
Article:
Rubin, Matthew, Brock, Marcus, Baker, Robert L. et al. (4 more authors) (2018) Circadian rhythms are associated with shoot architecture in natural settings. New Phytologist. pp. 246-258. ISSN 1469-8137

https://doi.org/10.1111/nph.15162

Circadian rhythms are associated with shoot architecture in multi-year field studies of *Arabidopsis thaliana*

Matthew J. Rubin[1,2,*], Marcus T. Brock[1] , Robert L. Baker[1†], Stephanie Wilcox[1], Kyle Anderson[1], Seth J. Davis[3,4] and Cynthia Weinig[1,2,5]

[1]Department of Botany, University of Wyoming, Laramie, WY 82071, USA.

[2]Program in Ecology, University of Wyoming, Laramie, WY 82071, USA.

[3]Department of Plant Developmental Biology, Max Planck Institute for Plant Breeding Research, Cologne 50829, Germany.

[4]Department of Biology, University of York, Heslington, York, YO10 5DD, UK.

[5]Department of Molecular Biology, University of Wyoming, Laramie, WY 82071, USA.

*Current address: Biology Department, Syracuse University, Syracuse, NY 13244, USA; Author for correspondence: mjrub100@syr.edu

† Current address: Biology Department, Miami University, Oxford OH 45056, USA

Summary

- Circadian rhythms are key regulators of diverse biological processes under controlled settings. Yet, the phenotypic and fitness consequences of quantitative variation in circadian rhythms remain largely unexplored in the field. As for other pathways, phenotypic characterization of circadian outputs in the field may reveal novel clock functions.
- Across multiple growing seasons, we test for associations between clock variation and flowering phenology, plant size, shoot architecture, and fruit set in clock mutants and segregating progenies of *Arabidopsis thaliana* expressing quantitative variation in circadian rhythms.
- Using structural equation modeling, we find that genotypic variation in circadian rhythms within a growing season is associated directly with branching, which in turn affects fruit production. Consistent with direct associations between the clock and branching in segregating progenies, cauline branch number is lower and rosette branch number higher in a short-period mutant relative to wild-type and long-period genotypes, independent of flowering time. Differences in branching arise from variation in meristem fate as well as leaf production rate prior to flowering and attendant increases in meristem number.
- Our results suggest that clock variation directly affects shoot architecture in the field, suggesting a novel clock function and means by which the clock affects performance.

Plants adjust their body plan, or architecture, over the course of their lifecycle in response to local environmental conditions. Architectural adjustment and the regulation of shoot production in response to the environment are feasible because post-embryonic development in plants proceeds from totipotent meristems (Wolff, 1774; Esau, 1953; Steeves & Sussex, 1989; reviewed in Tooke & Battey, 2003; Leyser, 2009). The primary shoot apical meristem arises during embryogenesis, and differentiates a series of modules later in development consisting of a section of stem, a leaf, and a leaf axillary meristem. The complexity of the mature shoot system depends on the number of axillary meristems as well as the fate of those meristems. Axillary meristems may differentiate to produce a new, indeterminate vegetative axis (a branch), a single determinant, reproductive axis (a flower) or remain quiescent. Early commitment of the shoot apical meristem to reproduction limits axillary meristem production and can lead to a simple architecture consisting of single primary shoot, whereas later reproduction and attendant increases in meristem number may enable greater architectural complexity (Watson, 1984; Bazzaz *et al.*, 1987; Geber, 1990; Huber *et al.*, 1999; Bonser & Aarssen, 2001; Bonser & Aarssen, 2003; Bonser & Aarssen, 2006).

The environmental and genetic influences on meristem number and fate are likely complex. Several developmental pathways have been identified that enable environmental sensing and regulate architectural phenotypes. For instance, phytochromes mediate neighbor perception, and phytochrome mutants differ from wild-type plants in stem elongation as well as branching patterns (Pierik & de Wit, 2013). Naturally segregating variation at phytochrome loci, signal transduction loci, and interacting factors are likewise associated with shifts in plant architecture (Borevitz *et al.*, 2002; Weinig *et al.*, 2006; Brock *et al.*, 2010; Finlayson *et al.*, 2010; de Montaigu *et al.*, 2015). The circadian clock is also a candidate pathway for directly or

indirectly regulating architectural responses to cues of growing season duration. Circadian clocks consist of three major components: an input pathway that detects environmental conditions, an oscillator that maintains the rhythm set by the environmental inputs, and an output pathway that coordinates downstream processes (McClung, 2006; Anwer & Davis, 2013; Hsu & Harmer, 2014). The circadian clock influences physiological, morphological, and life-history characters in plants under controlled settings, and is anticipated to enable synchronization of biological activities under natural diurnal cycles in the field (Kreps & Kay, 1997; Samach & Coupland, 2000; Dodd *et al.*, 2005; Izawa *et al.*, 2011; Rubin *et al.*, 2017). By affecting flowering time, the circadian clock could also determine axillary meristem numbers and fate, and thus indirectly affect the mature shoot phenotype. The clock also affects photosynthesis and vegetative size (Dodd *et al.*, 2005; Graf *et al.*, 2010) and hence the pool of resources available for shoot production, providing another means by which the clock could affect shoot architecture. The adaptive significance of segregating quantitative variation in the circadian clock remains largely untested in nature, although recent work suggests that the circadian clock is highly responsive to seasonally variable inputs and determines both survival and fecundity in the field (Rubin *et al.*, 2017). Whether the clock either indirectly regulates the shoot system or more directly regulates meristem number or fate remains likewise unknown.

The fitness consequences of different plant architectures vary depending on ambient conditions. For example, a highly elongated primary shoot axis with few branches is adaptive in settings with high neighbor density, while a more complex branched architecture is adaptive in low-density settings (Schmitt *et al.*, 1995; Dudley & Schmitt, 1996; Valladares & Pearcy, 1998; Dorn *et al.*, 2000; Weinig, 2000). A simple architecture consisting of a single primary shoot axis may also be advantageous in short growing seasons, because rapid commitment of meristems to

floral fates helps ensure fruit production prior to senescence (Duffy *et al.*, 1999; Bonser & Aarssen, 2003; Bonser & Aarssen, 2006; Baker & Diggle, 2011; Fournier-Level *et al.*, 2013; Remington *et al.*, 2013). Alternatively, a more complex shoot system with a greater number of branches and additional meristems for eventual fruit production leads to higher fitness in settings with longer growing seasons (Watson, 1984; Geber, 1990; Prusinkiewicz *et al.*, 2007; Friedman *et al.*, 2015). The potential expression of different shoot architectures in response to environmental variation depends on levels of segregating variation and the degree of plasticity for meristem number and fate, both of which may vary among populations (Bonser & Aarssen, 1996; Bonser & Aarssen, 2003; Baker *et al.*, 2014).

Here, we test for potential associations between the circadian clock and plant size, phenology, and branching patterns, and their contribution to fitness in the field in the annual plant species *Arabidopsis thaliana*. We address the following questions 1) does structural equation modeling suggest either direct or indirect associations between the circadian clock and plant architecture within a growing season, 2) do mutants with altered clock function differ from wild-type plants in size, phenology, or plant architecture, 3) do large differences in phenology across growing seasons alter bivariate associations between the clock and plant architecture, and 4) does branch number directly or indirectly affect overall plant fitness?

Methods

Genetic Lines: Arabidopsis thaliana (Brassicaceae) occurs in discrete and genetically differentiated populations in Europe and Asia as well as in recently naturalized populations in North America. *A. thaliana* grows vegetatively as a rosette and has one axillary meristem per

leaf axil (Grbić & Bleecker, 2000). After the main axis transitions to flowering, rosette leaf production ceases; therefore, flowering time affects the pool of available rosette axillary meristems. Once the main axis has bolted, it develops as a compound raceme. Leaves on the inflorescence are referred to as cauline leaves and have a single axillary meristem (Grbić & Bleecker, 2000) that may differentiate into a secondary racemose inflorescence (or paraclade; hereafter "cauline branch"), which may bear additional higher order cauline branches (Weberling, 1989). Flowers and fruits develop from axillary meristems that have subsumed their presumptive subtending leaves (Leyser & Day, 2003); they therefore appear to lack bracts. In *A. thaliana,* all branches develop after the transition to flowering. Typically cauline axillary meristems grow out into branches earlier in ontogeny than branches that originate from rosette axillary meristems (Hempel & Feldman, 1994). Compared to many other flowering plants, the fate of axillary meristems that are subtended by leaves appears restricted: they may remain quiescent or develop into branches, but do not develop into flowers.

We present results from three sets of RILs that contain the reporter gene *LUCIFERASE (LUC)*, the gene responsible for bioluminescence in the presence of the substrate luciferin in fireflies (*Photinus phralis*), linked to the native circadian gene, *COLD CIRCADIAN RHYTHM RNA BINDING 2 (CCR2)*. This reporter gene construct allows for quantification of circadian parameters (Miller *et al.* 1992), including in field-grown plants (Rubin et al. 2017). Three mapping populations were constructed by crossing natural accessions, where one parent in each cross harbored the *CCR2: LUC* reporter construct; as a result, all RILs within a set harbor the *CCR2: LUC* construct at the same genomic position (Boikoglou, 2008). Two sets of RILs were generated with Ws-2 (Russia) as the maternal parent and either C24 (Portugal; 84 RILs) or Ler (Germany; 91 RILs) as the paternal parent. The third set of RILs are the result of a cross between

Ler (Germany; 70 RILs) as the maternal parent and Ws-2 as the paternal parent. The RILs derived from reciprocal crosses of Ler to Ws-2 differ in their cytoplasmic and nuclear genotypes as a consequence of backcrossing generations (Boikoglou, 2008). The lines cannot therefore be used to assess maternal effects.

In addition to RILs, we tested the phenotypic effects of discrete clock mutations by growing clock mutants and their cognate wild-types in the field to compare phenotypes between genotypes with circadian cycles near 24 hrs *vs*. genotypes with altered cycles near 20 or 28 hrs. *timing of CAB expression1 (toc1)* mutants express a shortened clock cycle of 20 hrs under free-running conditions, while the *zeitlupe (ztl)* mutants express a 28 hr cycle under free-run. The mutant alleles used here, *toc1-1* and *ztl-2*, reside in the C24 background (Millar *et al.*, 1995; Somers *et al.*, 2000; Strayer *et al.*, 2000). We were specifically interested in testing if clock-branching associations observed in the RILs were also observed in clock mutants, a result which would be consistent with hypotheses of clock effects on branching.

Field Experiments: We planted RILs and parental genotypes in randomized blocks in three successive years at the University of Wyoming Agriculture Experimental Station Research and Extension Center Greenhouse Field Plots (Elevation: 2,226 meters; 41.32° N, 105.6° W). Specifically, on May 14th of year 1, 12 replicates of the Ws-2 × C24 RILs were planted into 5-cm diameter baskets filled with Sunshine Sungro LP-5 soil, cold-stratified for four days at 4°C, and transferred to the greenhouse to germinate. After 3 wk, plants were transplanted into twelve field blocks with 10 cm between adjacent pots. On May 7th of year 2, 14 replicates of the Ler × Ws-2 and Ws-2 × Ler RIL sets were planted, stratified, and then transplanted after 3 wk into the field. On May 14th of year 3, 14 replicates of 21 RILs from the Ler × Ws-2 set were planted using the same methodology, to validate associations in the path models identified in the year 2 plantings.

Because flowering time often varies dramatically among years, multiple plantings also enable tests of how large shifts in flowering time correlate with branching and other downstream phenotypes.

In addition to RILs, 16 replicates of the long- and short-period circadian clock mutants and their cognate wild-type genotypes were planted in years 2 and 3 in the same fashion as the RILs. Planting and phenotyping followed protocols described under APHIS BRS notifications 06-100-101n and 12-101-102n for RILs and 12-101-109n for mutants; the field sites were reviewed by APHIS personnel in 3 subsequent years, and found compliant (reports 12-037-103n and 14-091-111n).

The following traits were measured in all years: days from germination to flowering, days from germination to senescence, rosette size as estimated by the length of the longest leaf ~2.5 weeks post germination (prior to flowering), rosette leaf number at flowering, number of primary rosette branches (hereafter rosette branches), rosette axillary meristem fate (rosette branch number /rosette leaf number), cauline leaf number (on the primary axis), primary cauline branch number (hereafter cauline branches), cauline axillary meristem fate (cauline branch number/cauline leaf number), primary inflorescence fruit set (PI fruit set; number of siliques produced on the primary inflorescence, including primary cauline branches and higher order cauline branches), rosette branch fruit set (number of siliques produced on the primary and higher order rosette branches), and total fruit set (sum of PI and rosette branch fruit).

Circadian period and phase were measured for all three RIL sets at the same seasonal time as the field plantings. Seeds of each RIL were surface sterilized in 15% bleach and 0.1% Tween 20 and rinsed three times with sterile water. For each RIL set, eight replicates of each RIL were planted into white 96-well microtiter plates containing Murashige and Skoog mineral

plant growth media and sealed with clear polyolefin tape (Murashige & Skoog, 1962). The seeds were stratified for four days and germinated in a Percival PGC-9/2 growth chambers set to a 12-hour photoperiod, at 22°C and 50% relative humidity. Following germination, seedlings were moved into the field and entrained under natural conditions for 5 days. After entrainment, 20µl of a 100 mM D-luciferin monopotassium salt and 0.01% Triton X-100 solution were added to each well, and the plates were resealed and placed under a digital camera at 22°C (Hamamatsu Photonics C4742-98-24ER), which was close to the field temperature when plates were collected. Thirty-minute exposures of the seedlings were collected every hour for 3-4 days to quantify *LUC* light production. The images were analyzed using ImagePro/IandA software to determine the circadian period and phase of each RIL (Plautz *et al.*, 1997; McWatters *et al.*, 2000; Doyle *et al.*, 2002). The trait "period" estimates average cycle length, and the trait "phase" estimates the timing of peak expression. Because we were most interested in expression patterns relative to dawn rather than phase within a genotypic cycle, we did not statistically adjust phase values for genotypic period length, that is, we used sidereal phase.

We attribute differences in circadian phenotypes (period and phase) to entrainment conditions in the field for several reasons: first, using *LUC* as a proxy for the circadian clock, plants express a "memory" of entrainment akin to jetlag, in which endogenous cycles report the entraining environment for several cycles after transfer to free-running conditions (Boikoglou *et al.*, 2011). Second, we have not detected significant differences in period and phase among replicate growth-chamber experiments with similar programs (data not shown), that is, microenvironmental noise among temporal or spatial measurement blocks does not lead to differences in circadian traits.

Statistical Analyses:

Structural equation modeling

Line variance components were estimated using restricted maximum likelihood from models including RIL and block as random factors (PROC MIXED; SAS 2015). Best Linear Unbiased Predictors (BLUPs) were calculated for each RIL within each RIL set (Robinson, 1991). Standardized genotypic values were calculated from the BLUPs for each trait (mean of 0, standard deviation of 1) (Lande & Arnold, 1983; Rausher, 1992; SAS, 2015). Relative fitness was calculated by dividing each RIL estimate of fitness by the RIL population mean fitness.

We used Structural Equation Modeling (SEM) to identify direct and indirect selection on circadian rhythms, size, life-history traits, and branching among plants grown in seasonal settings using AMOS 5.0 (Arbuckle, 2006). Both circadian period and phase were included in the initial SEM, because it was not clear *a priori* which parameter would be more strongly related to the downstream traits. Size and time of flowering were included in the model, because the circadian clock is known to affect these two traits and they could mediate indirect selection on the clock. Further, size reflects carbon resources available for reproduction, which could affect either branch number or fruit set, while flowering time may affect leaf number as outlined in the Introduction and hence meristem numbers available for branching. Rosette leaf number (a proxy for meristem number) and meristem fate were excluded from the initial SEM due to their direct mathematical association with branch number (e.g., meristem fate is operationally defined as branch number / total rosette leaf number). These two traits were instead evaluated in separate bivariate tests of association, based on the preceding hypotheses of, for instance, flowering time effects on leaf number.

Replicates of each genotype were split and randomly assigned to one of two data sets, in which planting effects, environmental error during growth (effect of spatial block), and measurement error were independent. The first half of the data was used to identify the best fit model, and the second half was used to evaluate the best fit model and assign path coefficients. We first designed a null (or saturated) model, starting with all possible biologically meaningful connections among the traits for each RIL set within each season, and then permuted the model to identify the best-fit model using the Brown-Cudeck Criterion (BCC); we then evaluated the best-fit model with the second half of the data (Fig. 1A). A non-significant Root Mean Square Error of Approximation (RMSEA) and/or χ^2 test statistic indicates that the path model provides an improved fit to the data. We estimated percent variance explained (PVE) by each model. PVE was high (> 95%), because among other traits, we were interested in assessing the relative fitness contribution of fruits produced early (primary inflorescence and cauline branches) *vs*. later (rosette branches) in the season and these traits were very closely related to the response variable, relative fitness.

Inter-annual variation in flowering time in RILs

To test for inter-annual differences in the expression of circadian phenotypes, size, life-history traits, and branching in the Ler× Ws-2 RILs that were planted in multiple growing seasons, we partitioned variance among the random effects of block nested within year, RIL, and the RIL × year interaction and the fixed effect of year (PROC MIXED; SAS 2015). Significant effects of year were found on most traits including branching (see Results), and we therefore used ANCOVA to test if observed inter-annual differences in branching could be explained by

co-variation in size and flowering time, which were also included in the SEM, as well as rosette leaf number, which showed a pronounced response to year.

Phenotypic effects of clock function in circadian mutants

For clock mutant genotypes, we used ANOVA to partition variance attributable to the random effect of spatial block nested within planting year and the fixed effects of genotype, planting year, and the genotype × planting year interaction and (PROC MIXED; SAS, 2015).

Results

Structural equation modeling reveals clock and flowering time associations with branching

Genetic variation was detected for all measured traits in all three RIL sets with the exception of cauline axillary meristem fate (Tables 1, S1; Fig. 2, S1), which was invariant as RILs differentiated all or nearly all cauline meristems into branches (Fig. S2). RILs within a set showed significant variation in fitness as estimated by the number of siliques produced. (Tables 1, S1; Fig. 2, S1).

Fit statistics, both χ^2 and Root Mean Square Error of Approximation (RMSEA), for the reduced structural equation models indicate that in all three cases the model was a good fit to the data (Table S2A). Because fruit produced on different branches was closely related to our estimate of relative fitness, each model explained a minimum of 95.9 percent of the variance in relative fitness (PVE; Table S2A); excluding fruit set on the two different branch orders reduced the PVE to 65%.

Structural equation modeling revealed several associations between circadian parameters, plant architectural traits, branch fruit number and fitness (Figure 1B, S3A, S3B). In all RIL sets, circadian period and phase were positively correlated, and one of these two circadian parameters was directly related to aspects of plant architecture. Most notably, for the Ler × Ws-2 set, longer circadian period (or delayed phase in the other two RIL sets) was associated with an increase in cauline branches. Long circadian periods were also associated with a direct reduction in rosette branch number and an increase in relative fitness in the Ler × Ws-2 set. This association could have arisen from effects of the clock on rosette leaf number (and the number of axillary meristems) or meristem fate (the number of meristems differentiated into branches relative to the number of axillary meristems); circadian period was, however, not correlated with leaf number *(r* = 0.11, *P* = 0.37) but was associated with meristem fate *(r* = -0.27, *P* = 0.03), that is, long circadian cycles were associated with reduced differentiation of meristems into rosette branches.

Delayed flowering was associated with increased cauline branches in the Ler × Ws-2 and Ws-2 × C24 RIL sets, and with greater rosette branch number in all three RIL sets. This latter association was anticipated to arise from the relationship between flowering time and leaf (and hence axillary meristem) number; days to flowering was, correspondingly, strongly positively related to leaf number within each growing season and RIL set (Fig. 3A).

The number of cauline and rosette branches positively affected the number of fruit on each branch type, which is expected when the growing season is long enough to allow maturation of fruits initiated on later-developing branches. Primary inflorescence and rosette branch fruit set were positively associated in all RIL sets, presumably as a consequence of differences in genotypic variation in vigor, i.e., larger genotypes produced more fruit on all branches. Finally, variation in both primary inflorescence and rosette branch fruit set contributed

directly to fitness in all RIL sets, with rosette branch fruit set having approximately twice the effect of primary inflorescence fruit set (Fig. 1B, S3A, S3B).

Total selection was largely attributable to indirect effects, in part because fruit number by branch order was included in the model and was closely tied to relative fitness. For circadian parameters, flowering time, and rosette size, indirect selection gradients ranged from 0.01-0.13 in the Ler × Ws-2 set, indicating that a shift in mean circadian period (or phase) by 1 standard deviation unit leads to a 1-14% difference in relative fitness (Table S3). In sum, by statistically accounting for variation in other traits, SEM suggests that the circadian clock affects branch architecture partly independent of flowering time and size, and the magnitude of this direct clock effect is similar to the direct effect of flowering time (*cf.* Ler × Ws-2 paths from flowering time to branches *vs*. circadian period to branches; Fig. 1B).

Inter-annual variation in RILs

In comparison to the 2nd growing season, the subset of the Ler × Ws-2 RILs planted in the 3rd growing season produced 72% fewer fruit and survived 27% fewer days until senescence (Table 2A), suggesting that yr 3 was more stressful year for plant growth. Circadian period and phase, days to flower, rosette size at 2.5 wks, rosette leaf number and rosette branch number were also sensitive to year, such that flowering was accelerated and plants had fewer leaves and branches in yr 3; rosette size at 2.5 wks was larger in yr 3 (Table 2A) albeit size was not-significantly different at flowering (data not shown), indicating that plants grew more rapidly before flowering in the seemingly more stressful year. RILs differed in the sensitivity of circadian period, rosette size, rosette leaf number, and rosette meristem fate to year (significant line × year interaction, Table 2A).

Although days to flowering was earlier by approximately 33% (~13 days) on average in yr 3 relative to yr 2 (Table 2A), its effect and that of size on rosette branching were non-significant when these two covariates were tested in ANCOVA models of genotype and year effects on rosette branching (Table 2B). Meristem fate was unresponsive to year, and therefore not a contributor to inter-annual difference in rosette branch number. Rosette leaf number, by contrast, had significant effects on rosette branching (Table 2B). These results suggested that leaf production rates prior to flowering differed across years; although flowering time was correlated with leaf production rate within years (Fig. 3A), *across* years the relationship differed, with leaf production rate being higher in yr 2 ($F_1 = 3.76$, $P < 0.06$; Fig.3B). Thus, in the more favorable growing season genotypes differentiated leaves more rapidly (even if the pre-flowering leaf expansion was lower), and had more meristems available for branch production. We could not use ANCOVA to test statistically for differential clock effects across years because clock and branching phenotypes were measured on different replicate plants within a genotype. However, despite the lower sample of genotypes in yr 3 (N=23) relative to the yr 2 (N=70), we observed in multivariate regression with genotypic values that the negative association between rosette branch number and circadian period persisted in yr 3 ($R^2 = 0.19$, $P = 0.05$) after factoring out days to flowering and rosette size as in the original SEM for this RIL set in yr 2 (Fig. 1B).

Clock functionality in mutants affects flowering time, size, and branching

On average across years, both the short- and long-period clock mutants flowered approximately 2-4 days later and tended to be smaller than their cognate wild-type ("Line" effect, Table 3; Fig. 4A and B). As period length increased from short-period mutant to wild-type to long-period mutant, the number of cauline leaves and branches increased (Table 3), although

there was a more discrete difference between the short-period *vs*. wild-type and long-period genotypes after statistically factoring out leaf size and days to flowering (Fig. 4C). Genotypes differed in the number of rosette leaves at flowering, where the short-period and wild-type genotypes produced fewer leaves than the long-period mutant (Table 3, Fig. 4D). Thus, genotypes that flowered later did not necessarily produce more rosette leaves, indicating that clock perturbation independently affected flowering time and leaf production. Long-period and wild-type genotypes produced fewer rosette branches than the short-period mutant and had lower rates of rosette meristem deployment, after statistically accounting for differences in flowering time and size (Fig. 4E and F). Thus, genotypes with more rosette branches had higher rates of meristem differentiation to branches but fewer rosette leaves, indicating that the differences in branch number were likely mediated through meristem fate rather than meristem number. The observed clock associations, where long-period clock mutants have more cauline branches and fewer rosette branches than genotypes with shorter circadian cycles, resembles the direction of correlations observed in the SEM of the RILs.

As in the RILs, flowering was ~30% (or 12 days) earlier for plants grown in a second year on average over all wild-type and clock mutant genotypes (41.4 ± 0.59 days *vs*. 29.23 ± 0.50 days). In addition, plants in yr 3 had fewer rosette leaves (10.38 ± 0.31 *vs*. 7.70 ± 0.27), and fewer rosette branches were produced (6.80 ± 0.53 *vs*. 4.70 ± 0.47*;* "Year" effect, Table 3). The line × year interaction for rosette leaf number was attributable to a shift of the wild-type value closer to the *toc1-1* mutant in yr 2 and to the *ztl-2* mutant in yr 3 (yr 2 - *toc1-1*: 9.39 ± 0.46, C24: 9.47 ± 0.39, *ztl-2*: 12.30 ± 0.57 vs yr 3 - *toc1-1*: 6.89 ± 0.42, C24: 8.18 ± 0.39, *ztl-2*: 7.92 ± 0.38) not a shift in genotypic rank order. The lack of line × year interactions for all other traits indicates that the effect of clock function on branching was unchanged across years.

Discussion

Among genotypes within a species and across environments, shoot architecture can vary dramatically (Bonser & Aarssen, 1996; Bonser & Aarssen, 2001; Doust & Kellogg, 2006; Baker *et al.*, 2012). In some cases, environmental regulation of plant architecture and associated performance consequences are well-characterized, as in the case of phytochrome-regulated responses to controlled manipulations of red:far-red light cues of neighbor proximity (Smith & Whitelam, 1997; Franklin & Whitelam, 2005). Yet, many factors likely determine the shoot phenotype of plants in natural settings. The circadian clock is one key integrator of abiotic factors such as temperature and photoperiod, and several classes of branching genes are regulated by the circadian clock (Covington *et al.*, 2008; Michael *et al.*, 2008). However, no studies to date report a circadian clock association with plant architecture (branching) in controlled or field settings. Further, in controlled settings with favorable growth conditions, plants may differentiate all or most meristems to branching, but meristem fate may well be more variable in complex natural environments and the fate of different pools of higher-order meristems (e.g., rosette axillary *vs*. cauline axillary) may differ. Finally, it is commonly expected that early commitment to flowering delimits rosette leaf and meristem number, as leaf production ceases when the apical meristem differentiates to a bolting inflorescence. Genotypic or environmentally-mediated variation in leaf production rate could, however, upend the relationship to flowering time, with significant fitness consequences. Here, we examined the hierarchical interactions of the circadian clock, flowering time, plant architecture, and fitness in multiple genetic lines and growing seasons.

Hierarchical associations among the clock, flowering time, and plant architecture

Structural equation modeling provides a means to explore hierarchical relationships among traits, with the aim of identifying associations that maximally explain variation in a response variable such as fitness (Tonsor & Scheiner, 2007). Flowering time had significant direct effects on branching and thereby indirect effects on fitness, consistent with past studies (Fournier-Level *et al.*, 2013; Friedman *et al.*, 2015). Within a growing season, delayed flowering time was associated with increased cauline branch number (in 2 RIL sets) and increased rosette branch number (in all three RIL sets), the latter association presumably reflecting the consistent effect of flowering time on rosette leaf production within a growing season and that meristems were exposed to environmental conditions suitable for differentiation. Independent of flowering time, clock parameters were positively associated with cauline branch number (in all three RIL sets), and in the Ler × Ws-2 set, genotypes with longer period lengths produced fewer rosette branches. The unique association between clock period and rosette branching observed in the Ler × Ws-2 set may be attributable to the larger range of phenotypic variation in this RIL set (11-hr range for period) (Fig. 2) in comparison to the other 2 sets (4-hrs) (Fig. S1). Notably, although flowering time is well-known to correlate with branch number, the novel association between quantitative clock variation, cauline and rosette branching, and performance was of similar magnitude.

Comparisons between clock mutants and their cognate wild-type genotypes can be used to examine inter-relationships between the clock and other phenotypes in a genetically controlled manner (Young & Kay, 2001; McClung, 2006; Harmer, 2009). Consistent with the

results of the SEM, our analysis of clock mutants suggests that clock function affected branching patterns in a manner independent of flowering time within a growing season. In controlled settings, long-period mutants flower late relative to wild-type genotypes because *ztl* knock-out mutants have lower levels of *CONSTANS* and *FLOWERING LOCUS T,* which leads to late flowering (Somers *et al.*, 2004). *toc1* mutants likewise flower late under long-day growth chamber conditions. Under the prevailing long-day photoperiods of our field settings, the *toc1-1* and *ztl-2* mutants in fact flowered later than the cognate wild-type genotype, indicating that phenotypes characterized under controlled settings manipulating one factor (photoperiod) are expressed in like manner in the field settings used here.

In contrast to associations observed in the segregating RILs, branching patterns did not correspond to relative flowering time of the clock mutant and wild-type genotypes. Although the two clock period mutants flowered late in comparison to the wild-type, for cauline branches, the long-period and wild-type genotypes had more branches than short-period mutant (Figure 4C). For rosette branching, wild-type and long-period genotypes had fewer branches than the short-period mutant (Figure 4E). The results suggest that a major disruption in clock function via mutation affects branching, and seems to mask the flowering time effect on branching observed in RILs expressing quantitative clock variation, potentially because of the limited effect of clock misfunction on days to flowering observed in the mutants or because the clock affects branching and flowering time via different mechanisms. Notably, the Covington *et al.* (2008) transcriptomics database identifies several circadian-regulated genes implicated in branching phenotypes including hormone-signaling genes (auxin) and developmental genes (including members of the *MAX, PIN* and *TIR* families), and some of these could act independent of flowering. In sum, the associations observed between clock function and

branching in genotypes with discrete clock phenotypes were consistent with direct associations observed in the SEM, were consistent across multiple growing seasons (lack of significant line × yr effects in experiments with clock mutants, Table 3A), and were consistent with transcriptomic data showing that a range of branching genes are circadian-regulated (Covington *et al.*, 2008).

Environmental and genetic perturbations of trait integration and its effect on branching

Environmental perturbations are widely recognized as a means to study mechanisms of trait determination and trait integration (Alonso-Blanco & Koornneef, 2000; Weinig *et al.*, 2002; Donohue *et al.*, 2005; Fournier-Level *et al.*, 2013; Dechaine *et al.*, 2015). In the field site used here, yr 3 was much more severe with regard to plant performance. In experiments with RILs or mutants, trait integration and empirical trait associations differed across years, as some traits like rosette leaf number or meristem fate showed significant line × yr interactions while other traits such as flowering time did not (Table 2A). Further, while genotypic values of flowering time were, for instance, correlated with rosette branch number within a year, neither flowering time (nor rosette size) emerged as significant determinants of variation in the numbers of rosette branches across years in the subset of Ler x Ws-2 RILs grown in multiple growing seasons (Table 2B). That is, while average days to flowering was delayed and branching increased on average in yr 2 *vs*. 3, the RIL genotypes that flowered latest did not necessarily exhibit the greatest increase in rosette branching across years. Thus, while flowering time was associated with rosette branching variation *within* years, presumably by determining leaf and thus axillary meristem numbers (Fig. 3A), major environmental changes *across* years affected leaf production

rate independent of flowering time (Fig. 3B), thereby disrupting the association between flowering time and branch numbers.

In addition to the environmental disruption attributable to growing season, genetic perturbations also appeared to alter a commonly observed pattern of trait integration. In controlled settings used for genetic characterization of flowering time, leaf number is often used as a proxy for days to flowering (Gazzani *et al.*, 2003; Michaels *et al.*, 2003; Stinchcombe *et al.*, 2004). However, this relationship dissolves among clock mutants. Because the shoot apical meristem would have more time to differentiate leaves before transitioning to reproduction, late-flowering genotypes (clock mutants in this case) should have more leaves than early-flowering lines if leaf production rates were similar among all genotypes. However, the short-period mutant (*toc1-1*) is late-flowering relative to the wild-type in the field, but these two genotypes behave similarly (or tend to reverse rank order) with regard to leaf numbers (Fig. 4). Further, as described above, flowering time of the clock mutants and wild-type genotype was unrelated to their branching phenotypes. These observations raise the hypothesis that among panels of wild-type genotypes flowering time, rosette leaf number, and ultimately branching could be decoupled across some environments in part due to clock regulation of flowering time without parallel regulation of leaf production rate.

Effects of meristem fate and number on branching and fitness

Phenotypic associations observed in the SEM and among mutants raise the question of what meristem phenotypes are empirically connected to branching. In addition to traits included in the SEM, we also counted the number of cauline and rosette leaves in the RIL populations and

mutants in both growing seasons. These data allow an examination of meristem number *vs*. fate in determining branch number, assuming each axillary meristem is viable and that each axil harbors one meristem. Relevant to cauline branches, there was genetic variation in the number of cauline leaves (and hence in meristems available) in all RIL sets, but no genetic variation in the number cauline meristems that were differentiated into branches; all cauline meristems on the primary inflorescence must have been viable (and we never observed more than one branch per axil), and then differentiated into branches in all three RIL sets (Figure S3; Table 1) as was also the case in the clock mutants. These results suggest that regulation of variation in cauline branch number in our field settings is through the number of meristems and not through cauline meristem fate, because there is no variation in the latter trait.

The determination of rosette branch number appears more complex, and may be affected by different meristem behaviors. In the complete Ler × Ws-2 RIL population measured in yr 2 (Figure 1B), genetic variation existed for the number of rosette leaves and for meristem fate (Table 1) as was the case for the mutants (Table 3A). In the RILs, both rosette leaf number (as modulated by flowering time) and meristem fate (potentially modulated by the clock) were associated with rosette branch number within a growing season. However, in mutants, only meristem fate was associated with rosette branch number; the effect of leaf number was negative, that is, genotypes with fewer rosette leaves (and hence fewer meristems) had more branches (Fig. 3D, E for mutants), suggesting that meristem fate determined rosette branch number (Fig.3F). In sum, cauline *vs*. rosette branch numbers appear to be influenced by somewhat different meristem phenotypes, and these two branch types differ in their environmental sensitivity.

Differences in meristem regulation and environmental sensitivity have fitness implications. Cauline meristem fate appears canalized, as all meristems are committed to

branching, while rosette meristem fate is more complex and plastic (Bonser & Aarssen, 2001; Bonser & Aarssen, 2003). From an evolutionary perspective, it may be advantageous to deploy all meristems on the primary inflorescence, because this ensures increased early season fitness if the growing season is short. Flexibility in the fate of rosette axillary meristems provides an opportunity to capitalize on favorable conditions, or limit branch number if growing season conditions are poor.

Conclusions

Circadian rhythms, or internal time-keeping mechanisms that are set by environmental inputs, are predicted to be adaptive when the biological processes are best aligned with an organisms' external environment. There is a rich empirical history indicating that 24-hour clocks confer highest fitness (Dodd *et al.*, 2005); this observation likely results from a complicated interplay between clock parameters and other traits with opposing directions and magnitudes of fitness effects. Despite the putative value of a 24-hr clock cycle, significant standing genetic variation in clock parameters is observed for natural populations, and the maintenance of genetic variation in general remains an evolutionary conundrum. One possibility is that genetic variation is maintained as a result from variable selection, where the relationship between circadian period and adaptive output phenotypes varies (e.g., variable effects of period length on cauline *vs*. rosette branch numbers) and where the adaptive value of these output phenotypes (cauline *vs*. rosette branches) depends on environment (e.g., depends on season length). Further mechanistic experiments are needed using additional mutants and different clock mutations introgressed into diverse backgrounds to ascertain the mechanism by which clock functionality may affect meristems numbers and fate, and ultimately cauline and rosette branch production.

Acknowledgments

The authors are grateful for the assistance in field preparation and plant care of the AES GH staff, C Seals and R Pendleton, as well as the APHIS site reviewer, L Cain. Research was supported by NSF grant IOS-0923752 and IOS-1025965 to CW.

References

Alonso-Blanco C, Koornneef M. 2000. Naturally occurring variation in *Arabidopsis*: an underexploited resource for plant genetics. *Trends in plant science* **5**(1): 22-29.

Anwer MU, Davis SJ 2013. An overview of natural variation studies in the *Arabidopsis thaliana* circadian clock. *Seminars in cell & developmental biology*: Elsevier. 422-429.

Arbuckle JL. 2006. Amos (version 5.0)[computer program]. *Chicago: SPSS*.

Baker RL, Diggle PK. 2011. Node-specific branching and heterochronic changes underlie population-level differences in *Mimulus guttatus* (Phrymaceae) shoot architecture. *American Journal of Botany* **98**(12): 1924-1934.

Baker RL, Hileman LC, Diggle PK. 2012. Patterns of shoot architecture in locally adapted populations are linked to intraspecific differences in gene regulation. *New Phytologist* **196**(1): 271-281.

Baker RL, Scherbatskoy E, Lay CR, Diggle PK. 2014. Developmental Plasticity of Shoot Architecture: Morphological Expression and Ecologically Relevant Onset in Locally Adapted Populations of *Mimulus guttatus*. *International Journal of Plant Sciences* **175**(1): 59-69.

Bazzaz FA, Chiariello NR, Coley PD, Pitelka LF. 1987. Allocating resources to reproduction and defense. *BioScience*: 58-67.

Boikoglou E. 2008. *Quantitative genetic analysis of temperature entrainment in the Arabidopsis thaliana circadian clock.* Max-Planck-Institut für Pflanzenzüchtungsforschung.

Boikoglou E, Ma ZS, von Korff M, Davis AM, Nagy F, Davis SJ. 2011. Environmental memory from a circadian oscillator: The *Arabidopsis thaliana* clock differentially integrates perception of photic vs. thermal entrainment. *Genetics* **189**(2): 655-U796.

Bonser SP, Aarssen LW. 1996. Meristem allocation: a new classification theory for adaptive strategies in herbaceous plants. *Oikos*: 347-352.

Bonser SP, Aarssen LW. 2001. Allometry and plasticity of meristem allocation throughout development in Arabidopsis thaliana. *Journal of Ecology* **89**(1): 72-79.

Bonser SP, Aarssen LW. 2003. Allometry and development in herbaceous plants: functional responses of meristem allocation to light and nutrient availability. *American Journal of Botany* **90**(3): 404-412.

Bonser SP, Aarssen LW. 2006. Meristem allocation and life-history evolution in herbaceous plants. *Botany* **84**(1): 143-150.

Borevitz JO, Maloof JN, Lutes J, Dabi T, Redfern JL, Trainer GT, Werner JD, Asami T, Berry CC, Weigel D. 2002. Quantitative trait loci controlling light and hormone response in two accessions of *Arabidopsis thaliana*. *Genetics* **160**(2): 683-696.

Brachi B, Faure N, Horton M, Flahauw E, Vazquez A, Nordborg M, Bergelson J, Cuguen J, Roux F. 2010. Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. *PLoS genetics* **6**(5): e1000940.

Brock MT, Maloof JN, Weinig C. 2010. Genes underlying quantitative variation in ecologically important traits: *PIF4* (phytochrome interacting factor 4) is associated with variation in internode length, flowering time, and fruit set in *Arabidopsis thaliana*. *Molecular ecology* **19**(6): 1187-1199.

Covington MF, Maloof JN, Straume M, Kay SA, Harmer SL. 2008. Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development. *Genome biology* **9**(8): R130.

de Montaigu A, Giakountis A, Rubin M, Tóth R, Cremer F, Sokolova V, Porri A, Reymond M, Weinig C, Coupland G. 2015. Natural diversity in daily rhythms of gene expression contributes to phenotypic variation. *Proceedings of the National Academy of Sciences* **112**(3): 905-910.

Dechaine JM, Brock MT, Weinig C. 2015. Maternal environmental effects of competition influence evolutionary potential in rapeseed (*Brassica rapa*). *Evolutionary Ecology*: 1-15.

Dodd AN, Salathia N, Hall A, Kevei E, Toth R, Nagy F, Hibberd JM, Millar AJ, Webb AAR. 2005. Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. *Science* **309**(5734): 630-633.

Donohue K, Dorn L, Griffith C, Kim E, Aguilera A, Polisetty CR, Schmitt J, Galloway L. 2005. Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. *Evolution* **59**(4): 740-757.

Dorn LA, Pyle EH, Schmitt J. 2000. Plasticity to light cues and resources in *Arabidopsis thaliana*: testing for adaptve value and costs *Evolution* **54**(6): 1982-1994.

Doust AN, Kellogg EA. 2006. Effect of genotype and environment on branching in weedy green millet (Setaria viridis) and domesticated foxtail millet (Setaria italica) (Poaceae). *Molecular ecology* **15**(5): 1335-1349.

Doyle MR, Davis SJ, Bastow RM, McWatters HG, Kozma-Bognar L, Nagy F, Millar AJ, Amasino RM. 2002. The *ELF4* gene controls circadian rhythms and flowering time in *Arabidopsis thaliana*. *Nature* **419**(6902): 74-77.

Dudley SA, Schmitt J. 1996. Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *American Naturalist*: 445-465.

Duffy NM, Bonser SP, Aarssen LW. 1999. Patterns of variation in meristem allocation across genotypes and species in monocarpic Brassicaceae. *Oikos*: 284-292.

Esau K. 1953. Plant anatomy. *Soil Science* **75**(5): 407.

Finlayson SA, Krishnareddy SR, Kebrom TH, Casal JJ. 2010. Phytochrome Regulation of Branching in Arabidopsis. *Plant Physiology* **152**(4): 1914-1927.

Fournier-Level A, Wilczek AM, Cooper MD, Roe JL, Anderson J, Eaton D, Moyers BT, Petipas RH, Schaeffer RN, Pieper B. 2013. Paths to selection on life history loci in

different natural environments across the native range of *Arabidopsis thaliana*. *Molecular ecology* **22**(13): 3552-3566.
Franklin KA, Whitelam GC. 2005. Phytochromes and shade-avoidance responses in plants. *Annals of Botany* **96**(2): 169-175.
Friedman J, Twyford AD, Willis JH, Blackman BK. 2015. The extent and genetic basis of phenotypic divergence in life history traits in *Mimulus guttatus*. *Molecular ecology* **24**(1): 111-122.
Gazzani S, Gendall AR, Lister C, Dean C. 2003. Analysis of the molecular basis of flowering time variation in *Arabidopsis* accessions. *Plant Physiology* **132**(2): 1107-1114.
Geber MA. 1990. The cost of meristem limitation in *Polygonum arenastrum*: negative genetic correlations between fecundity and growth. *Evolution*: 799-819.
Graf A, Schlereth A, Stitt M, Smith AM. 2010. Circadian control of carbohydrate availability for growth in *Arabidopsis* plants at night. *Proceedings of the National Academy of Sciences* **107**(20): 9458-9463.
Grbić V, Bleecker AB. 2000. Axillary meristem development in Arabidopsis thaliana. *The Plant Journal* **21**(2): 215-223.
Harmer SL. 2009. The circadian system in higher plants. *Annual review of plant biology* **60**: 357-377.
Hempel F, Feldman L. 1994. Bi-directional inflorescence development inArabidopsis thaliana: Acropetal initiation of flowers and basipetal initiation of paraclades. *Planta* **192**(2): 276-286.
Hsu PY, Harmer SL. 2014. Wheels within wheels: the plant circadian system. *Trends in plant science* **19**(4): 240-249.
Huber H, Lukács S, Watson MA. 1999. Spatial structure of stoloniferous herbs: an interplay between structural blue-print, ontogeny and phenotypic plasticity. *Plant Ecology* **141**(1-2): 107-115.
Izawa T, Mihara M, Suzuki Y, Gupta M, Itoh H, Nagano AJ, Motoyama R, Sawada Y, Yano M, Hirai MY. 2011. *Os-GIGANTEA* confers robust diurnal rhythms on the global transcriptome of rice in the field. *The Plant Cell Online* **23**(5): 1741-1755.
Kreps JA, Kay SA. 1997. Coordination of plant metabolism and development by the circadian clock. *The Plant Cell* **9**(7): 1235.
Lande R, Arnold SJ. 1983. The measurement of selection on correlated characters. *Evolution*: 1210-1226.
Leyser O. 2009. The control of shoot branching: an example of plant information processing. *Plant, Cell & Environment* **32**(6): 694-703.
Leyser O, Day S. 2003. *Mechanisms in plant development*. Malden, MA: Blackwell Science Ltd.
Malmberg RL, Held S, Waits A, Mauricio R. 2005. Epistasis for fitness-related quantitative traits in *Arabidopsis thaliana* grown in the field and in the greenhouse. *Genetics* **171**(4): 2013-2027.
McClung CR. 2006. Plant circadian rhythms. *The Plant Cell Online* **18**(4): 792-803.
McWatters HG, Bastow RM, Hall A, Millar AJ. 2000. The *ELF3* zeitnehmer regulates light signalling to the circadian clock. *Nature* **408**(6813): 716-720.
Michael TP, Mockler TC, Breton G, McEntee C, Byer A, Trout JD, Hazen SP, Shen R, Priest HD, Sullivan CM. 2008. Network discovery pipeline elucidates conserved time-of-day–specific cis-regulatory modules. *PLoS genetics* **4**(2): e14.

Michaels SD, He Y, Scortecci KC, Amasino RM. 2003. Attenuation of FLOWERING LOCUS C activity as a mechanism for the evolution of summer-annual flowering behavior in Arabidopsis. *Proceedings of the National Academy of Sciences* **100**(17): 10102-10107.
Millar AJ, Carre IA, Strayer CA, Chua NH, Kay SA. 1995. Circadian clock mutants in *Arabidopsis* identified by luciferase imaging. *Science* **267**(5201): 1161-1163.
Murashige T, Skoog F. 1962. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum* **15**(3): 473-497.
Pierik R, de Wit M. 2013. Shade avoidance: phytochrome signalling and other aboveground neighbour detection cues. *Journal of Experimental Botany* **65**(11): 2815-2824.
Plautz JD, Straume M, Stanewsky R, Jamison CF, Brandes C, Dowse HB, Hall JC, Kay SA. 1997. Quantitative analysis of *Drosophila* period gene transcription in living animals. *Journal of Biological Rhythms* **12**(3): 204-217.
Prusinkiewicz P, Erasmus Y, Lane B, Harder LD, Coen E. 2007. Evolution and development of inflorescence architectures. *Science* **316**(5830): 1452-1456.
Rausher MD. 1992. The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution*: 616-626.
Remington DL, Leinonen PH, Leppälä J, Savolainen O. 2013. Complex genetic effects on early vegetative development shape resource allocation differences between *Arabidopsis lyrata* populations. *Genetics* **195**(3): 1087-1102.
Robinson GK. 1991. That BLUP is a Good Thing: The Estimation of Random Effects. *Statistical Science* **6**(1): 15-32.
Rubin MJ, Brock MT, Davis AM, German ZM, Knapp M, Welch SM, Harmer SL, Maloof JN, Davis SJ, Weinig C. 2017. Circadian rhythms vary over the growing season and correlate with fitness components. *Molecular ecology*.
Samach A, Coupland G. 2000. Time measurement and the control of flowering in plants. *Bioessays* **22**(1): 38-47.
SAS. 2015. *Base SAS 9.4 Procedures Guide*: SAS Institute.
Schmitt J, McCormac AC, Smith H. 1995. A test of the adaptive plasticity hypothesis using transgenic and mutant plants disabled in phytochrome-mediated elongation responses to neighbors. *American Naturalist*: 937-953.
Smith H, Whitelam G. 1997. The shade avoidance syndrome: multiple responses mediated by multiple phytochromes. *Plant, Cell & Environment* **20**(6): 840-844.
Somers DE, Kim W-Y, Geng R. 2004. The F-box protein *ZEITLUPE* confers dosage-dependent control on the circadian clock, photomorphogenesis, and flowering time. *The Plant Cell Online* **16**(3): 769-782.
Somers DE, Schultz TF, Milnamow M, Kay SA. 2000. *ZEITLUPE* encodes a novel clock-associated PAS protein from *Arabidopsis*. *Cell* **101**(3): 319-329.
Steeves TA, Sussex IM. 1989. *Patterns in plant development*: Cambridge University Press.
Stinchcombe JR, Weinig C, Ungerer M, Olsen KM, Mays C, Halldorsdottir SS, Purugganan MD, Schmitt J. 2004. A latitudinal cline in flowering time in Arabidopsis thaliana modulated by the flowering time gene FRIGIDA. *Proceedings of the National Academy of Sciences of the United States of America* **101**(13): 4712-4717.
Strayer C, Oyama T, Schultz TF, Raman R, Somers DE, Más P, Panda S, Kreps JA, Kay SA. 2000. Cloning of the *Arabidopsis* clock gene *TOC1*, an autoregulatory response regulator homolog. *Science* **289**(5480): 768-771.

Tonsor SJ, Scheiner SM. 2007. Plastic trait integration across a CO_2 gradient in *Arabidopsis thaliana*. *The American Naturalist* **169**(5): E119-E140.
Tooke F, Battey N. 2003. Models of shoot apical meristem function. *New Phytologist* **159**(1): 37-52.
Valladares F, Pearcy RW. 1998. The functional ecology of shoot architecture in sun and shade plants of Heteromeles arbutifolia M. Roem., a Californian chaparral shrub. *Oecologia* **114**(1): 1-10.
Watson MA. 1984. Developmental constraints: effect on population growth and patterns of resource allocation in a clonal plant. *American Naturalist*: 411-426.
Weberling F. 1989. *Morphology of flowers and inflorescences*. Great Britain: Cambridge University Press.
Weinig C. 2000. Differing selection in alternative competitive environments: shade-avoidance responses and germination timing. *Evolution* **54**(1): 124-136.
Weinig C, Johnston J, German ZM, Demink LM. 2006. Local and Global Costs of Adaptive Plasticity to Density in *Arabidopsis thaliana*. *The American Naturalist* **167**(6): 826-836.
Weinig C, Ungerer MC, Dorn LA, Kane NC, Toyonaga Y, Halldorsdottir SS, Mackay TF, Purugganan MD, Schmitt J. 2002. Novel loci control variation in reproductive timing in *Arabidopsis thaliana* in natural environments. *Genetics* **162**(4): 1875-1884.
Wolff CF. 1774. *Theoria generationis. Cum 2 tab. aen*: Hendelius.
Young MW, Kay SA. 2001. Time zones: a comparative genetics of circadian clocks. *Nature Reviews Genetics* **2**(9): 702-715.

Table 1. Quantitative genetic models partitioning variance between the main effects of line and spatial block for circadian, phenological, and architectural traits in the Ler × WS-2 RIL set grown under field conditions. z-values are reported for random effects of line and block. Significance levels (*P*-value): **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, $^{\Delta} P < 0.10$. Model terms denoted as 'ne' were not estimable.

	Line	Block
Circadian Period	3.63***	0.5
Circadian Phase	1.43^{Δ}	0.91
Rosette Size	3.22***	ne
Days to Flower	2.44**	0.75
Cauline Leaf Number	2.53**	0.24
Cauline Branches	2.14*	0.69
Cauline Meristem Fate	ne	ne
Rosette Leaf Number	4.33****	0.54
Rosette Branches	2.97**	Ne
Rosette Meristem Fate	3.63***	0.57
PI Fruit Set	2.96**	0.72
Rosette BR Fruit Set	2.07*	1.1
Total Fruit Set	1.69*	ne

Table 2. Quantitative genetic models for a subset of RILs from the Ler × Ws-2 recombinant population (n=23) grown in the field for two years. Means and standard errors for year are reported (A). ANCOVA for rosette branch number with days to flower, rosette size and rosette leaf number included as covariates (B). z-values are reported for random effects and f-values for fixed effects†. Significance levels (*P*-value): **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, $^{\Delta}$ $P < 0.10$.

A.

	Line	Line × Year	Block (Year)	Year†	Year 2 (mean ± se)	Year 3 (mean ± se)
Circadian Period	ne	2.52**	ne	15.99***	21.81 ± 0.49	24.42 ± 0.44
Circadian Phase	0.07	0.17	0.73	5.76*	12.26 ± 0.81	16. 70 ± 0.70
Rosette Size	1.16	2.23*	1.77*	168.77****	6.35 ± 0.27	10.68 ± 0.27
Days to Flower	2.35**	ne	0.76	2161.37****	37.8 ± 0.18	24.9 ± 0.17
Cauline Leaf Number	0.25	0.13	0.11	0.27	2.5 ± 0.54	2.88 ± 0.51
Cauline Branches	0.28	0.11	0.10	0.32	2.5 ± 0.52	2.9 ± 0.48
Cauline Meristem Fate	ne	ne	ne	2.98	0.98 ± 0.01	0.99 ± 0.01
Rosette Leaf Number	1.69*	2.08*	1.02	116.77****	9.8 ± 0.25	6.8 ± 0.25
Rosette Branches	1.66*	0.67	3.12***	34.88****	5.6 ± 0.08	3.8 ± 0.08
Rosette Meristem Fate	1.30	1.89*	3.16***	0.17	0.60 ± 0.01	0.58 ± 0.01
PI Fruit Set	0.59	0.41	3.43***	0.18	150.7 ± 25.29	135.9 ± 24.14
Rosette BR Fruit Set	0.13	1.03	1.12	508.96****	523.1 ± 14.76	58.0 ± 14.70
Total Fruit Set	0.67	0.34	3.29***	98.14****	700.2 ± 37.68	193.4 ± 35.14
Lifespan	ne	2.10*	3.51***	20.12***	67.1 ± 2.91	49.2 ± 2.91

B.

	Line	Line × Year	Block (Year)	Year†	Days to Flower†	Rosette Size†	Rosette Leaf Number†
Rosette Branches	1.61 $^{\Delta}$	0.88	3.13***	13.88****	0.12	0.09	21.22****

Table 3. Quantitative genetic models for circadian mutants and their cognate wild-type grown across 2 years in the field with means and standard errors by genotype (A). Means with different letters are significantly different from each other with a *P*-value < 0.05 after Tukey-Kramer correction for multiple tests. z-values are reported for random effects and f-values for fixed effects†. Significance levels (*P*-value): **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, $^{\Delta}$ $P < 0.10$.

A.

	Line†	Year†	Line × Year†	Block (Year)	*toc1-1* (mean ± se)	C24 (mean ± se)	*ztl-2* (mean ± se)
Rosette Size	20.43****	4.32*	1.21	2.66**	5.79 ± 0.37 [a]	8.04 ± 0.34 [b]	7.46 ± 0.39 [b]
Days to Flower	8.77***	248.53****	1.96	0.36	35.8 ± 0.66 [a]	33.4 ± 0.55 [b]	36.9 ± 0.70 [a]
Cauline Leaf Number	9.85***	3.54	0.17	0.00	1.93 ± 0.23 [a]	2.74 ± 0.22 [b]	3.44 ± 0.25 [b]
Cauline Branches	12.66****	2.72	0.06	0.00	1.79 ± 0.22 [a]	2.54 ± 0.21 [b]	3.40 ± 0.24 [c]
Cauline Meristem Fate	1.72	1.00	1.18	1.09	0.91 ± 0.04 [a]	0.95 ± 0.03 [a]	1.00 ± 0.04 [a]
Rosette Leaf Number	10.43***	44.84****	7.25**	1.09	8.14 ± 0.31 [a]	8.83 ± 0.28 [a]	10.11 ± 0.34 [b]
Rosette Branches	1.52	8.82**	0.33	1.41	6.3 ± 0.54 [a]	5.91 ± 0.48 [a]	5.03 ± 00.59 [a]
Rosette Meristem Fate	4.87*	0.03	2.12	0.67	0.82 ± 0.07 [a]	0.68 ± 0.06 [a/b]	0.50 ± 0.08 [b]

Supplementary Table 1. Quantitative genetic models partitioning variance between the main effects of line and spatial block for circadian, phenological, and architectural traits for the Ws-2 × C24 and Ws-2 × Ler RIL sets grown under field conditions. z-values are reported for random effects of line and block. Parameters in gray were originally reported in Rubin *et al.* 2017. Significance levels (*P*-value): **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, $^{\Delta} P < 0.10$. Model terms denoted as 'ne' were not estimable.

	Year 1: Ws-2 × C24		Year 2: Ws-2 × Ler	
	Line	Block	Line	Block
Circadian Period	3.04**	0.61	3.94****	0.81
Circadian Phase	3.19***	1.01	1.87*	1.27
Rosette Size	3.21***	1.92*	4.34****	ne
Days to Flower	6.14****	2.06*	4.58****	0.36
Cauline Leaf Number	4.79****	0.86	2.00*	ne
Cauline Branches	4.74****	0.83	1.91*	0.33
Cauline Meristem Fate	ne	ne	0.04	ne
Rosette Leaf Number	5.48****	1.72*	5.42****	0.29
Rosette Branches	5.72****	1.96*	4.39****	ne
Rosette Meristem Fate	3.80****	1.82*	1.22	ne
PI Fruit Set	4.97****	0.76	3.66***	ne
Rosette BR Fruit Set	4.38****	1.81*	1.67*	0.59
Total Fruit Set	4.80****	1.69*	2.61**	ne

Supplementary Table 2. Fit statistics and percent variance explained (PVE) for the best-fit path models for theWs-2 × C24, Ws-2 × Ler, and Ler×Ws-2 RIL sets (A). Partial regression coefficients for each significant path within each RIL set (B). Non-significant paths are denoted with 'ns'.

A.	Year 1: Ws-2 × C24	Year 2: Ws-2 × Ler	Year 2: Ler ×Ws-2
χ2, df	21.84, 19	10.82, 22	22.34, 19
χ2 p-value	0.29	1.00	0.27
RMSEA	0.04	0.00	0.05
RMSEA 90% Confidence Interval	0.0-0.11	0.0-0.0	0.0-0.12
RMSEA p-value	0.50	0.99	0.45
PVE in Total Fruit Set by Model	96.9	96.6	95.9

B. Path	Year 1: Ws-2 × C24	Year 2: Ws-2 × Ler	Year 2: Ler × Ws-2
Circadian Period ↔ Circadian Phase	0.82	0.64	0.31
Circadian Period → Rosette Size	0.24	NS	0.22
Circadian Period → Days to Flower	0.29	ns	ns
Circadian Period → Cauline Branches	ns	ns	0.24
Circadian Period → Rosette Branches	ns	ns	-0.26
Circadian Period → PI Fruit Set	ns	ns	ns
Circadian Period → Rosette BR Fruit Set	ns	ns	ns
Circadian Period → Total Fruit Set	ns	ns	0.01
Circadian Phase → Rosette Size	ns	-0.08	ns
Circadian Phase → Days to Flower	ns	0.34	ns
Circadian Phase → Cauline Branches	0.12	0.21	ns
Circadian Phase → Rosette Branches	ns	ns	ns
Circadian Phase → PI Fruit Set	ns	ns	ns
Circadian Phase → Rosette BR Fruit Set	ns	ns	ns
Circadian Phase → Total Fruit Set	ns	ns	ns
Rosette Size → Days to Flower	ns	-0.42	ns
Rosette Size → Cauline Branches	0.12	ns	ns
Rosette Size → Rosette Branches	ns	ns	0.25
Rosette Size → PI Fruit Set	0.40	ns	0.18
Rosette Size → Rosette BR Fruit Set	ns	ns	ns
Rosette Size → Total Fruit Set	ns	ns	ns
Days to Flower → Cauline Branches	0.74	ns	0.26
Days to Flower → Rosette Branches	0.46	0.44	0.23
Days to Flower → PI Fruit Set	NS	-0.18	NS
Days to Flower → Rosette BR Fruit Set	-0.28	ns	0.39
Days to Flower → Total Fruit Set	ns	ns	ns
Cauline Branches → Rosette Branches	0.50	ns	ns
Cauline Branches → PI Fruit Set	0.19	0.49	0.53
Cauline Branches → Total Fruit Set	ns	0.01	0.03
Rosette Branches → Rosette BR Fruit Set	0.31	0.68	0.46
Rosette Branches → Total Fruit Set	0.01	-0.02	-0.01
PI Fruit Set → Rosette Branches	-0.26	ns	ns
PI Fruit Set → Rosette BR Fruit Set	0.65	0.23	0.25
PI Fruit Set → Total Fruit Set	0.12	0.02	0.03
Rosette BR Fruit Set → Total Fruit Set	0.20	0.18	0.18

Supplementary Table 3. Summary of direct, number of indirect paths to fitness originating from each trait and total indirect selection for each RIL set.

	Year 1: Ws-2 × C24			Year 2: Ws-2 × Ler			Year 3: Ler × Ws-2		
	Direct	Paths	Indirect	Direct	Paths	Indirect	Direct	Paths	Indirect
Circadian Period	0.00	19	0.07	0.00	0	0.00	0.01	9	0.01
Circadian Phase	0.00	6	0.01	0.00	11	0.02	0.00	0	0.00
Rosette Size	0.00	8	0.10	0.00	4	-0.02	0.00	4	0.03
Days to Flower	0.00	9	0.11	0.00	4	0.04	0.00	6	0.13
Cauline Branches	0.00	6	0.08	0.01	2	0.03	0.03	2	0.04
Rosette Branches	0.01	1	0.06	-0.02	1	0.12	-0.01	1	0.08
Cauline BR Fruit Set	0.12	3	0.11	0.02	1	0.04	0.03	1	0.05
Rosette BR Fruit Set	0.20	-	-	0.18	-	-	0.18	-	-

Figure 1. Saturated path model showing all possible trait interactions (A) and reduced, best-fit path model for the Ler × Ws-2 RIL set (B). Double-sided arrows indicate a correlation between two traits. Single-sided arrows indicate hypothesized directionality of the effect. Solid lines indicate a positive coefficient between the two traits and dashed lines indicate a negative coefficient. The width of the line illustrates the magnitude of the coefficient. Colors are included for clarity of the paths and denote the trait where the arrow originates.

Figure 2. Density plots for each trait included in the structural equation modeling for the Ler × Ws-2 RIL sets.

Figure 3. Relationship between days to flower and rosette leaf number for all three RIL set (A) and across years for the Ler × Ws-2 RIL set. RIL means are plotted. The regression coefficients (b ± SE) for panel A: Ws-2 × C24 RIL set: 1.35 ± 0.07, $P < 0.0001$, Ws-2 × Ler RIL set: 1.05 ± 0.14, $P < 0.0001$, Ws-2 × C24 RIL set: 0.67 ± 0.08, $P < 0.0001$ and panel B: Year 2: 0.46 ± 0.14, $P = 0.0045$ and Year 3: 1.16 ± 0.37, $P = 0.0047$.

Figure 4. Means and standard errors for short (*toc1-1*) and long (*ztl-2*) circadian period mutants and their wild-type background (C24) for days to flowering (A), rosette size (B), cauline branch number (C), rosette leaf number (D), rosette branch number (E) and rosette meristem fate (F). Panels A, B, and D are adjusted means for each genotype grown across two years (see Table 2A for full models). Panels C, E, F are adjusted means for each genotype grown across two years including days to flower and rosette size as covariates to be consistent with the SEM modeling of these traits.

Figure 1

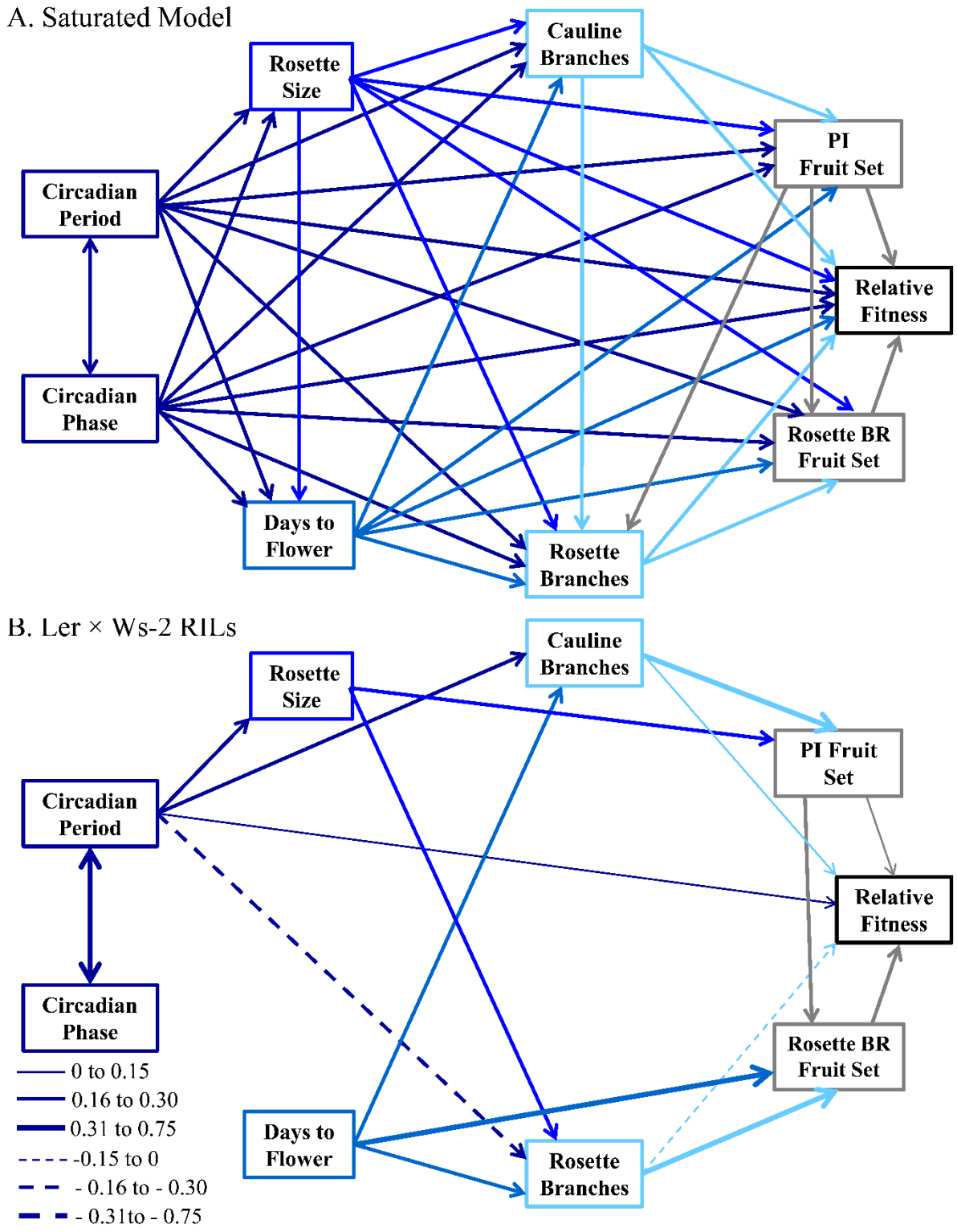

A. Saturated Model
Rosette Size
Cauline Branches
PI Fruit Set
Circadian Period
Relative Fitness
Circadian Phase
Rosette BR Fruit Set
Days to Flower
Rosette Branches
B. Ler × Ws-2 RILs
Rosette Size
Cauline Branches
PI Fruit Set
Circadian Period
Relative Fitness
Circadian Phase
Rosette BR Fruit Set
Days to Flower
Rosette Branches
0 to 0.15
0.16 to 0.30
0.31 to 0.75
-0.15 to 0
- 0.16 to - 0.30
- 0.31to - 0.75

Figure 2

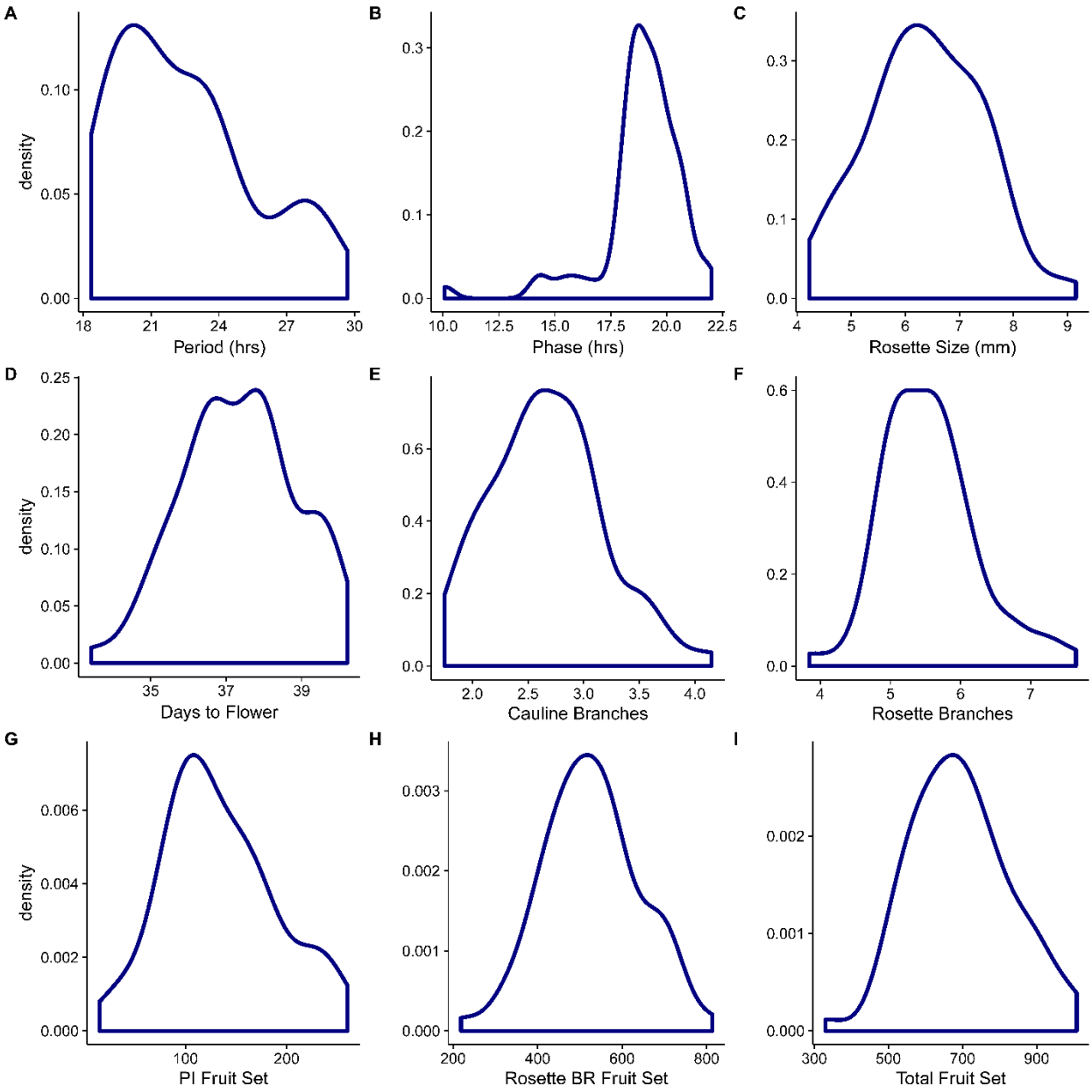

A
density
0.10
0.05
0.00
18
21
24
27
30
Period (hrs)
B
0.3
0.2
0.1
0.0
10.0
12.5
15.0
17.5
20.0
22.5
Phase (hrs)
C
0.3
0.2
0.1
0.0
4
5
6
7
8
9
Rosette Size (mm)
D
density
0.25
0.20
0.15
0.10
0.05
0.00
35
37
39
Days to Flower
E
0.6
0.4
0.2
0.0
2.0
2.5
3.0
3.5
4.0
Cauline Branches
F
0.6
0.4
0.2
0.0
4
5
6
7
Rosette Branches
G
density
0.006
0.004
0.002
0.000
100
200
PI Fruit Set
H
0.003
0.002
0.001
0.000
200
400
600
800
Rosette BR Fruit Set
I
0.002
0.001
0.000
300
500
700
900
Total Fruit Set

Figure 3

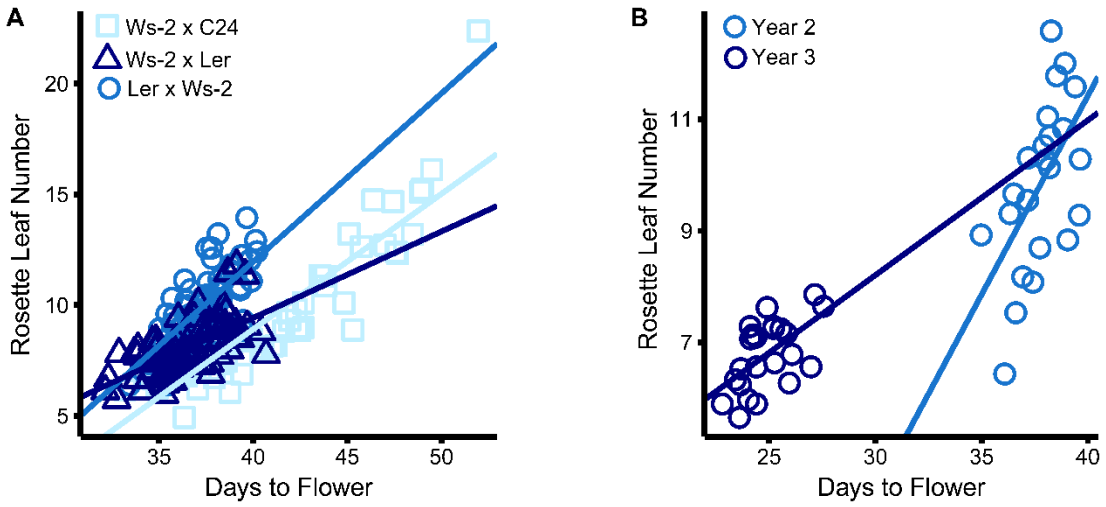
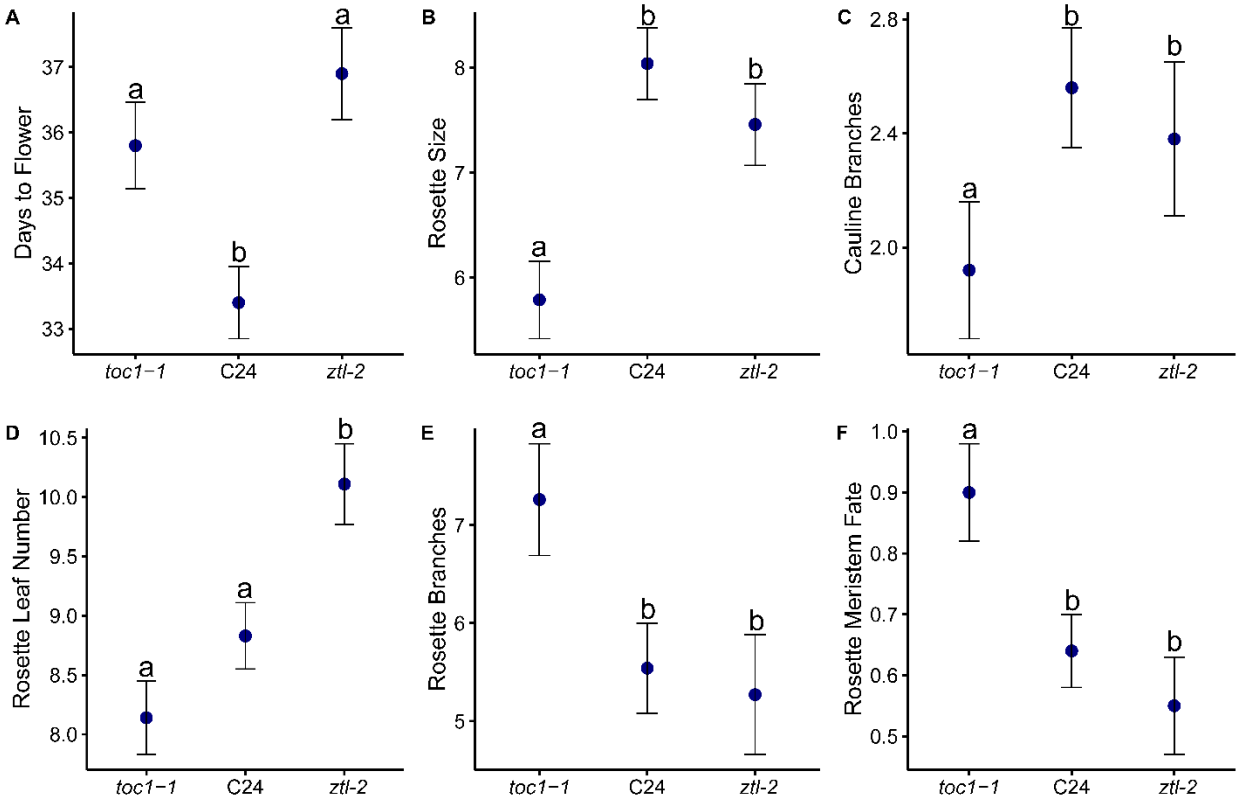

A
Ws-2 x C24
Ws-2 x Ler
Ler x Ws-2
Rosette Leaf Number
20
15
10
5
35
40
45
50
Days to Flower
B
Year 2
Year 3
Rosette Leaf Number
11
9
7
25
30
35
40
Days to Flower

Figure 4

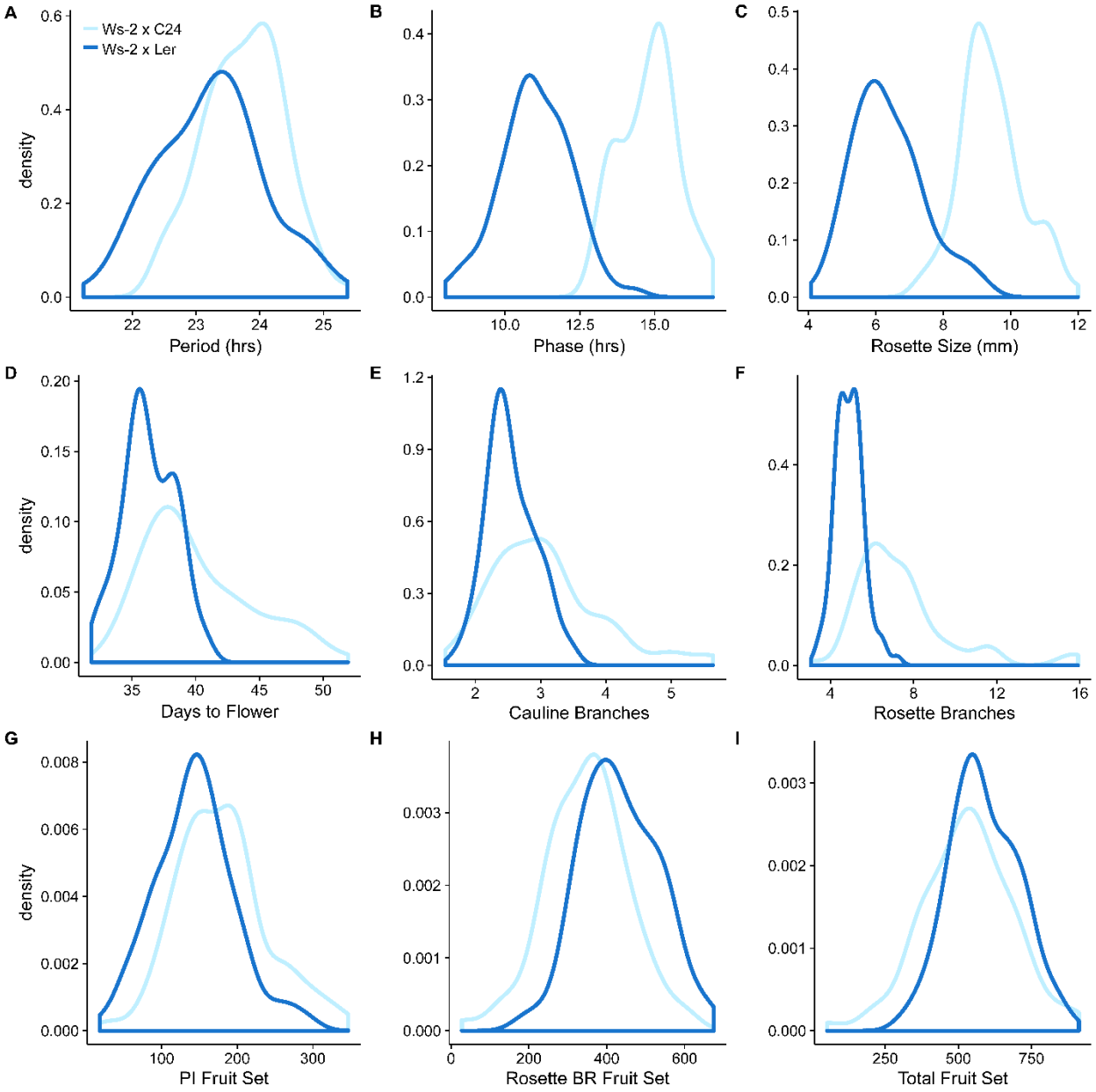

A
Days to Flower
37
36
35
34
33
a
b
a
toc1−1
C24
ztl-2
B
Rosette Size
8
7
6
a
b
b
toc1−1
C24
ztl-2
C
Cauline Branches
2.8
2.4
2.0
a
b
b
toc1−1
C24
ztl-2
D
Rosette Leaf Number
10.5
10.0
9.5
9.0
8.5
8.0
a
a
b
toc1−1
C24
ztl-2
E
Rosette Branches
7
6
5
a
b
b
toc1−1
C24
ztl-2
F
Rosette Meristem Fate
1.0
0.9
0.8
0.7
0.6
0.5
a
b
b
toc1−1
C24
ztl-2

Supplementary Figure 1. Bivariate plots of genotypic means for cauline leaf number with cauline branch number in theWs-2 × C24 (A), Ws-2 × Ler (B) and Ler × Ws-2 (C) RIL sets. *r* and *P*-values for each correlation are: (A) $r = 0.99$, $P < 0.0001$, (B) $r = 0.80$, $P < 0.0001$, and (C) $r = 0.83$, $P < 0.0001$.

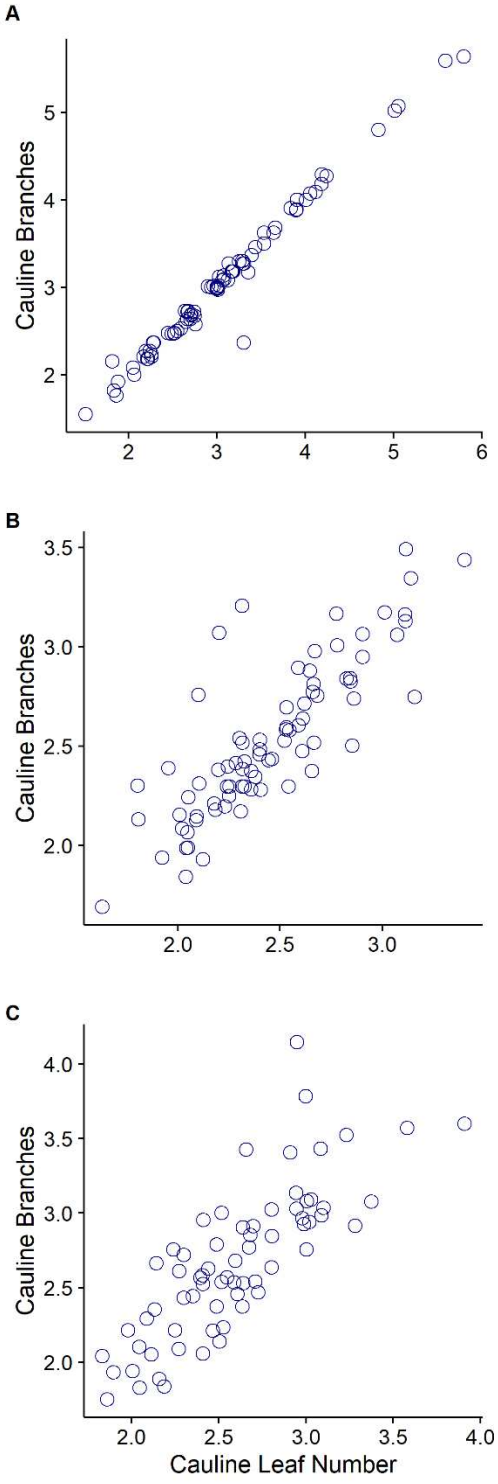
Supplementary Figure 2. Density plots for each trait included in the structural equation modeling for the Ws-2 × C24 and Ws-2 × Ler RIL sets.

Supplementary Figure 3. Reduced, best-fit path model for the Ws-2 × C24 RIL set (A) and Ws-2 × Ler RIL set (B). Double-sided arrows indicate a correlation between two traits. Single-sided arrows indicate directionality of the effect. Solid lines indicate a positive coefficient between the two traits and dashed lines indicate a negative coefficient. The width of the line illustrates the magnitude of the coefficient. Colors are included for clarity of the paths and represent the trait where the arrow originates.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3