and long day). Given that the distributions of *LS* is generally far from (multivariate) normal (44), empirical *P* values were estimated via 1,000 permutations of the data by using a custom script in R. For alleles for which the MANOVA was consistent with a significant effect, univariate models were examined to determine whether variation for particular traits was largely responsible.

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