

# Environmental memory from a circadian oscillator



# Environmental Memory from a Circadian Oscillator: The *Arabidopsis thaliana* Clock Differentially Integrates Perception of Photic *vs.* Thermal Entrainment

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**ABSTRACT** The constraint of a rotating earth has led to the evolution of a circadian clock that drives anticipation of future environmental changes. During this daily rotation, the circadian clock of *Arabidopsis thaliana* (*Arabidopsis*) intersects with the diurnal environment to orchestrate virtually all transcriptional processes of the plant cell, presumably by detecting, interpreting, and anticipating the environmental alternations of light and temperature. To comparatively assess differential inputs toward phenotypic and physiological responses on a circadian parameter, we surveyed clock periodicity in a recombinant inbred population modified to allow for robust periodicity measurements after entrainment to respective photic *vs.* thermal cues, termed *zeitgebers*. Lines previously thermally entrained generally displayed reduced period length compared to those previously photically entrained. This differential *zeitgeber* response was also detected in a set of diverse *Arabidopsis* accessions. Thus, the *zeitgebers* of the preceding environment direct future behavior of the circadian oscillator. Allelic variation at quantitative trait loci generated significant differences in *zeitgeber* responses in the segregating population. *These were important for periodicity variation dependent on the nature of the subsequent entrainment source*. Collectively, our results provide a genetic paradigm for the basis of environmental memory of a preceding environment, which leads to the integrated coordination of circadian periodicity.

WITHIN 1 day, environmental changes in light and temperature predictably oscillate. Many organisms have evolved a circadian clock as an adaptive mechanism to maximize fitness through the predictions of these anticipated environmental conditions. This clock allows for the rhythmic coordination of a wide range of developmental and metabolic processes, and this occurs with a period close to 24 hr. In plants, as photoautotrophic organisms, the clock has a particularly dominant role in mediating the photosynthetic-metabolic reactions of light capture and carbon fixation. This clock is a regulator of abiotic and biotic responses and developmental decisions (Mcclung 2006; Harmer 2009; Shin and Davis 2010). The proper timing of these processes has consequences on plant physiology and reproductive fitness (Michael *et al.* 2003b; Nozue *et al.* 2007; Resco *et al.* 2009; Yerushalmi and Green 2009). Ultimately, this enhancement occurs through the integrated coordination of the oscillator with the daily changes in light and temperature, which serve as *zeitgebers* (time givers).

Over the past decade, the molecular-genetic basis for the light-entrained plant circadian oscillator has been established in *Arabidopsis thaliana* (*Arabidopsis*). It was proposed that two morning-expressed Myb transcription factors, *Circadian Clock Associated 1* (*CCA1*) and *Late Elongated Hypocotyl* (*LHY*), and an evening-expressed gene that encodes a protein of unknown biochemical function, called *Timing of CAB expression 1* (*TOC1*), work in a feedback loop to drive overt rhythmicity (Alabadi *et al.* 2001). Mutations in any of these three genes caused decreases in periodicity of the circadian rhythm, and rhythmicity was found to be arrested in the triple-mutant background (Ding *et al.* 2007). Computational


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methods expanded this proposal and predicted that the clock is composed of at least three interconnected feedback loops (Locke *et al.* 2006; Zeilinger *et al.* 2006; Shin and Davis 2010). This interconnected feedback system was confirmed in molecular-genetic tests of these computational models (Ding *et al.* 2007; Niwa *et al.* 2007). One can wonder if the complexity of the interconnected loops allows for different entry points for environmental clock resetting, a process termed entrainment (Boikoglou and Davis 2009; Troein *et al.* 2009; Dalchau *et al.* 2010; Edwards *et al.* 2010).

Oscillations in light–dark and in warm–cool are both capable of resetting the circadian oscillator (Somers *et al.* 1998). These input systems are not completely understood, but mechanisms of light input is starting to be elucidated (Kim *et al.* 2007). In contrast, the mechanism of daily temperature entrainment of the plant oscillator is poorly understood (Michael *et al.* 2003a; Diernfellner *et al.* 2005; Glaser and Stanewsky 2005; Boikoglou and Davis 2009). What is known is that *Arabidopsis* can be entrained by as little as a 4° temperature oscillation (Somers *et al.* 1998; Mcclung and Davis 2010). A major entrainment finding was that two oscillators can be physiologically distinguished on the basis of differential sensitivity to temperature (Michael *et al.* 2003a). Several core-oscillator genes have been shown to be sensitive to both inputs. Interestingly, when the triple mutant *cca1 lhy toc1* was entrained to temperature, rhythms were severely compromised after the first day of free run (Ding *et al.* 2007). Further genetic data implicate the clock components *PRR7* and *PRR9* as genetic responders to temperature entrainment (Salome and Mcclung 2005; Salome *et al.* 2010). Despite such progress, we are only beginning to understand the functions and interactions of the known genes in the interplay of temperature and light as integrating *zeitgebers* in the synchronization of the core oscillator (Mcclung and Davis 2010).

In contrast to light input, the input mechanism of temperature entrainment could be more complicated to resolve due to its temperature-compensated nature. Notably, circadian rhythms are buffered from changes in mean ambient temperature in a process termed temperature compensation (Gould *et al.* 2006; Mcclung 2006; Mcclung and Davis 2010; Salome *et al.* 2010). Furthermore, the stress effect of prolonged exposure to chilling cold that occurs over winter, a process termed vernalization, acts on clock periodicity (Salathia *et al.* 2006). The conflict between temperature changes being capable of entraining the oscillator *vs.* the oscillators compensated capacity to resist the effects of temperature changes is an enigma. What is known is that the genetic control of temperature compensation and temperature entrainment mechanism can be partially overlapping (Gould *et al.* 2006; Salome *et al.* 2010).

Along the latitudinal cline, environmental changes in light and temperature can be highly differential. Expectedly, plants originating from different environments displayed differential genetic variation that had been shaped and maintained by natural selection in response to these environmental changes (Michael *et al.* 2003b; Dodd *et al.* 2005; Jimenez-Gomez *et al.* 2010). To explore the genetic variation present in populations that derive these differences, natural variation on circadian-clock parameters has been successfully assayed to map quantitative trait loci (QTL) for circadian rhythmicity after light–dark entrainment (Swarup *et al.* 1999; Edwards *et al.* 2005; Darrah *et al.* 2006). In most of these studies, circadian periodicity was measured. Known clock genes colocalize with many of the QTL identified to control circadian periodicity of leaf movement of light–dark entrained seedlings (Swarup *et al.* 1999). The phased peak position of a clock output in three different photoperiods was also determined (Darrah *et al.* 2006). According to this study, different QTL mediate photoperiod information to the oscillator. From these results, differential allelic variation was readily detectable and led to the detection of novel loci.

As the genetic architecture of thermal-entrainment input is not understood for the plant clock, it was of interest for us to determine circadian-oscillator responses in an *Arabidopsis* mapping population subjected to daily oscillations of temperature, with a comparison to circadian responses after subjection to daily oscillations of light. Since the analysis of natural variation is an established approach for studying circadian rhythmicity, we assessed free-running circadian periodicity after photic *vs.* thermal entrainment in a recombinant inbred line (RIL) population. The identification of loci and their interactions could be provided. This led us to an expanded view on the molecular-genetic responses to a preceding photic entrainment from that of thermal environment, as the dominant *zeitgebers*. From this, we concluded that quantitative variation in response to photic and thermal inputs have partially separable genetic bases. Interestingly, we found that thermally entrained plants commonly displayed reduced periodicity. A survey of natural *Arabidopsis* accession confirmed this period-shortening effect from thermal *vs.* photic entrainment. Collectively, this work establishes differential integration paths of *zeitgeber* responses to circadian periodicity within the oscillator and establishes in plants a memory response of the preceding environment.

## Materials and Methods

### *Plant material*

The RILs used were from Landsberg *erecta* (L*er*) by Cape Verde Islands (Cvi) collection (Alonso-Blanco *et al.* 1998), termed here CvL. This population was chosen as it was previously reported to display QTL for circadian periodicity (Swarup *et al.* 1999; Edwards *et al.* 2005) and because the Cvi and L*er* parental accessions come from differing photoperiodic and thermal environments (Alonso-Blanco *et al.* 1998). Multiple, independent T1 transgenic *CCR2*::*LUC* (Doyle *et al.* 2002) reporter lines were obtained for 41 separate RILs of the CvL collection after floral dipping (Davis *et al.* 2009) by selecting for hygromycin-resistant plants grown in 1.1 g/liter MS hygromycin containing and followed by confirmation of luciferase expression in given

transgenics. These T1 lines were transferred to soil and self-fertilized. The 41 RILs assayed were selected from the larger collection to maximize genetic diversity and to allow for balanced allele frequencies at each marker locus. As such, the selected core of 41 lines is representative of the original population. Additionally, these 41 lines were ensured to harbored multiple, independent transformants (more than two independent T1 transformants per RIL). This choice was made to reduce the possibility for positional effects of the transgene insertion site and, thus, to increase statistical power. T2 segregant progeny were used for circadian-rhythm experiments. The total number of transformants assayed is described (Supporting Information, Table S1, Table S2). In addition to the CvL RIL population, 44 natural accessions modified with the *CCR2*::*LUC* transgene were assayed (Table S3, Table S4). The accessions were selected to maximize genetic and geographic diversity. The same transformation and selection procedure was followed, as above. Here, two to seven T1 transformants were selected per accession. Both CvL lines and natural accessions were kindly provided by M. Koornneef.

### *Growth conditions and rhythm-data analysis*

Reporter imaging was as described (Hanano *et al.* 2006; Kolmos *et al.* 2009). For *zeitgeber*-entrainment experiments, lines were synchronized either to 12 hr light::12 hr darkness at 22° for the photic entrainment (LD) or to 12 hr 22°::12 hr 16°, at constant light for thermal entrainment (TMP), respectively. Entrainment light intensity was 35 μmol/m$^2$/sec. We note that under the free-run measurements, in all cases, light and temperature were the same 22° under constant light. Luminescence levels were quantified and data processed, as described (Plautz *et al.* 1997; Southern and Millar 2005). Briefly, period estimates were performed using BRASS (Southern and Millar 2005), which included the FAST FOURIER TRANSFORMATION NONLINEAR LEAST SQUARES (FFT-NLLS) curve-estimation method (Plautz *et al.* 1997). A time window corresponding to 90 hr was used, within the range of 30–120 hr.

### *Statistic analysis*

The package SPSS version 14.0.0 was used (SPSS, Chicago, IL) for statistical analyses. Univariate analysis was selected as it allowed both uni- and multivariate F-tests. To test for genetic and environmental variation in period, a GLM Univariate analysis was conducted with period as a dependent variable, environment as fixed factor, and RIL and transformants as random factors. Pearson correlation coefficients were determined using the bivariate assay. Broad sense heritability, coefficient of genetic variation, and genetic correlation were calculated, as previously reported (Keurentjes *et al.* 2007; Reymond *et al.* 2006).

### *QTL mapping and analysis*

In total, 41 CvL and 44 accessions were assayed for *CCR2* rhythmic periodicity, after light and temperature entrainment, respectively. All primary data are provided (Table S1, Table S2, Table S3, Table S4). Period mean was subsequently used for QTL mapping, performed with MapQTL 5.0 (B.V. Kyazma, Wageningen, The Netherlands). Interval mapping (IM) and Multiple QTL Mapping (MQM) were used to detect QTL. During the IM, putative QTL were detected, and during MQM, markers nearby were taken as cofactors, to detect the presence of additional QTL. Walking speed was set at 0.5 cM. LOD threshold was determined by the averaged LOD, after thrice performing 1000 permutations. QTL detected from MQM mapping were those distinguished from interaction, modifying QTL identified by statistic analysis. Two-way interactions among the QTL identified for period were tested using the corresponding two markers as fixed factors and the period as dependent variable, using the general linear model (GLM). Additive effects represent the effect of the replacement of the L*er* allele as compared to the Cvi allele, at a particular locus for the respective population.

## Results

### *The entrainment nature of the preceding environment directs circadian periodicity*

Although temperature entrainment has been identified as an important *zeitgeber*, natural variation in temperature-entrainment responses has not yet been described in plants. We analyzed the effect of temperature entrainment and compared this to defined effects after photic entrainment, in a luciferase-modified *Arabidopsis* inbred line population to reveal the genetics of natural-variation effects of temperature entrainment. For this, we adapted lines from the Cvi/L*er* (Alonso-Blanco *et al.* 1998) (hereafter CvL) RIL population, by systematically transforming them with the circadian-regulated reporter, *COLD CIRCADIAN RHYTHM RNA BINDING 2* promoter (*CCR2*::*LUC*) (also termed *GRP7* (Heintzen *et al.* 1997; Doyle *et al.* 2002), to make them suitable to assess the phenotypic response of circadian periodicity after a differential preceding *zeitgebers* of entrainment. This reporter was chosen as it is robustly rhythmic under a wide range of physiological conditions (Doyle *et al.* 2002; Mcwatters *et al.* 2007; Kolmos *et al.* 2009).

Free-running period of *CCR2*-derived bioluminescence was assayed under constant light and temperature after photic or after thermal entrainment for the modified CvL RILs. Importantly, the assay conditions themselves were identical and the difference in circadian physiology was thus a memory response of the preceding *zeitgeber* cue. We found extensive variation in *CCR2* period within the examined CvL RILs. A representative example of *CCR2* rhythmicity of two CvL lines, CvL6 and CvL47, is shown, where CvL6 displayed a shorter periodicity under both entrainment protocols compared to the CvL47 (Figure 1). Moreover, various lines displayed near-negligible period difference after the two-entrainment protocols, as typically exemplified by CvL5

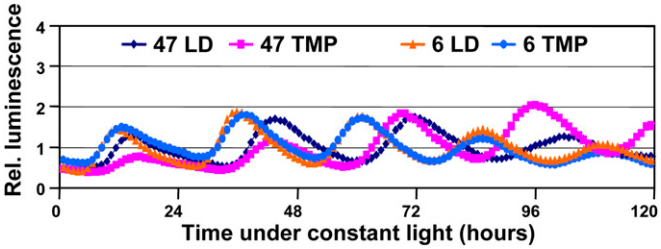


**Figure 1** Quantitative features of *CCR2* periodicity after photic *vs.* after thermal entrainment. Variation of free-running period of *CCR2* after entrainment to the two different protocols exemplified by two representative RILs: CvL6 and CvL47. LD denotes the free running rhythmicity of luminescence driven from the *CCR2* promoter after entrainment by 12 hr light:12 hr dark at a constant 22°, and TMP denotes the free-running rhythmicity after entrainment to constant light with thermal cycles of 12 hr at 22°:12 hr 16°. Note that all assay conditions were under constant light at 22°. Relative luminescence is depicted. Assay started at time 0 and is the onset of lights for photic entrainment, or the onset of warm temperature for thermal entrainment. Note that CvL6 has smaller differences in free-running period than CvL47, after the two-entrainment protocols. Period variation of *CCR2* period in CvL6 and CvL47 RILs. Line names are indicated. Dark blue, averaged period of *CCR2* after photic entrainment in CvL47; pink, averaged period of *CCR2* after thermal entrainment in CvL47; orange, averaged period of CvL6 after photic entrainment; light blue, averaged period of CvL6 after thermal entrainment.

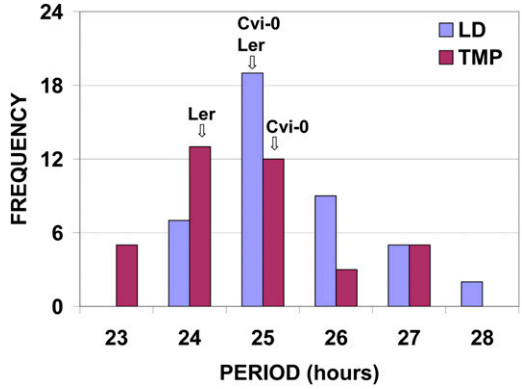


**Figure 2** Frequency distribution of *CCR2* periodicity after photic *vs.* after thermal entrainment. Normal frequency distribution of *CCR2* periodicity in individuals of the CvL population. Blue-colored bars represent periodicity after photic entrainment and pink-colored bars represent periodicity after thermal entrainment. Cvi and L*er* denote the periodicity of *CCR2* in the parental genotypes. Note the skew of temperature-entrained plants to shorter periodicity, when compared to photic-entrained plants.

(Δ 0.17 hr), whereas other lines, after photic entrainment displayed a larger period difference compared to after thermal entrainment, as exemplified by CvL49 (Δ 0.82 hr). The period of *CCR2* in all additionally assayed CvL RILs was found to display such differential responses, and this was often in greater magnitude than the typical, with up to 2.5 hr periodicity difference (Figure 1 and Figure 2). These results suggested the effect of differential genetic variation for thermal and photic entrainment. Extending from this, thermal entrainment caused significantly shorter periodicity than photic entrainment in the RIL population (Table 1). Interestingly, the two parental lines of the CvL population did not differ in mean periodicity after each entrainment. The periodicity differences between the two entrainments found in the RILs resulted thus from transgressive allelic combinations at different quantitative loci for thermal and photic entrainments (Table 1).

There were multiple RILs in the CvL population with highly significant positive period correlations between the different environments (Table 1). We thus examined the degree of covariance of *CCR2* periodicity after the photic or thermal entrainment. Moderate levels of covariance between the two traits were found (Table 1). Although moderate, the variance among RILs was quite high for each entrainment, and this resulted in the reduction in genetic correlations (Table 1). Collectively, these results suggested that different *zeitgeber*-periodicity QTL, with pleiotropic effects and/or linked QTL, as well as opposite effect alleles, would be expected and are anticipated in the CvL population.

Circadian period was found to follow a normal distribution regardless of the entrainment *zeitgeber* (Figure 2). For either entrainment, there was a greater variation in periodicity in the RILs than between the parental ecotypes (Figure 3). The transgressive variation found indicates that the parental periodicity is a result of balancing effects of alleles that increase or decrease oscillator speed. Furthermore, analysis of variance of the *CCR2* periodicity between environments showed highly significant differences, due to the genotypes (Table 2). The phenotypic variation between RILs was also highly significant (Table 2). Importantly, highly statistically significant genotype-by-environment interactions were found, implying a resultant phenotypic plasticity (Table 2). In addition, there were significant differences of transformants within genotypes, and negligible transformant (genotype) × environment interactions of CvL lines (Table 2). From these results, we anticipated a partial degree of shared genetic control of periodicity after either entrainment.

We assessed the genotype effect of RILs after each entrainment. The phenotypic variation was highly significant in each environment, due to the underlying genetic variation within RILs, compared to the transformant or Trans(genotype) assessments (Table 2). The coefficient of genetic variation was generally similar for CvL when compared between both entrainment environments (Table 2). Our statistical analysis collectively revealed that the quantitative and differential kinetic effect of rhythm generation depended on a memory of prior entrainment in a context to the genetic architecture.

### *Most accessions display a "memory" preference in the zeitgeber response*

Expanding on the physiology of thermal entrainment, a collection of ~40 accessions was measured for differential *zeitgeber* responses to circadian periodicity (Figure 4). The mean periodicity after photic entrainment was longer compared to after thermal periodicity (Tables 1 and 2), confirming the findings in the CvL population. After either entrainment protocol, many ecotypes exhibited delayed periodicity when compared to the behavior of the CvL

**Table 1 Periodicity analysis of the CvL population, and natural accessions after different entrainment cues**

| | | | 95% Confidence Interval | |
|---|---|---|---|---|
| *Zeitgeber* | Mean[a] | S.E. of the mean | Lower bound | Upper bound |
| CvL | | | | |
| LD | 26.087 | 0.032 | 26.024 | 26.151 |
| TMP | 25.329 | 0.033 | 25.264 | 25.395 |
| Ler | | | | |
| LD | 25.81 | 0.201 | 25.398 | 26.220 |
| TMP | 24.87 | 0.178 | 24.500 | 25.236 |
| Cvi | | | | |
| LD | 25.90 | 0.156 | 25.578 | 26.212 |
| TMP | 25.28 | 0.123 | 25.030 | 25.527 |
| Accessions | | | | |
| LD | 27.032 | 0.051 | 26.932 | 27.133 |
| TMP | 26.552 | 0.059 | 26.437 | 26.667 |

| LD-TMP | CvL | Accessions |
|---|---|---|
| Pairwise comparisons | | |
| Mean difference (hours) | 0.758 | 0.488 |
| S. E | 0.045 | 0.078 |
| Significance | <0.001 | <0.001 |
| Period Correlations | | |
| Correlation coefficient | 0.129 | 0.064 |
| Significance | <0.001 | 0.029 |
| Covariance | 0.284 | 0.296 |
| Genetic correlation | 0.0082 | 0.007 |

LD stands for photic-*zeitgeber* and TMP for thermal-*zeitgeber*. S.E. denotes standard error.
LD-TMP denotes the pairwise comparisons such that TMP period is subtracted from LD period.
[a] The modified population marginal mean for the 95% Confidence Interval.

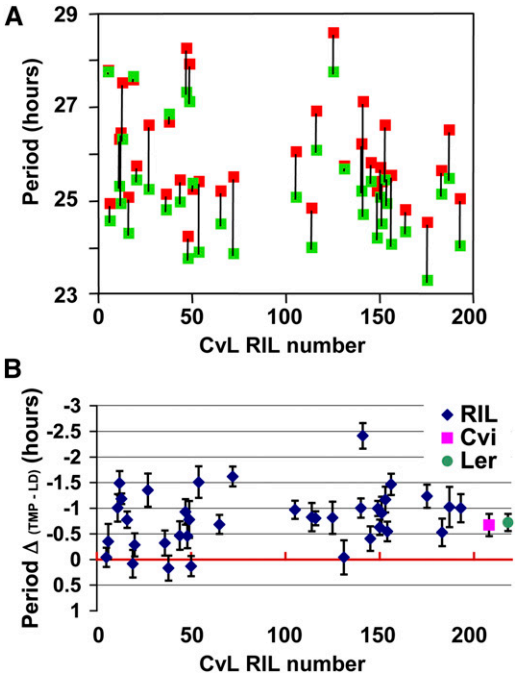


**Figure 3** Free-running-period differences in CvL lines. (A) The free-running period estimates of each RIL was plotted for oscillator speed after photic entrainment (red squares) or thermal entrainment (green squares). Note that the vast majority of RILs have a faster running oscillator after thermal, compared to after photic, entrainment. (B) The period difference for RILs depicted in A, with the parental lines included for comparison. Pairwise differences (in hours) were calculated by extracting periodicity after photic from after thermal entrainment. *x*-axis displays the RILs, named on the basis of its defined number (Alonso-Blanco *et al.* 1998), and the *y*-axis displays the difference (in hours) in periodicity after subtracting TMP periodicity from LD periodicity.

population (Table 1). Moreover, this mean difference of periodicity in the two-entrainment protocols was highly significant (Table 1). The free-running periodicity of *CCR2* in the assayed accessions varied continuously. Interestingly, there were only marginally significant correlations of *CCR2* periodicity (Table 1). Nevertheless, the periodicity after photic entrainment, and after thermal entrainment, displayed a moderate degree of covariance (Table 1). Similarly to the genetic correlations of the CvL population, natural accessions displayed a lower genetic correlation, when comparing periodicity of *CCR2* after the differing entrainment protocols (Table 1). These results for *CCR2* periodicity assayed in the natural accessions support the notion revealed by the CvL analysis that differential genetic control of thermal *vs.* photic entrainment exists.

Further statistical analysis in natural accessions was in concordance with the CvL RILs, for between-, as well as within-, entrainment protocols. Between the two-entrainment protocols, the factor analysis revealed an equivalence to the highly significant main effects of genotype and environment and to the interaction of genotype by environment. Furthermore, we found a significant effect of transformants nested to genotypes and the interaction of environment by the transformants nested to genotypes (Table 2). After either entrainment, variation in periodicity was found to be highly significantly attributed to the genotypes. To a lesser degree, but also highly significant, it could also be attributed to the transformants nested to genotypes (Table 2). Either between or within the different entrainments, the periodicity variation in the CvL population was found to be much higher than in the natural accessions.

### *Quantitative genetic analysis of circadian periodicity after light–dark and temperature-entrained seedlings*

Our next efforts were to determine a genomic foundation for the detected environmental *zeitgeber* memory. The continuous distribution of *CCR2* periodicity suggests that multiple loci controlled phenotypic variation. Using the respective *CCR2* free-running period for each line, after assaying the response to two distinctive *zeitgeber* signals, QTL mapping revealed differential allelic variation in the CvL population, which was associated with these discriminating responses. Large effect QTL and interaction-effect QTL were revealed.

QTL with a large effect in the CvL collection were identified for both entrainment protocols in the first and fifth chromosome for photic and thermal entrainment. The fifth chromosome QTL for photic entrainment colocalized with the QTL after the temperature entrainment. In contrast, the

**Table 2 Statistical analysis of *CCR2* period after the two *zeitgeber* protocols**

| Overall model factor | Univariate CvL | | | Univariate accessions | | |
|---|---|---|---|---|---|---|
| | SS | F | P | SS | F | P |
| Genotype | 2161.483 | 14.690 | <0.001 | 2709.308 | 5.175 | <0.001 |
| Environment | 291.835 | 104.446 | <0.001 | 121.507 | 11.453 | 0.002 |
| Genotype × environment | 111.172 | 3.245 | <0.001 | 440.396 | 2.655 | <0.001 |
| Trans(genotype) | 217.432 | 2.280 | <0.001 | 767.053 | 1.526 | 0.015 |
| Trans(genotype) × Environment | — | — | NS | 427.644 | 1.411 | 0.006 |

| | Univariate LD | | | Univariate TMP | | |
|---|---|---|---|---|---|---|
| | F | P | CV LD | F | P | CV TMP |
| CvL | | | | | | |
| Genotype | 31.793 | <0.001 | 21.614 | 34.092 | <0.001 | |
| Trans | — | NS | | 3.743 | 0.001 | 24.233 |
| Trans(genotype) | 2.125 | <0.001 | | 1.436 | 0.007 | |
| Accessions | | | | | | |
| Genotype | 8.088 | <0.001 | 24.616 | 7.450 | <0.001 | 23.914 |
| Trans(genotype) | 1.658 | <0.001 | | 1.970 | <0.001 | |

F shows the variation explained by each factor relative to the error variation. $P$ = significance value of the F-ratio. Genotype denotes RIL, Environment denotes the different entrainments, Trans denotes independent transformants of each genotype. SS, type III Sum of Squares. CV, coefficient of genetic variation shown as %. LD, photic. TMP, thermal entrainment. NS, non-significance. *, the testing of an interaction between two factors; B(A), the testing main factor A in which a factor B is nested to.

detected QTL in the first chromosome after the two-entrainment protocols did not colocalize. Moreover, the QTL-additive effects, which contributed to alternations in *CCR2* periodicity, varied from 1.083 to 0.755 hr for the photic entrainment, and from 1.475 to 1.49 hr for the thermal entrainment (Table 3). Interestingly, these opposite-additive effects on periodicity of *CCR2* resulted from only two QTL with alleles of opposing effects, as detected in the thermal-entrainment protocol. These findings support the theory of balancing effect on *CCR2* periodicity from the parental Cvi and L*er* accessions.

The current model of the *Arabidopsis* circadian system is based on molecular-genetic data after preceding light–dark entrainment and consists of multiple interlocking feedback loops (Locke *et al.* 2006; Zeilinger *et al.* 2006; Edwards *et al.* 2010). These widespread interconnections suggest that complex connections between genetic components fine tune the circadian system in response to environmental changes. Consistent with this, we found allele-specific interactions (Tables 4–5). Specifically, a significant interaction was found between the interacting-effect QTL at the PW4 locus at chromosome 1 and the QTL at the CC.262C locus at chromosome 5, after light entrainment. Another distinct allelic interaction between the QTL at CH.160L-Col locus at chromosome 1 and the QTL at the CC.262C at chromosome 5 was found, after thermal entrainment. In both cases, the interaction of the L*er* allele of the $CHR1^{PW4}$, or $CHR1^{CH.160L\text{-}Col}$, with the Cvi allele of the $CHR5^{CC.262C}$ displayed longer period than any other allelic interaction of these two QTL (Tables 4–5). These detected epistasic interactions are in support, with transgressive variation of *CCR2* period found in CvL population being explained by the genetic interactions of QTL.

**Table 3 Localization of the main QTL and the interactions found between them for the CvL population after photic *vs.* thermal *zeitgeber* protocols**

| *Zeitgeber* | $h^2$ | Chromosome | Position (cM) | Candidate genes | LOD score[a] | % expl. variance | *F* | *P* value | 2*a* (*hr*) |
|---|---|---|---|---|---|---|---|---|---|
| LD | 0.79 | I | 62 | Novel | 2.8 | 10 | 9.526 | <0.001 | 0.829 |
| | | IV | 8 | Novel | 3.45 | 12.9 | 13.474 | 0.001 | 0.755 |
| | | V | 90 | TOC1, PRR3, SRR1 | 3.65 | 14 | 17.175 | <0.001 | −1.083 |
| | | I • V | 0 • 90 | | — | — | 9.61 | 0.004 | — |
| TMP | 0.85 | I | 26 | Novel or GI | 4.53 | 25.8 | 9.825 | <0.001 | 1.475 |
| | | V | 95 | TOC1, PRR3, SRR1 | 4.1 | 24 | 12.037 | <0.001 | −1.49 |
| | | I • V | 26 • 95 | | — | — | 6.243 | 0.018 | — |
| Difference QTL | | V | 97 | TOC1, PRR3, SSR1 | 2.52 | 22.9 | 6.303 | 0.017 | 0.456 |

% expl. variance is the percentage of explained variance. F shows the variation explained by each factor relative to the error variation. $h^2$, broad sense heritability. cM, centiMorgan. *P*, the significance value of the *F* ratio. 2*a*, the additive effect of L*er* allele, measured in hr, when the effect of Cvi allele on period is subtracted. *hr*, the effect in hours. —, the Cvi displayed longer period than Ler allele.

[a]LOD-score threshold was determined at 2.6.

**Table 4 LD interaction**

| Chr 1 (0 cM) | Chr 5 (90 cM) | Period |
|---|---|---|
| 1 | 1 | 25.729 |
| 1 | 2 | 25.566 |
| 2 | 1 | 27.504 |
| 2 | 2 | 26.021 |

**Table 5 TMP interaction**

| Chr 1 (26 cM) | Chr 5 (95 cM) | Period |
|---|---|---|
| 1 | 1 | 25.311 |
| 1 | 2 | 27.537 |
| 2 | 1 | 24.725 |
| 2 | 2 | 25.435 |

The significant differences between genotypes resulted in high trait heritabilities, which were seen after either environment protocol (Table 3). This prompted us to check for the periodicity difference as a measure of given genotypic variation between the different RILs assayed. Interestingly, after calculating periodicity differences in a pairwise comparison ($Per_{LD}$-$Per_{TMP}$), a statistically significant differential-memory QTL was mapped in the CvL population. The differential-memory QTL colocalized with the large-effect periodicity QTL at chromosome 5, which was identified under both entrainments (Table 3). The additive result of the differential-memory QTL for CvL equals that of the difference between the respective parents (Table 1), supporting further the notion that balancing-effect alleles defined a given *zeitgeber* response.

In addition to the QTL that generated large effects, we detected interaction QTL. These modifier QTL genetically interacted with the major QTL, both positively and negatively. Such modifier QTL are exemplified by one located on chromosome 5 (located at 20 cM), after temperature entrainment, and on chromosomes 1, 2, and 5 (located at 0, 15, and 20 cM, respectively), after photic entrainment. We thus detected that the number of QTL that generated large effects and modifier QTL was higher for the photic entrainment than for the thermal entrainment.

## Discussion

Over 24 hr, plants generally experience both a light–dark cycle and a warm–cool cycle, where warmth coincides in time with light and coolness with darkness. Although light is the major factor responsible for resetting the plant circadian oscillator, temperature cycles are also a robust *zeitgeber* to the oscillator (Somers *et al.* 1998; Barak *et al.* 2000; Mcclung 2006; Mcclung and Davis 2010). To study the effect of temperature in the entrainment of the *Arabidopsis* oscillator, we monitored the kinetic expression of the promoter of *CCR2*. In a derived RIL population, circadian periodicity was measured under free-running conditions after thermal entrainment, and these results were compared to the effect of these populations after entrainment to light–dark cycles. In parallel, we extended this survey in a population of natural accessions. Our data showed that temperature differentially entrains the oscillator, when compared to photic entrainment. The memory response to the oscillator could explain a benefit of a multiloop system that defines the plant circadian clock. The dominant effect of temperature to speed the oscillator indicated that the variation in periodicity relies on different loci variation, in response to such a differential *zeitgeber* input.

Mutant analysis has revealed genes that colocalize with several of the QTL we report here. *GIGANTEA* (*GI*), *FLOWERING LOCUS C (FLC)*, and *TIME OF CAB2 EXPRESSION* (*TOC1*) were among the candidates for the detected QTL. The genomic regions identified also overlap with previously described allelic variation (Swarup *et al.* 1999; Edwards *et al.* 2005; Darrah *et al.* 2006). Taken together, both known clock genes and genes not yet known may contribute to circadian function as QTL candidates for the *zeitgeber*-memory QTL reported here.

Temperature-compensation studies in other model organisms—*e.g.*, *Drosophila* and *Neurospora*—have shown that clock components that respond to light–dark entrainment also play a role in response to temperature compensation and to temperature entrainment (Diernfellner *et al.* 2005; Glaser and Stanewsky 2005). In analogous comparisons, one could expect that genetic components involved in temperature compensation in *Arabidopsis* could also be involved in temperature entrainment. This is intriguing as temperature compensation is a thermal resistance, whereas temperature entrainment is a positive input mediating clock resetting. Our findings that *Arabidopsis* thermal-entrainment responses do not exclusively overlay with previous reports for compensation effects (Gould *et al.* 2006; Salome *et al.* 2010) supports the idea that allelic variation exists as a consequence of a transfer from one temperature regime to another. Thus, even if similar components are used in resistance that generates compensation compared to the thermal activation that mediates entrainment, temperature compensation and thermal entrainment must be mechanistically different in the plant oscillator.

Photic and thermal periodicity QTL were identified, and the different detected QTL depended on the nature of the given *zeitgeber* (Table 3). This result was a strong indication that the *Arabidopsis* circadian clock is a mechanism under selective pressure from thermal and photic cues. Overall, the CvL set was found to display diverse genetic control in response to the two-entrainment inputs (Figure 3, Table 2, and Table 3). The two accessions of this RIL population were collected from distinct habitats, and therefore, natural variation present in a given accession could have been specific to a given environmental condition.

Numerous response modes were detected with pairwise differences that approximate that of the CvL RIL lines

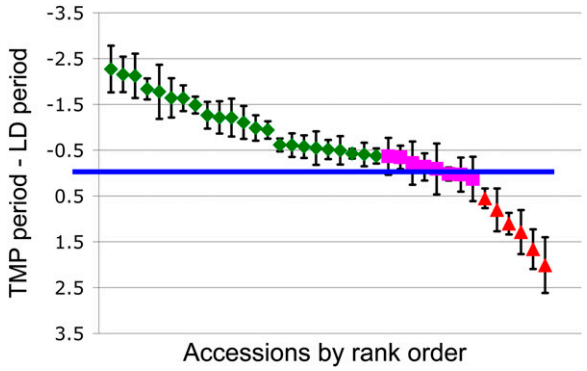


**Figure 4** Free-running pairwise-period differences of *CCR2:LUC* in natural accessions. Accessions oscillator speed was plotted as the difference after thermal *vs.* after photic entrainment, as in Figure 3B. Accessions were ordered on the basis of their TMP periodicity from LD periodicity difference. Note extensive positive and several negative differences. Most lines ran a faster oscillator after thermal entrainment ($P > 0.01$). Green represents accessions with a statistically significant thermal enhancement difference, pink represents accessions with no statistically significant difference, and red represents accessions with a statistically significant photic enhancement.

(Figure 3). The majority of RILs and accessions displayed longer periodicity after photic compared to after thermal entrainment. Moreover, the mean pairwise difference between the RILs was 80% greater than that between the natural accessions. Additionally, the mean periodicity in natural accessions after both entrainments was on average longer by an hour compared to the CvL population (Table 1). These findings suggest that there is a great likelihood that the alleles identified in the CvL collection represent only a fraction of the balancing-effect alleles that exist within *Arabidopsis* genomes.

Cvi and L*er* accessions both displayed a positive difference in periodicity. They were markedly detected as those with the least standard errors of the difference between the means of periodicity between the two entrainments. As shown in Table 1 and Figures 2 and 3, the phenotypic variation of Cvi and L*er* periodicity was comparable between the two environments. Interestingly, the RILs displayed wider differences than the parental lines, suggesting that additive effects of multiple QTL alleles underlie the periodicity variation between the two entrainments. Thus, assaying this CvL population revealed previously unknown QTL alleles that displayed opposing effects and allelic interactions, and these alleles likely would not have been identified in an accession survey (Figure 4).

The temporal pattern of memory in plants has been detected at the generational (Molinier *et al.* 2006), the developmental (Bastow *et al.* 2004), and here we show, the daily level. The most described memory system in plants, where the genetic mechanism is essentially understood, is a seasonal memory termed vernalization that shapes a developmental pattern in response to a preceding chilling winter (Amasino 2010). Memory of the nature of previous entrainment has previously been established in an animal system, as a determinant of reproduction. The authors assessed the persistence of photoperiodic history information and determined the duration of previous exposure required for memory to photoperiod (Prendergast *et al.* 2000). Interestingly, recent entrainment changes, and not older ones, influenced reproduction. Thus, *zeitgeber* memory might be a generic phenomenon for many circadian organisms.

Plants originating from different latitudes display differential genetic variation that had been shaped and maintained by natural selection in response to the environmental changes (Davis 2002; Mcclung and Davis 2010). This selected genetic variation accounts for the differential responses to the two-entrainment protocols, as shown in the natural accessions. Thus, when natural variation is assayed, a memory of the prior light and temperature regimes is reflected, and this is differentially fine tuned. One can speculate that as the *zeitgebers* of light and temperature differentially act on subsequent clock speed, the natures of these entrainment signals could in themselves be a buffer against weather variation to ensure robust entrainment. Modeling supports this notion (Troein *et al.* 2009). Our finding that plants remember whether their oscillator was entrained by a photic *vs.* a thermal *zeitgeber* cue provides a paradigm under which to explore the genetic basis for ambient-temperature perception and to differential, daily *zeitgeber* memory in plants.

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# Environmental Memory from a Circadian Oscillator: The *Arabidopsis thaliana* Clock Differentially Integrates Perception of Photic *vs.* Thermal Entrainment

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**Table S1 RIL periodicity of *CCR2::LUC* in CvL after photic entrainment**

| CvL | Period LD | N | SEM | # transformants |
|---|---|---|---|---|
| 5 | 27.88 | 51 | 0.13 | 3 |
| 6 | 24.89 | 23 | 0.16 | 4 |
| 11 | 26.31 | 17 | 0.27 | 2 |
| 12 | 26.42 | 60 | 0.14 | 4 |
| 13 | 27.43 | 20 | 0.17 | 3 |
| 16 | 25.03 | 39 | 0.14 | 4 |
| 19 | 27.59 | 25 | 0.14 | 4 |
| 20 | 25.73 | 24 | 0.16 | 4 |
| 27 | 26.63 | 23 | 0.14 | 2 |
| 36 | 25.18 | 53 | 0.11 | 2 |
| 38 | 27.22 | 31 | 0.17 | 4 |
| 44 | 25.43 | 18 | 0.24 | 3 |
| 47 | 28.21 | 44 | 0.15 | 4 |
| 48 | 24.50 | 26 | 0.23 | 4 |
| 49 | 27.98 | 22 | 0.18 | 2 |
| 50 | 26.43 | 33 | 0.22 | 4 |
| 54 | 25.41 | 23 | 0.27 | 3 |
| 59 | 24.39 | 16 | 0.24 | 2 |
| 61 | 25.04 | 20 | 0.21 | 2 |
| 65 | 25.55 | 15 | 0.15 | 2 |
| 69 | 26.50 | 28 | 0.21 | 2 |
| 72 | 25.44 | 17 | 0.14 | 3 |
| 105 | 26.01 | 32 | 0.20 | 3 |
| 114 | 24.85 | 31 | 0.15 | 4 |
| 116 | 26.92 | 46 | 0.16 | 6 |
| 125 | 28.47 | 21 | 0.22 | 3 |
| 131 | 26.85 | 22 | 0.23 | 3 |
| 140 | 26.17 | 52 | 0.17 | 3 |
| 141 | 27.08 | 23 | 0.31 | 3 |
| 145 | 26.00 | 13 | 0.21 | 2 |
| 149 | 25.11 | 46 | 0.16 | 5 |
| 150 | 25.68 | 43 | 0.17 | 6 |
| 151 | 25.37 | 31 | 0.19 | 4 |
| 153 | 26.57 | 48 | 0.15 | 5 |
| 154 | 25.76 | 22 | 0.23 | 3 |

| | | | | |
|---|---|---|---|---|
| 156 | 25.52 | 35 | 0.19 | 3 |
| 164 | 25.80 | 27 | 0.25 | 4 |
| 175 | 24.61 | 18 | 0.24 | 3 |
| 183 | 25.80 | 29 | 0.28 | 4 |
| 187 | 26.60 | 30 | 0.13 | 3 |
| 193 | 25.01 | 41 | 0.28 | 5 |
| Cvi | 25.90 | 38 | 0.16 | 3 |
| L*er* | 25.81 | 30 | 0.20 | 2 |

CvL denotes RIL number, Period LD denotes period in hours after photic entrainment, N denotes the individuals assayed per RIL, SEM denotes Standard Error of the Mean, # denotes number of independent transformants

**Table S2 RIL periodicity of *CCR2::LUC* in CvL after thermal entrainment**

| CvL | Period TMP | N | SEM | # transformants |
|---|---|---|---|---|
| 5 | 27.71 | 65 | 0.11 | 4 |
| 6 | 24.49 | 25 | 0.19 | 4 |
| 11 | 25.28 | 31 | 0.09 | 2 |
| 12 | 25.01 | 37 | 0.12 | 4 |
| 13 | 26.28 | 14 | 0.17 | 2 |
| 16 | 24.28 | 33 | 0.15 | 4 |
| 19 | 27.62 | 41 | 0.12 | 4 |
| 20 | 25.40 | 30 | 0.14 | 4 |
| 27 | 25.15 | 25 | 0.17 | 2 |
| 36 | 24.86 | 66 | 0.13 | 2 |
| 38 | 26.82 | 30 | 0.21 | 4 |
| 44 | 24.95 | 26 | 0.20 | 3 |
| 47 | 27.70 | 26 | 0.23 | 4 |
| 48 | 23.89 | 36 | 0.12 | 6 |
| 49 | 27.16 | 39 | 0.13 | 2 |
| 50 | 26.33 | 27 | 0.17 | 6 |
| 54 | 23.95 | 20 | 0.15 | 3 |
| 65 | 25.04 | 19 | 0.15 | 2 |
| 72 | 23.85 | 20 | 0.25 | 3 |
| 105 | 25.13 | 19 | 0.16 | 2 |
| 114 | 23.96 | 26 | 0.12 | 4 |
| 116 | 26.06 | 43 | 0.09 | 6 |
| 125 | 27.67 | 27 | 0.16 | 4 |
| 131 | 25.67 | 23 | 0.16 | 3 |
| 140 | 25.15 | 24 | 0.16 | 3 |
| 141 | 25.26 | 23 | 0.24 | 3 |
| 145 | 25.34 | 15 | 0.13 | 2 |
| 149 | 24.16 | 44 | 0.09 | 5 |
| 150 | 24.98 | 34 | 0.13 | 5 |
| 151 | 24.33 | 21 | 0.24 | 4 |
| 153 | 25.44 | 29 | 0.25 | 5 |
| 154 | 24.96 | 23 | 0.13 | 3 |
| 156 | 24.14 | 33 | 0.12 | 3 |
| 164 | 26.64 | 20 | 0.38 | 4 |
| 175 | 23.34 | 31 | 0.13 | 4 |

| | | | | |
|---|---|---|---|---|
| 183 | 24.91 | 23 | 0.21 | 3 |
| 187 | 26.12 | 23 | 0.26 | 3 |
| 193 | 24.37 | 18 | 0.32 | 5 |
| Cvi | 25.28 | 44 | 0.12 | 3 |
| L*er* | 24.87 | 24 | 0.18 | 2 |

CvL denotes RIL number, Period TMP denotes period in hours after thermal entrainment, N denotes the individuals assayed per RIL, SEM denotes Standard Error of the Mean, # denotes number of independent transformants

**Table S3 Periodicity of *CCR2::LUC* in natural Accessions after photic entrainment**

| Accessions | Independent transformants | # of plants | Period LD | SEM |
|---|---|---|---|---|
| En-2 | 1 | 6 | 26.27 | 0.45 |
| | 2 | 9 | 26.16 | 0.36 |
| | 3 | 3 | 26.51 | 0.12 |
| **Total** | **3** | **18** | **26.26** | **0.23** |
| Kas-2 | 1 | 5 | 25.34 | 0.60 |
| | 2 | 8 | 25.99 | 0.50 |
| | 3 | 7 | 25.06 | 1.22 |
| | 4 | 7 | 25.69 | 0.76 |
| **Total** | **4** | **27** | **25.55** | **0.40** |
| Sei-0 | 1 | 9 | 25.01 | 0.61 |
| | 2 | 9 | 26.12 | 0.47 |
| | 3 | 6 | 25.48 | 0.41 |
| | 4 | 9 | 25.75 | 0.74 |
| **Total** | **4** | **33** | **25.60** | **0.30** |
| KL-PW-1 | 1 | 11 | 26.13 | 0.30 |
| | 2 | 12 | 25.64 | 0.25 |
| | 3 | 12 | 25.61 | 0.25 |
| | 4 | 10 | 24.94 | 0.20 |
| | 5 | 11 | 25.35 | 0.32 |
| | 6 | 11 | 25.15 | 0.20 |
| **Total** | **6** | **67** | **25.48** | **0.11** |
| Sed-1 | 1 | 12 | 25.73 | 0.19 |
| | 2 | 12 | 25.65 | 0.28 |
| **Total** | **2** | **24** | **25.69** | **0.17** |
| Be-0 | 1 | 6 | 26.92 | 1.01 |
| | 2 | 6 | 26.73 | 1.32 |
| | 3 | 9 | 26.32 | 0.49 |
| **Total** | **3** | **21** | **26.61** | **0.49** |
| Bur-0 | 1 | 9 | 25.38 | 0.25 |
| | 2 | 10 | 24.69 | 0.20 |
| | 3 | 12 | 24.90 | 0.14 |
| | 4 | 11 | 25.39 | 0.37 |
| | 5 | 11 | 26.90 | 0.63 |
| **Total** | **5** | **53** | **25.46** | **0.19** |

| | | | | | |
|---|---|---|---|---|---|
| Cal-0 | | 1 | 5 | 27.30 | 0.49 |
| | | 2 | 10 | 25.54 | 0.33 |
| | | 3 | 8 | 26.93 | 0.68 |
| | **Total** | **3** | **23** | **26.40** | **0.33** |
| Ei-2 | | 1 | 10 | 26.60 | 0.72 |
| | | 2 | 7 | 26.50 | 0.37 |
| | | 3 | 8 | 27.35 | 1.06 |
| | | 4 | 4 | 27.36 | 1.07 |
| | **Total** | **4** | **29** | **26.89** | **0.41** |
| Eil-0 | | 1 | 10 | 25.84 | 0.26 |
| | | 2 | 11 | 25.67 | 0.23 |
| | | 3 | 11 | 25.16 | 0.26 |
| | | 4 | 11 | 25.82 | 0.15 |
| | **Total** | **4** | **43** | **25.62** | **0.12** |
| Es-0 | | 1 | 11 | 29.53 | 0.58 |
| | | 2 | 11 | 28.62 | 0.35 |
| | | 3 | 9 | 27.89 | 0.67 |
| | | 4 | 9 | 28.17 | 0.31 |
| | | 5 | 10 | 28.68 | 0.31 |
| | **Total** | **5** | **50** | **28.62** | **0.22** |
| Ka-0 | | 1 | 12 | 26.51 | 0.80 |
| | | 2 | 16 | 26.40 | 0.54 |
| | | 3 | 11 | 25.76 | 0.62 |
| | | 4 | 20 | 27.03 | 0.62 |
| | | 5 | 22 | 26.05 | 0.41 |
| | | 6 | 9 | 26.13 | 0.43 |
| | **Total** | **6** | **90** | **26.36** | **0.24** |
| Mh-1 | | 1 | 12 | 26.11 | 0.22 |
| | | 2 | 11 | 26.71 | 0.18 |
| | | 3 | 10 | 26.74 | 0.18 |
| | **Total** | **3** | **33** | **26.50** | **0.12** |
| Rsh-0 | | 1 | 8 | 24.69 | 0.16 |
| | | 2 | 12 | 25.17 | 0.31 |
| | | 3 | 11 | 24.82 | 0.22 |
| | | 4 | 11 | 24.87 | 0.28 |
| | **Total** | **4** | **42** | **24.91** | **0.13** |
| Te-0 | | 1 | 9 | 27.95 | 0.61 |

| | | | | | |
|---|---|---|---|---|---|
| | | 2 | 8 | 28.75 | 0.66 |
| | | 3 | 8 | 27.46 | 0.42 |
| | | 4 | 5 | 26.71 | 0.42 |
| | **Total** | **4** | **30** | **27.82** | **0.30** |
| Lim | | 1 | 7 | 27.98 | 0.99 |
| | | 2 | 9 | 28.51 | 1.18 |
| | | 3 | 8 | 29.00 | 0.69 |
| | **Total** | **3** | **24** | **28.52** | **0.56** |
| Driel | | 1 | 7 | 28.94 | 0.72 |
| | | 2 | 8 | 27.13 | 0.83 |
| | | 3 | 4 | 28.07 | 0.20 |
| | | 4 | 7 | 28.64 | 0.47 |
| | | 5 | 4 | 27.59 | 1.00 |
| | **Total** | **5** | **30** | **28.09** | **0.33** |
| Jea | | 1 | 5 | 28.81 | 0.97 |
| | | 2 | 6 | 26.85 | 0.47 |
| | | 3 | 10 | 28.11 | 0.52 |
| | **Total** | **3** | **21** | **27.91** | **0.38** |
| Fuk | | 1 | 7 | 28.58 | 0.88 |
| | | 2 | 10 | 27.57 | 0.77 |
| | | 3 | 6 | 29.34 | 1.30 |
| | **Total** | **3** | **23** | **28.34** | **0.54** |
| Vil-0 | | 1 | 6 | 25.49 | 0.50 |
| | | 2 | 17 | 26.99 | 0.65 |
| | | 3 | 15 | 27.01 | 0.56 |
| | | 4 | 18 | 27.05 | 0.45 |
| | **Total** | **4** | **56** | **26.85** | **0.29** |
| Bl-1 | | 1 | 4 | 30.68 | 1.37 |
| | | 2 | 4 | 28.30 | 1.13 |
| | | 3 | 8 | 27.76 | 0.58 |
| | | 4 | 6 | 29.02 | 0.90 |
| | **Total** | **4** | **22** | **28.73** | **0.48** |
| Bla-10 | | 1 | 13 | 28.39 | 0.70 |
| | | 2 | 19 | 27.89 | 0.57 |
| | | 3 | 15 | 28.29 | 0.88 |
| | | 4 | 5 | 29.28 | 1.20 |
| | **Total** | **4** | **52** | **28.26** | **0.38** |

| | | | | | |
|---|---|---|---|---|---|
| Pak-3 | | 1 | 7 | 27.44 | 0.29 |
| | | 2 | 11 | 27.51 | 0.71 |
| | | 3 | 10 | 26.55 | 0.24 |
| | **Total** | **3** | **28** | **27.15** | **0.30** |
| YK | | 1 | 14 | 27.94 | 0.64 |
| | | 2 | 14 | 28.98 | 0.84 |
| | | 3 | 10 | 27.89 | 0.54 |
| | **Total** | **3** | **38** | **28.31** | **0.41** |
| AK | | 1 | 12 | 27.27 | 0.45 |
| | | 2 | 7 | 26.57 | 0.30 |
| | | 3 | 11 | 26.80 | 0.76 |
| | | 4 | 9 | 27.22 | 0.30 |
| | **Total** | **4** | **39** | **27.00** | **0.26** |
| Sij-1 | | 1 | 4 | 28.71 | 1.40 |
| | | 2 | 5 | 26.16 | 0.58 |
| | | 3 | 10 | 26.92 | 0.54 |
| | | 4 | 9 | 27.86 | 0.81 |
| | **Total** | **4** | **28** | **27.34** | **0.40** |
| Pyl | | 1 | 6 | 27.16 | 0.54 |
| | | 2 | 5 | 26.58 | 0.41 |
| | | 3 | 4 | 26.63 | 0.50 |
| | | 4 | 5 | 27.29 | 0.47 |
| | **Total** | **4** | **20** | **26.94** | **0.24** |
| Wha-2 | | 1 | 8 | 28.89 | 0.81 |
| | | 2 | 5 | 28.02 | 1.17 |
| | | 3 | 8 | 28.08 | 0.99 |
| | | 4 | 10 | 28.69 | 0.66 |
| | **Total** | **4** | **31** | **28.48** | **0.42** |
| Sapporo | | 1 | 7 | 26.44 | 0.76 |
| | | 2 | 6 | 26.25 | 0.60 |
| | | 3 | 8 | 27.34 | 0.67 |
| | | 4 | 9 | 28.29 | 0.69 |
| | **Total** | **4** | **30** | **27.20** | **0.36** |
| Ang | | 1 | 9 | 27.02 | 0.64 |
| | | 2 | 10 | 27.18 | 0.28 |
| | | 3 | 9 | 25.82 | 0.37 |
| | | 4 | 11 | 26.17 | 0.60 |

| | | | | | |
|---|---|---|---|---|---|
| | **Total** | **4** | **39** | **26.54** | **0.26** |
| Rome-1 | | 1 | 6 | 29.91 | 1.41 |
| | | 2 | 8 | 29.77 | 0.70 |
| | | 3 | 9 | 27.95 | 0.53 |
| | | 4 | 10 | 27.00 | 0.51 |
| | **Total** | **4** | **33** | **28.46** | **0.41** |
| Amel-1 | | 1 | 4 | 28.14 | 2.23 |
| | | 2 | 22 | 25.62 | 0.29 |
| | **Total** | **2** | **26** | **26.01** | **0.43** |
| Baa-1 | | 1 | 6 | 29.25 | 0.97 |
| | | 2 | 10 | 28.26 | 1.12 |
| | | 3 | 6 | 29.11 | 1.37 |
| | | 4 | 5 | 25.51 | 0.75 |
| | | 5 | 9 | 29.27 | 1.11 |
| | | 6 | 7 | 28.75 | 1.04 |
| | **Total** | **6** | **43** | **28.49** | **0.47** |
| Hey | | 1 | 11 | 25.97 | 0.31 |
| | | 2 | 8 | 26.42 | 0.71 |
| | | 3 | 6 | 27.82 | 0.80 |
| | | 4 | 5 | 29.02 | 1.28 |
| | **Total** | **4** | **30** | **26.97** | **0.39** |
| Wag-1 | | 1 | 6 | 27.59 | 0.86 |
| | | 2 | 10 | 27.64 | 0.59 |
| | | 3 | 11 | 28.09 | 0.60 |
| | | 4 | 8 | 28.44 | 0.81 |
| | **Total** | **4** | **35** | **27.95** | **0.33** |
| Gd-1 | | 1 | 11 | 24.86 | 0.28 |
| | | 2 | 10 | 24.58 | 0.30 |
| | | 3 | 7 | 26.35 | 0.55 |
| | **Total** | **3** | **28** | **25.13** | **0.24** |
| Cvi | | 1 | 7 | 25.55 | 0.50 |
| | | 2 | 20 | 25.97 | 0.19 |
| | | 3 | 11 | 25.98 | 0.29 |
| | **Total** | **3** | **38** | **25.90** | **0.16** |
| Ler | | 1 | 9 | 25.89 | 0.32 |
| | | 2 | 21 | 25.77 | 0.26 |
| | **Total** | **2** | **30** | **25.81** | **0.20** |
| Shakdara | | 1 | 17 | 27.34 | 0.44 |

| | | | | | |
|---|---|---|---|---|---|
| | | 2 | 3 | 27.72 | 1.77 |
| | | 3 | 18 | 26.85 | 0.32 |
| | **Total** | **3** | **38** | **27.14** | **0.28** |
| Bay-0 | | 1 | 9 | 27.52 | 0.62 |
| | | 2 | 10 | 27.48 | 0.69 |
| | **Total** | **2** | **19** | **27.50** | **0.45** |

Accessions denotes accession common name, # of plants denotes the individuals assayed per independent transformant, Period LD denotes period in hours after photic entrainment, SEM denotes Standard Error of the Mean.
Bold letters denote the total number of: independent transformants per accession, individuals assayed per accession, mean period of the total number of individuals per accession, and the averaged standard error

**Table S4 Periodicity of *CCR2::LUC* in natural Accessions after thermal entrainment**

| Accessions | | Independent transformants | # of plants | Period LD | SEM |
|---|---|---|---|---|---|
| En-2 | | 1 | 4 | 25.28 | 0.66 |
| | | 2 | 13 | 26.11 | 0.30 |
| | **Total** | **2** | **17** | **25.91** | **0.28** |
| Gd-1 | | 1 | 9 | 25.83 | 0.36 |
| | | 2 | 10 | 26.49 | 0.68 |
| | | 3 | 4 | 24.85 | 0.43 |
| | | 4 | 20 | 25.65 | 0.30 |
| | | 5 | 9 | 26.07 | 0.84 |
| | **Total** | **5** | **52** | **25.85** | **0.23** |
| Sei-0 | | 1 | 8 | 24.80 | 0.44 |
| | | 2 | 9 | 26.14 | 0.38 |
| | | 3 | 8 | 24.80 | 0.14 |
| | | 4 | 3 | 24.45 | 0.31 |
| | **Total** | **4** | **28** | **25.19** | **0.22** |
| St-0 | | 1 | 5 | 29.35 | 1.37 |
| | | 2 | 4 | 28.22 | 1.19 |
| | | 3 | 7 | 27.28 | 1.11 |
| | | 4 | 8 | 27.18 | 0.88 |
| | **Total** | **4** | **24** | **27.83** | **0.55** |
| KL-PW-1 | | 1 | 8 | 24.99 | 0.20 |
| | | 2 | 10 | 24.94 | 0.29 |
| | | 3 | 12 | 25.22 | 0.23 |
| | | 4 | 10 | 24.94 | 0.24 |
| | | 5 | 8 | 25.48 | 0.34 |
| | | 6 | 12 | 24.85 | 0.17 |
| | **Total** | **6** | **60** | **25.06** | **0.10** |
| Sed-1 | | 1 | 9 | 25.78 | 0.23 |
| | | 2 | 10 | 24.63 | 0.35 |
| | **Total** | **2** | **19** | **25.17** | **0.25** |
| Be-0 | | 1 | 6 | 25.52 | 0.72 |
| | | 2 | 6 | 27.08 | 0.40 |
| | | 3 | 3 | 28.46 | 1.25 |
| | **Total** | **3** | **15** | **26.73** | **0.48** |
| Bur-0 | | 1 | 8 | 25.17 | 0.38 |

| | | | | | |
|---|---|---|---|---|---|
| | | 2 | 9 | 24.84 | 0.18 |
| | | 3 | 9 | 24.99 | 0.18 |
| | | 4 | 11 | 25.58 | 0.33 |
| | | 5 | 6 | 24.57 | 0.23 |
| | **Total** | **5** | **43** | **25.08** | **0.13** |
| Cal-0 | | 1 | 8 | 24.77 | 0.37 |
| | | 2 | 9 | 25.26 | 0.27 |
| | | 3 | 8 | 25.37 | 0.64 |
| | **Total** | **3** | **25** | **25.14** | **0.25** |
| Ei-2 | | 1 | 6 | 26.78 | 0.76 |
| | | 2 | 6 | 25.24 | 0.77 |
| | | 3 | 6 | 28.38 | 1.59 |
| | **Total** | **3** | **18** | **26.80** | **0.68** |
| Eil-0 | | 1 | 9 | 25.26 | 0.48 |
| | | 2 | 9 | 24.77 | 0.43 |
| | | 3 | 9 | 25.22 | 1.09 |
| | | 4 | 5 | 24.59 | 0.15 |
| | **Total** | **4** | **32** | **25.01** | **0.34** |
| Es-0 | | 1 | 9 | 26.51 | 0.14 |
| | | 2 | 12 | 27.60 | 0.27 |
| | | 3 | 11 | 26.82 | 0.39 |
| | | 4 | 9 | 27.22 | 0.39 |
| | | 5 | 8 | 27.47 | 0.25 |
| | **Total** | **5** | **49** | **27.13** | **0.15** |
| Est-0 | | 1 | 11 | 26.14 | 0.23 |
| | | 2 | 11 | 26.40 | 0.58 |
| | | 3 | 8 | 25.34 | 0.19 |
| | **Total** | **3** | **30** | **26.02** | **0.24** |
| Ka-0 | | 1 | 3 | 25.41 | 0.26 |
| | | 2 | 7 | 24.22 | 0.52 |
| | | 3 | 11 | 24.09 | 0.37 |
| | | 4 | 6 | 26.93 | 0.58 |
| | | 5 | 16 | 25.60 | 0.39 |
| | | 6 | 12 | 26.92 | 0.84 |
| | | 7 | 12 | 26.89 | 0.48 |
| | **Total** | **7** | **67** | **25.79** | **0.25** |
| Mh-1 | | 1 | 9 | 26.91 | 0.43 |

| | | | | | |
|---|---|---|---|---|---|
| | | 2 | 7 | 27.71 | 0.60 |
| | | 3 | 10 | 26.71 | 0.44 |
| | **Total** | **3** | **26** | **27.05** | **0.28** |
| Rsh-0 | | 1 | 5 | 24.66 | 0.28 |
| | | 2 | 9 | 24.94 | 0.32 |
| | | 3 | 9 | 24.93 | 0.31 |
| | | 4 | 11 | 25.03 | 0.30 |
| | **Total** | **4** | **34** | **24.93** | **0.15** |
| Te-0 | | 1 | 6 | 27.19 | 0.39 |
| | | 2 | 7 | 27.10 | 0.53 |
| | | 3 | 9 | 26.40 | 0.36 |
| | **Total** | **3** | **22** | **26.84** | **0.25** |
| Lim | | 1 | 6 | 26.71 | 0.47 |
| | | 2 | 4 | 26.06 | 1.47 |
| | | 3 | 4 | 25.74 | 0.48 |
| | **Total** | **3** | **14** | **26.25** | **0.46** |
| Driel | | 1 | 9 | 27.74 | 0.72 |
| | | 2 | 6 | 25.12 | 0.66 |
| | | 3 | 5 | 27.09 | 1.33 |
| | | 4 | 4 | 25.99 | 0.87 |
| | | 5 | 6 | 26.55 | 0.87 |
| | | 6 | 5 | 29.07 | 0.51 |
| | **Total** | **6** | **35** | **26.99** | **0.38** |
| Terlet | | 1 | 2 | 25.37 | 0.38 |
| | | 2 | 6 | 24.11 | 0.61 |
| | | 3 | 3 | 25.08 | 1.28 |
| | | 4 | 3 | 25.39 | 0.90 |
| | | 5 | 4 | 24.40 | 0.33 |
| | **Total** | **5** | **18** | **24.69** | **0.33** |
| Jea | | 1 | 12 | 27.16 | 0.63 |
| | | 2 | 5 | 28.51 | 1.15 |
| | | 3 | 4 | 28.30 | 1.82 |
| | **Total** | **3** | **21** | **27.70** | **0.55** |
| Fuk | | 1 | 5 | 25.89 | 1.11 |
| | | 2 | 6 | 26.48 | 1.11 |
| | | 3 | 4 | 27.53 | 1.17 |
| | **Total** | **3** | **15** | **26.56** | **0.63** |
| Vil-0 | | 1 | 12 | 25.31 | 0.42 |

| | | | | | |
|---|---|---|---|---|---|
| | | 2 | 9 | 27.36 | 1.29 |
| | | 3 | 4 | 26.30 | 0.51 |
| | | 4 | 14 | 26.99 | 1.01 |
| | **Total** | **4** | **39** | **26.49** | **0.49** |
| Bl-1 | | 1 | 7 | 26.86 | 1.11 |
| | | 2 | 8 | 25.93 | 0.50 |
| | | 3 | 5 | 27.32 | 0.99 |
| | **Total** | **3** | **20** | **26.61** | **0.49** |
| Bla-10 | | 1 | 11 | 27.41 | 0.61 |
| | | 2 | 8 | 27.78 | 0.94 |
| | | 3 | 6 | 29.13 | 1.06 |
| | | 4 | 12 | 27.25 | 0.44 |
| | **Total** | **4** | **37** | **27.72** | **0.35** |
| Pak-3 | | 1 | 7 | 25.92 | 0.34 |
| | | 2 | 7 | 25.11 | 0.34 |
| | **Total** | **2** | **14** | **25.51** | **0.26** |
| YK | | 1 | 8 | 27.54 | 1.29 |
| | | 2 | 14 | 26.85 | 0.60 |
| | | 3 | 10 | 27.09 | 0.34 |
| | **Total** | **3** | **32** | **27.10** | **0.42** |
| AK | | 1 | 2 | 27.90 | 0.55 |
| | | 2 | 6 | 26.31 | 0.29 |
| | | 3 | 10 | 27.61 | 1.03 |
| | | 4 | 8 | 26.86 | 0.23 |
| | | 5 | 9 | 26.19 | 0.37 |
| | **Total** | **5** | **35** | **26.87** | **0.32** |
| Pyl | | 1 | 3 | 27.10 | 0.77 |
| | | 2 | 5 | 26.54 | 0.90 |
| | | 3 | 4 | 27.13 | 0.91 |
| | | 4 | 8 | 25.80 | 0.53 |
| | **Total** | **4** | **20** | **26.44** | **0.37** |
| Wha-2 | | 1 | 7 | 26.65 | 0.34 |
| | | 2 | 8 | 27.28 | 0.81 |
| | | 3 | 8 | 27.94 | 0.42 |
| | | 4 | 9 | 27.12 | 0.44 |
| | **Total** | **4** | **32** | **27.26** | **0.27** |
| KZ-13 | | 1 | 8 | 28.46 | 1.09 |

| | | | | | |
|---|---|---|---|---|---|
| | | 2 | 4 | 27.89 | 1.64 |
| | | 3 | 5 | 29.35 | 1.76 |
| | **Total** | **3** | **17** | **28.59** | **0.78** |
| Sapporo | | 1 | 5 | 26.27 | 0.76 |
| | | 2 | 5 | 28.93 | 1.09 |
| | | 3 | 9 | 29.43 | 1.16 |
| | | 4 | 6 | 28.53 | 1.06 |
| | **Total** | **4** | **25** | **28.48** | **0.58** |
| Ang | | 1 | 11 | 27.61 | 0.44 |
| | | 2 | 9 | 26.93 | 0.15 |
| | | 3 | 5 | 28.64 | 0.85 |
| | | 4 | 9 | 27.84 | 0.26 |
| | **Total** | **4** | **34** | **27.65** | **0.22** |
| Cerv-1 | | 1 | 2 | 29.12 | 3.85 |
| | | 2 | 3 | 29.09 | 2.31 |
| | | 3 | 7 | 29.22 | 0.79 |
| | **Total** | **3** | **12** | **29.17** | **0.81** |
| Rome-1 | | 1 | 3 | 32.09 | 1.46 |
| | | 2 | 4 | 33.36 | 0.56 |
| | | 3 | 2 | 27.26 | 0.42 |
| | | 4 | 7 | 29.04 | 1.02 |
| | **Total** | **4** | **16** | **30.47** | **0.75** |
| Amel-1 | | 1 | 4 | 27.10 | 0.55 |
| | | 2 | 16 | 25.78 | 0.33 |
| | **Total** | **2** | **20** | **26.04** | **0.30** |
| Baa-1 | | 1 | 2 | 28.28 | 1.05 |
| | | 2 | 6 | 25.98 | 0.18 |
| | | 3 | 9 | 25.97 | 0.46 |
| | | 4 | 8 | 27.34 | 0.73 |
| | | 5 | 3 | 26.44 | 0.70 |
| | | 6 | 4 | 28.75 | 2.25 |
| | **Total** | **6** | **32** | **26.85** | **0.38** |
| Hey | | 1 | 3 | 28.40 | 0.60 |
| | | 2 | 9 | 27.62 | 0.81 |
| | | 3 | 2 | 27.50 | 0.89 |
| | **Total** | **3** | **14** | **27.77** | **0.54** |
| Wag-1 | | 1 | 3 | 31.18 | 0.54 |
| | | 2 | 5 | 28.26 | 0.61 |

| | | | | | |
|---|---|---|---|---|---|
| | | 3 | 7 | 28.81 | 0.71 |
| | | 4 | 6 | 30.90 | 1.28 |
| | **Total** | **4** | **21** | **29.61** | **0.51** |
| Kas-1 | | 1 | 6 | 24.87 | 0.16 |
| | | 2 | 2 | 25.62 | 0.22 |
| | | 3 | 7 | 25.80 | 0.52 |
| | **Total** | **3** | **15** | **25.40** | **0.27** |
| Cvi | | 1 | 10 | 25.72 | 0.20 |
| | | 2 | 11 | 24.88 | 0.23 |
| | | 3 | 23 | 25.28 | 0.18 |
| | **Total** | 3 | **44** | **25.28** | **0.12** |
| Ler | | 1 | 12 | 25.00 | 0.16 |
| | | 2 | 12 | 24.74 | 0.32 |
| | **Total** | 2 | **24** | **24.87** | **0.18** |
| Shakdara | | 1 | 12 | 25.62 | 0.36 |
| | | 2 | 9 | 24.43 | 0.40 |
| | | 3 | 10 | 24.41 | 0.26 |
| | | 4 | 28 | 25.75 | 0.24 |
| | **Total** | **4** | **59** | **25.30** | **0.17** |
| Bay-0 | | 1 | 13 | 25.68 | 0.51 |
| | | 2 | 17 | 25.09 | 0.40 |
| | **Total** | **2** | **30** | **25.35** | **0.31** |

Accessions denotes accession common name, # of plants denotes the individuals assayed per independent transformant, Period TMP denotes period in hours after thermal entrainment, SEM denotes Standard Error of the Mean.
Bold letters denote the total number of: independent transformants, individuals assayed per accession, mean period of the total number of individuals per accession, and the averaged standard error