

# Genetic architecture of the circadian clock and flowering time in *Brassica rapa*

P. Lou · Q. Xie · X. Xu · C. E. Edwards ·  
M. T. Brock · C. Weinig · C. R. McClung

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**Abstract** The circadian clock serves to coordinate physiology and behavior with the diurnal cycles derived from the daily rotation of the earth. In plants, circadian rhythms contribute to growth and yield and, hence, to both agricultural productivity and evolutionary fitness. *Arabidopsis thaliana* has served as a tractable model species in which to dissect clock mechanism and function, but it now becomes important to define the extent to which the *Arabidopsis* model can be extrapolated to other species, including crops. Accordingly, we have extended our studies to the close *Arabidopsis* relative and crop species, *Brassica rapa*. We have investigated natural variation in circadian function and flowering time among multiple *B. rapa* collections. There is wide variation in clock function, based on a robust rhythm in cotyledon movement,

within a collection of *B. rapa* accessions, wild populations and recombinant inbred lines (RILs) derived from a cross between parents from two distinct subspecies, a rapid cycling Chinese cabbage (ssp. *pekinensis*) and a Yellow Sarson oilseed (ssp. *trilocularis*). We further analyzed the RILs to identify the quantitative trait loci (QTL) responsible for this natural variation in clock period and temperature compensation, as well as for flowering time under different temperature and day length settings. Most clock and flowering-time QTL mapped to overlapping chromosomal loci. We have exploited micro-synteny between the *Arabidopsis* and *B. rapa* genomes to identify candidate genes for these QTL.

## Introduction

Circadian rhythms are endogenously generated and self-sustaining, and exhibit periods of ~24 h. These rhythms are temperature compensated, meaning that the period is maintained relatively constant over a broad range of temperatures (Johnson et al. 2004). Circadian rhythms are widespread from cyanobacteria to mammals and allow the coordination of biological functions with the environmental oscillation of day and night imposed by the earth's rotation (Zhang and Kay 2010). The ability to use a circadian clock to anticipate the transitions associated with light/dark cycles confers a fitness advantage as shown in cyanobacteria, *Arabidopsis*, and chipmunks (DeCoursey et al. 2000; Dodd et al. 2005; Graf et al. 2010; Green et al. 2002; Michael et al. 2003; Ouyang et al. 1998; Yerushalmi et al. 2011). In *Arabidopsis*, biomass and yield are adversely affected in clock mutants or when the environmental period deviates from 24 h (Dodd et al. 2005). This has been attributed to adverse effects on photosynthetic

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P. Lou, Q. Xie and X. Xu contributed equally to this work.

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P. Lou · Q. Xie · X. Xu · C. R. McClung (✉)  
Department of Biological Sciences, 6044 Gilman Laboratories,  
Dartmouth College, Hanover, NH 03755, USA  
e-mail: c.robertson.mcclung@dartmouth.edu

C. E. Edwards · M. T. Brock · C. Weinig  
Department of Botany, University of Wyoming, Laramie,  
WY 82071, USA

**Present Address:**

X. Xu  
Hebei Key Laboratory of Molecular Cell Biology,  
College of Biological Sciences, Hebei Normal University,  
Shijiazhuang 050016, Hebei, China

rates (Dodd et al. 2005) and also to suboptimal starch utilization during the night (Graf et al. 2010). In addition, the analysis of allopolyploids and hybrids in *Arabidopsis* has suggested that clock function may contribute to hybrid vigor (Ni et al. 2009). These results impel the study of the genetics and physiology of circadian rhythms in crops to develop tools of use for increasing yield.

The *Arabidopsis* clock is composed of multiple interlocked feedback loops (Harmer 2009; McClung and Gutiérrez 2010; Pruneda-Paz and Kay 2010). Two single Myb domain transcription factors, CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), together with TIMING OF CAB EXPRESSION 1 (TOC1) form a central feedback loop. CCA1 and LHY also participate in the second loop as positive regulators of *PSEUDO RESPONSE REGULATOR 7* (*PRR7*) and *PRR9*, which encode negative regulators of *CCA1* and *LHY* (Nakamichi et al. 2010). TOC1 participates in a third loop as a negative regulator of *GIGANTEA* (*GI*) and possibly *PRR5* (Harmer 2009). Little is known of how variation in these components contributes to functional differences in clock function among natural populations. Several studies have identified allelic variation in clock function, including period length and temperature compensation, based on QTL analysis in *Arabidopsis* (Darrah et al. 2006; Edwards et al. 2006; Edwards et al. 2005; Michael et al. 2003; Swarup et al. 1999), and a number of known clock genes have been suggested as candidates underlying some of these QTL.

It is important to address the extent to which the circadian clock model elucidated in *Arabidopsis* will apply to other plants. Orthologs to *Arabidopsis* clock genes have been identified in a number of species, and phylogenetic analysis suggests that the common ancestor of monocots and eudicots had the components to construct clocks consisting of multiple interlocked feedback loops (McClung 2010; Takata et al. 2010).

*Brassica* species include a number of crops adapted to a broad range of agricultural and climatic conditions. *Brassica* and *Arabidopsis* lineages have only recently, ~43 Mya, diverged (Beilstein et al. 2010). Extensive studies on the syntenic relationships between *Arabidopsis* and *Brassica* at different levels have revealed that conserved colinearity is found in several but not in all genomic regions (Cheung et al. 2009; Kim et al. 2007, 2009; Parkin et al. 2005; Schranz et al. 2006; Trick et al. 2009). *B. rapa* orthologs of the *TOC1/PRR* clock genes have been identified by comparative genomics with *Arabidopsis* (Kim et al. 2007), but functional studies of the genetic and molecular bases of natural variation in circadian clock function are in their infancy. Quantitative trait loci (QTL) for cotyledon movement have been detected in *B. oleracea* (Salathia et al. 2007), and we have recently described a

circadian rhythm in cotyledon movement in *B. rapa* and shown that *B. rapa* tissue culture expresses circadian rhythms in gene expression (Xu et al. 2010). Here, we identify natural variation in clock function as well as correlated effects on flowering time among natural accessions and cultivars of *B. rapa* and identify QTL for these traits. *B. rapa*, like *B. oleracea*, displays a robust circadian rhythm in cotyledon movement over a broad range of temperatures, allowing the identification of loci that contribute to period length and temperature compensation. In addition, we identify QTL for flowering time and explore the correlation between circadian clock function and flowering time.

## Materials and methods

### *Brassica rapa* cultivars, accessions and recombinant inbred lines

Genotypes used in this study include a collection of 50 *B. rapa* accessions, provided by Genetic Resources Centre (CGN) in the Netherlands that includes traditional cultivars and breeding materials from different latitudes (Fig. S1 and Table S1). In addition, eight wild *B. rapa* populations were sampled from southern California (Fig. S2). Finally, we used a *Brassica rapa* mapping population, BraIRRI (*Brassica rapa* International Reference Recombinant Inbred), consisting of 159 RILs derived from a cross between a rapid cycling Chinese cabbage (ssp. *pekinensis*) genotype (IMB211; ♀ parent) and an annual Yellow Sarson seed oil (ssp. *trilocularis*) genotype (R500; ♂ parent). The F1 generation was selfed and the progeny were advanced to the S6 generation by single seed descent (Iniguez-Luy et al. 2009). A linkage map of 1125 cM and a mean distance between marker loci of 5.7 cM was used. However, for the current work the north–south orientations of Chr. A5, A8, A9 and A10 are inverted relative to Iniguez-Luy et al. (Iniguez-Luy et al. 2009) to be consistent with the orientation defined by Parkin et al. (Parkin et al. 2005).

### Plant growth conditions for cotyledon movement measurement

Seeds were sown in a standard soil mixture (Pro-mix “BX,” Premier, Rivière-du-Loup, Québec, Canada) in 1.5-inch diameter pots and stratified at 4°C for 4 days to synchronize germination, then released into growth chambers with 12-h light (120  $\mu\text{mol m}^{-2} \text{s}^{-1}$  white light provided by fluorescent and incandescent bulbs)/12-h dark cycles at constant 18°C temperature for 5–7 days, until most individuals had fully emerged cotyledons. Ten individual plants per RIL were grown in a completely randomized

design. For the cotyledon movement assay, white polystyrene balls were glued to the end of the cotyledon blade to improve the tracking of cotyledon position (Salathia et al. 2007), and then seedlings were transferred into a growth chamber under continuous white light at different (12, 18 or 24°C) temperatures to determine the effects of temperature on circadian parameters.  $Q_{10}$  is the increase in rate (the inverse of period) for a 10°C increase in temperature and was calculated as  $Q_{10} = (1/\text{period}_{T+10}) / (1/\text{period}_T)$ , where  $\text{period}_{T+10}$  is the period at temperature  $T + 10$  and  $\text{period}_T$  is the period at temperature  $T$ . Seedlings were imaged using a Lariion Digital Video Surveillance System (<http://www.lariion.com>) (Xu et al. 2010). Images were recorded every 10 min for at least 5 days. Cotyledon position was recovered from the time series images using nktrace (Onai et al. 2004) and Metamorph software (Molecular Devices).

#### Growth conditions and trait estimates for flowering time

One individual of each of the RILs and the two parental genotypes were grown in each of six growth chamber compartments. For all growth chambers, light intensity during light cycles was maintained at a photosynthetic photon flux density (PPFD) of  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  and the vapor pressure deficit was maintained below 1.7. The growth chambers were set to the following conditions: (1) WL: warm temperature (24°C), long photoperiod (14 h/10 h light/dark cycles); (2) CL: cool temperature (12°C), long photoperiod (14 h/10 h light/dark cycles); and (3) CS: cool temperature (12°C), short photoperiod (10 h/14 h light/dark cycles). These treatments simulate conditions encountered by crop and wild populations. Each of these treatments occupied two of the six growth chambers at one time, and this was temporally replicated three times so that a total of six replicates of each genotype were grown in each of the three treatments. The treatments rotated among growth chambers such that each treatment occupied each growth chamber compartment once.

For each genotype, three seeds were planted in  $360 \text{ cm}^3$  pots filled with 1 ml of Osmocote 18-6-12 N-P-K fertilizer (Scotts Miracle Grow, Marysville, OH, USA), and a 50/50 combination of metromix 200 soil (Sun-Gro Horticulture, Vancouver, BC, Canada) and Fafard custom mix (Conrad Fafard Inc., Agawam, MA, USA). After planting, the seeds were lightly covered with vermiculite and then cold/dark stratified for 3 days at 4°C. The pots were then placed into growth chambers set to the three treatments, with genotypes fully randomized within each chamber. Once seeds had germinated, seedlings were thinned to one plant closest to the center of the pot. Plants were watered regularly to maintain moist soil conditions.

Plants were checked daily for bolting, which was defined as the date when buds 1 mm in length were visible. Once a plant had bolted, it was checked each morning for flowering. After flowering, each plant was checked daily at 2 p.m. for the presence of a mature flower; this additional measure of flowering was collected because some genotypes aborted several buds before producing the first viable flower. To estimate phenology, we recorded planting to first mature flower (PtoF), and bolting to flowering (BtoF).

#### Statistical analysis

Circadian clock parameters were analyzed from leaf movement data using fast Fourier transform non-linear least squares (FFT-NLLS) (Plautz et al. 1997) in BRASS software (<http://millar.bio.ed.ac.uk/PEBrown/BRASS/BrassPage.htm>). For the estimation of mean circadian periods, results were restricted to period values within the range of 18–32 h; values outside that range were considered to be non-circadian and excluded from further analysis. Phase estimates were normalized for differences in period and are presented in circadian time (CT = phase/period  $\times$  24 h). Amplitude measures the distance that a cotyledon moves in the y-axis over one period; amplitude therefore estimates the magnitude of the cotyledon movement but does not represent an estimate of amplitude of the circadian oscillator.

We carried out separate statistical analyses on the following four data sets: (1) circadian data from the CGN accessions, (2) circadian data from the wild California populations, (3) circadian data from the RILs, and (4) flowering-time data from the RILs. For each of these four data sets, we used restricted maximum likelihood to estimate the random effect of genotype on each phenotypic trait in each treatment (PROC MIXED, SAS ver. 9.2). The variance components estimated from this analysis were used to estimate broad-sense heritability for each trait,  $V_G/V_P$ , where  $V_G$  is the among-genotype variance component in each treatment and  $V_P$  is the sum of all variance components for a trait in each treatment.

We next carried out analysis of variance to evaluate the fixed effect of treatment and the random effects of genotype and the genotype  $\times$  treatment interaction (PROC MIXED, SAS ver. 9.2). While a fully randomized design was used to measure circadian traits, the growth chamber experiment that measured phenological traits was spatially and temporally blocked. Chamber nested within treatment and temporal block were therefore included as an additional factor in the model for phenological traits; however, the blocking factors were never significant and are not reported further. The preceding models were also used to estimate variance components attributable to each factor and the genotypic least-square means of each trait in each

treatment, the latter of which were used in QTL mapping (see “QTL analysis” below). To determine the source of significant genotype  $\times$  treatment interactions, we removed one treatment at a time from the data set and re-ran the two-way ANOVA model.

Using the genotypic estimates from above, bivariate correlations were estimated between circadian clock parameters and the geographic origin of the 50 accessions, between clock parameters and  $Q_{10}$ , and between clock parameters and flowering-time parameters (SPSS Version18).

### QTL analysis

The linkage map used in this study consisted of 224 RFLP and microsatellite markers covering ten linkage groups, with an average marker density of 5.7 cM/marker (Iniguez-Luy et al. 2009). Composite interval mapping in QTL Cartographer (Wang et al. 2007) was utilized for all QTL mapping. Genome-wide significance thresholds were estimated using 1,000 permutations in QTL cartographer with a type-I error rate of 0.05. The output from QTL Cartographer was further verified using the fitQTL model in the R/QTL software package. We also performed analyses of the epistatic interactions on each trait using the scantwo procedure in R/QTL (R Development Core Team 2009). The final QTL results are graphically displayed including 1-LOD and 2-LOD support intervals using Mapchart 2.1 (Voorrips 2002). We considered QTL to co-localize when their 2-LOD support intervals overlapped.

For individual QTL, we tested for significant QTL  $\times$  treatment interactions. For these analyses, we evaluated the fixed effects of treatment, the genotype at the marker nearest to each detected QTL and the marker  $\times$  treatment interaction on the genotypic values of each trait (PROC GLM, SAS ver. 9.2). To differentiate the effects of temperature, for instance, on the expression of circadian QTL, these analyses were carried out for all pairwise combinations of treatments in which the QTL was detected (for example, if the QTL was detected in 12°C, we evaluated QTL  $\times$  treatment interactions across 12/18°C and across 12/24°C).

## Results

### Natural variation in circadian clock parameters within *Brassica rapa*

To investigate the natural variation of circadian clock function within *B. rapa*, we measured clock parameters by cotyledon movement using three different genetic materials: 50 *B. rapa* accessions including breeding material and

landraces, 8 wild populations collected from southern California, USA, and a population of 159 RILs derived from parents representing two subspecies, *pekinensis* and *trilocularis*.

The 50 accessions represent different morphotypes and geographic origins primarily from Europe (Table S1; Fig. S1). The 50 accessions exhibited considerable variation in circadian period at each of the three temperatures tested (Table S2), with period length ranging from  $\sim 22$  to 28 h and with the majority of accessions showing a period length of 25–26 h (Fig. 1a and S3). There was very little difference in average period length between 12 and 18°C, but periods were shorter by about 1 h at 24°C (Fig. 1a; significant effect of treatment, Table S3).

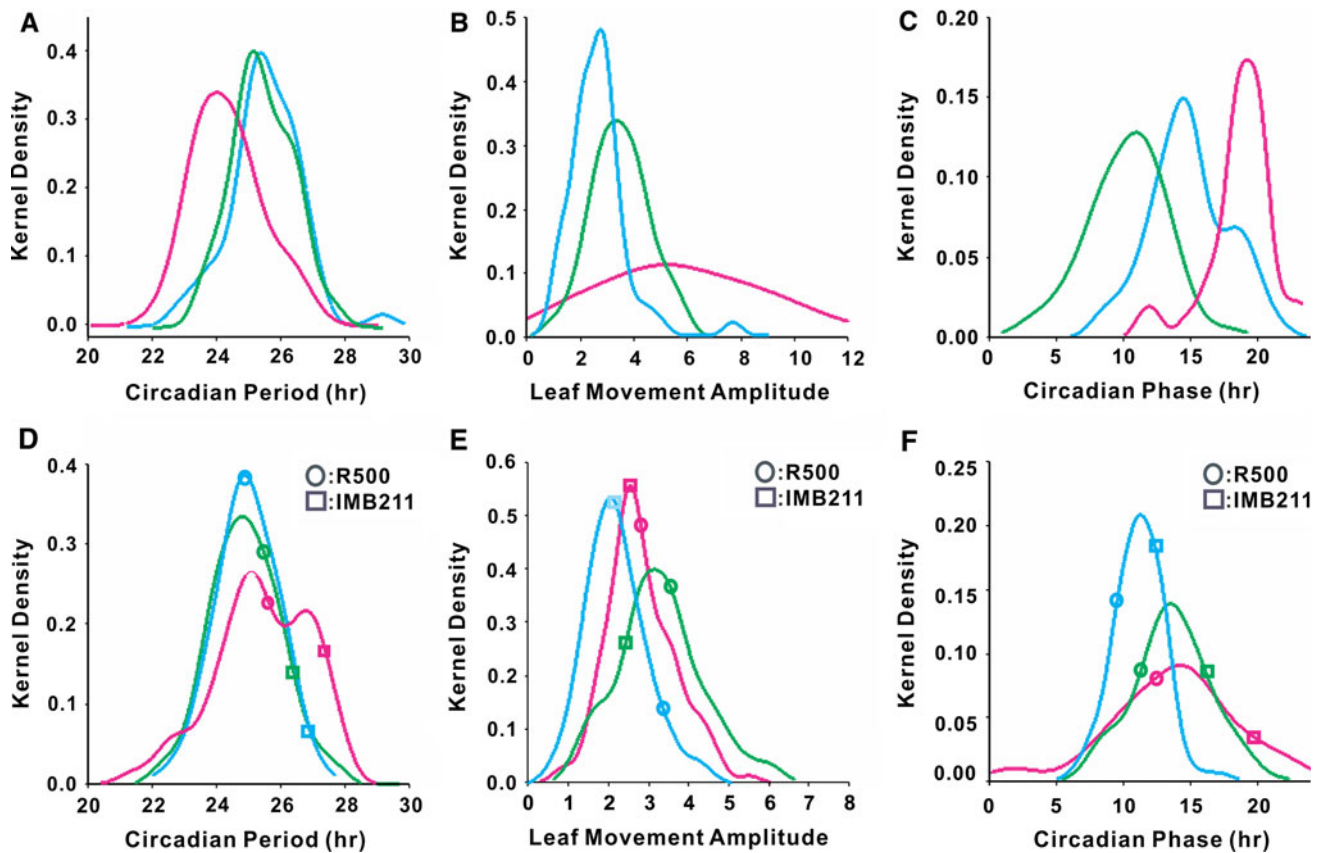
Among the 50 accessions, there was considerable variation in phase, defined as the peak or acrophase of the cotyledon movement rhythm, within each treatment (Table S2), and the distributions in phase responded to temperature, with the earliest phases at 24°C and the latest phases at 18°C (Fig. 1c; significant effect of treatment, Table S3). Amplitude, defined as one-half the peak to trough distance of cotyledon movement, does not refer to amplitude of circadian oscillator cycle, but instead gives an indication of the magnitude of cotyledon movement. The amplitude was weakest at 24°C (Fig. 1b and S3), which apparently approached the upper temperature limit at which this rhythmic output could be detected. We asked if there was evidence for systematic variation in clock parameters with geographic location, but detected no significant correlation between clock parameters and either latitude or longitude.

For the eight wild populations from southern California, USA (Fig. S2), we detected significant (or marginally significant) effects of population on period at 12 and 18°C ( $p = 0.04$  and  $0.07$ , respectively), but not at 24°C (Table S2). It is also worth noting that there was substantial phenotypic variation within each population, with differences in period ranging from as little as 1.0 h to as much as 4.3 h at all three temperatures (Fig. S4).

There was considerable variation in clock parameters between the parents of the RIL collection, oil type Yellow Sarson (R500) and Rapid Cycling line (IMB211) at each of the three temperatures tested (Fig. 1d–f and S5). The period of the cotyledon movement rhythm of IMB211 was 1–2 h longer than that of R500 at each temperature. Correspondingly, RILs differed significantly in period length (Table S2), and the variation in period among the RILs showed transgressive inheritance, ranging from  $\sim 22$  to 27 h (range of 5.3 h) at 12°C, from  $\sim 22$  to 28 h (range of 5.8 h) at 18°C, and from  $\sim 21$  to 28 h (range of 6.7 h) at 24°C (Fig. 1d and S5).

Each of the clock parameters showed a continuous distribution at all three temperatures, demonstrating multi-genic control of the clock function in *B. rapa* and





**Fig. 1** Distribution of clock parameters among accessions and recombinant inbred lines. Kernel density, a non-parametric estimate of frequency (Parzen 1962; Rosenblatt 1956), of the circadian parameters period (a, d), amplitude (b, e) and phase of the oscillation

(c, f), as determined by cotyledon movement, among natural accessions (a–c) and RILs (d–f). Cotyledon movement was measured at three temperatures: 12°C (cyan), 18°C (green), and 24°C (magenta)

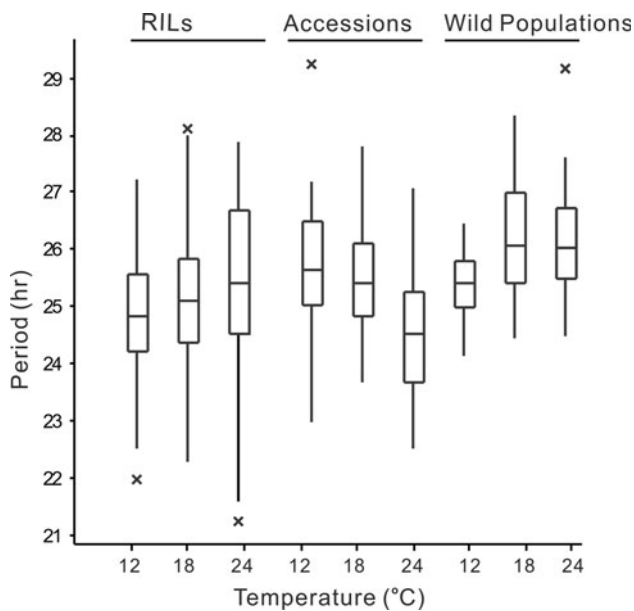
suggesting that there are multiple period-shortening and period-lengthening loci (Fig. 1a, d; and S5). As noted, the IMB211 period was consistently longer than that of R500 and that of most RILs, suggesting that R500 alleles tend to shorten period. In general, the ranges in period were greater among the RILs (6.5 h) than among the accessions (5 h) or the wild California populations (4 h), which may suggest a homeostatic effect of natural selection acting against phenotypic extremes. Phase and amplitude also showed transgressive variation at all temperatures (Fig. 1e, f; and S5), but expressed values both higher and lower than the parental ones, suggesting that both parents contain loci that increased or decreased these phenotypes. For circadian phase, the IMB211 alleles tended to have a lagging (greater phase value) effect and for amplitude, while the R500 alleles tended to increase the amplitude of movement.

#### Natural variation in temperature compensation within *B. rapa*

We tested the responsiveness of all the *B. rapa* genotypes to three different temperatures (12, 18 and 24°C) in order

to characterize temperature compensation. All the genotypes expressed robust rhythms in cotyledon movement at 12 and 18°C, but a number of lines failed to express rhythms in cotyledon movement at 24°C. Among the 50 accessions period generally shortened with increasing temperature, as alluded to above, whereas in the RILs period generally lengthened with increasing temperature. The wild California populations showed the longest period at higher temperatures of 18 and 24°C (Fig. 2).

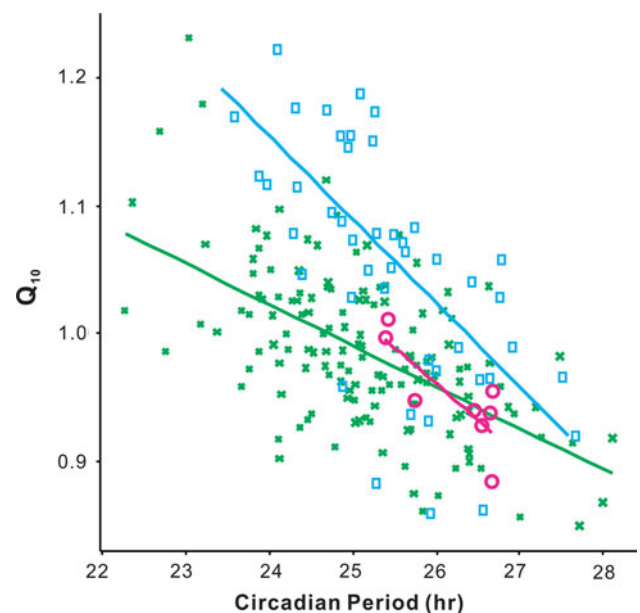
Although period varied with temperature, all the lines examined showed strong temperature compensation.  $Q_{10}$ , the temperature coefficient, is the change in rate in response to an increase of 10°C, and is  $\sim 2$  for typical biochemical reactions. Most of the RIL lines and accessions showed  $Q_{10}$  between 0.9 and 1.2 and the wild California populations showed a narrow range in  $Q_{10}$  of 0.9–1.0, indicating that clock function was fairly constant between 12 and 18°C (Fig. 3).  $Q_{10}$  values calculated for 12 versus 24°C and for 18 versus 24°C were consistent with those for 12 versus 18°C. Because a number of the genotypes were arrhythmic at 24°C, we have emphasized  $Q_{10}$  values calculated for the lower range of temperatures.



**Fig. 2** Response to temperature in clock parameters of RILs, accessions and wild populations. The box spans the interquartile range (25–75%) of the values in the variate, so the middle 50% of the data lie within the box, with a horizontal line indicating the median. Whiskers extend beyond the ends of the box as far as the minimum and maximum values. X indicates variate outside of boundary (95%)

There is a negative correlation between  $Q_{10}$  and period in RIL populations ( $r = -0.60$ ,  $p < 0.001$ ) and accessions ( $r = -0.62$ ,  $p < 0.001$ ). We observed a more negative correlation in wild California populations ( $r = -0.80$ ,  $p = 0.31$ ), although this correlation was not statistically significant because of small sample size.  $Q_{10}$  was not correlated with geographic origin of the accessions.

For the accessions and RILs, the genotype  $\times$  treatment interaction for circadian period was significant (Table S3), indicating that genotypes differed in the response of circadian period to increasing temperatures. Despite the significant genotype  $\times$  treatment interaction, most genotypes, including the RIL parents (IMB211 and R500), the accessions and the wild populations, showed relatively little change in period across the three temperatures (Fig. S6A and S7A, Table S4). However, some genotypes differed from this pattern; for example, several genotypes showed similar period lengths at low and high temperatures, but either slightly longer or shorter periods at the intermediate temperature (Fig. S6, E and F, S7E). Other genotypes showed a slight period shortening at higher temperatures (Fig. S6, D and G, S7, D and G), but in every case the  $Q_{10}$  was less than 1.2, indicating that temperature compensation was maintained. Only one accession, a Chinese cabbage (CC-048), showed a progressive shortening of period with increasing temperature, suggestive of a loss of temperature compensation (Fig. S6, I); however, the  $Q_{10}$  for this accession was 1.18, indicating that period length was



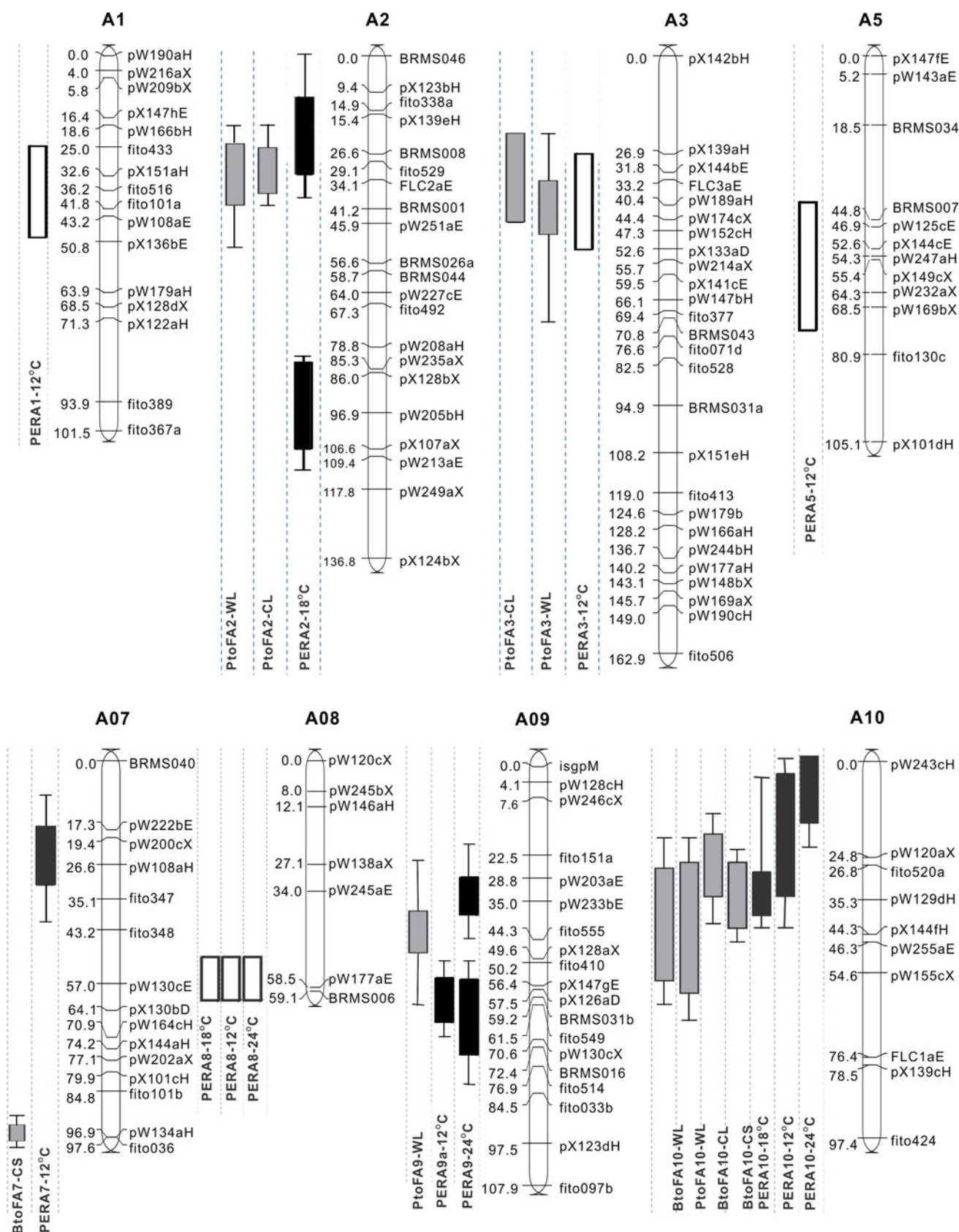
**Fig. 3** Relationship of temperature compensation with period length. Temperature response ( $Q_{10}$ ) of circadian period from 12 to 18°C plotted against period at 18°C. RILs green X, accessions cyan squares, wild populations magenta circles

nevertheless compensated. A number of genotypes showed a slight period lengthening with increasing temperature (Fig. S6, B and C, Fig. S7B), which was most pronounced for one RIL (RIL109; Fig. S6H) and for one accession (a vegetable turnip variety from the former Soviet Union, VT-007; Fig. S7H). This is consistent with weak over-compensation, as described in some long-period mutants in *Neurospora crassa* (Gardner and Feldman 1981; Mehra et al. 2009), *Drosophila melanogaster* (Huang et al. 1995; Matsumoto et al. 1999) and *Arabidopsis* (Salomé et al. 2010).

#### QTL analysis of clock parameters and temperature compensation

We detected QTL for circadian period and amplitude in the RIL populations at each temperature (Fig. 4; Table 1). QTL were named PER for period and AMP for amplitude, followed by the chromosome position of the QTL.

**Fig. 4** Period and phenological (flowering-time) quantitative trait loci. Location of quantitative trait loci (QTL) of clock parameters and flowering time detected in the *Brassica rapa* IRR1 RIL population at different environmental settings. LOD profiles are graphically displayed using Map Chart, including the LOD minus 1/LOD minus 2 support interval to approximate a 95% confidence interval. Black bars QTL for clock parameters, open bar nonsignificant QTL for clock parameters, gray bars QTL for flowering time. QTL nomenclature: PtoF, planting to first mature flower; BtoF, bolting to flowering; WL, warm (24°C) long (14-h light) days; CL, cool (12°C) long (14 h light) days; PER, circadian period at the indicated temperature





We identified a total of six significant and four additional marginally significant ( $p = 0.075$ ) period QTL in the RIL population; collectively, these QTL explained 55.8, 34.8 and 38.9% of the genetically determined variation among the RILs at 12, 18 and 24°C, respectively. We detected no significant QTL for circadian phase in this study.

The major period QTL, PERA10, was observed at all three temperatures and explained from 11.6 to 14.2% of the variation in period length, depending on the treatment; the R500 allele shortened the period, consistent with the shorter period of R500 at all three temperatures (Table 1). A second QTL, PERA8, was also observed at all three temperatures, with the R500 allele increasing the period. However, the expression of many QTL showed temperature specificity (i.e., the QTL  $\times$  environment interaction was significant; Table 1). Even when the interaction effect was not formally significant, QTL mapped in only one environment may well be temperature-specific, given the missing genotypic information for some RILs and the generally low power to test for environmental interactions. Several QTL were mapped only in a single environment; for example, two QTL, PERA2a and PERA2b, were detected only at 18°C, with the R500 alleles increasing the period length. The observed QTL effects are consistent with a model of multigenic inheritance in which period is determined by the additive effects of multiple period-lengthening and period-shortening alleles. Four QTL, PERA1, PERA3, PERA5 and PERA7, were specific to low (12°C) temperature and one QTL, PERA9b, was specific at high (24°C) temperature. One QTL, PERA9a, was detected at both 12 and 24°C, suggesting sensitivity to temperature extremes.

QTL for flowering time in different environmental settings

Flowering time, measured both as planting to first mature flower (PtoF) and bolting to flowering (BtoF), was accelerated by increased day length and higher temperature (Table S2). Among the RILs, there was a strong genotypic component to this variation, as indicated by the highly significant ( $p < 0.0001$ )  $V_G$  values (Table S2). Flowering-time QTL were mapped in the RIL populations grown under three different conditions in growth chambers: cool short days (CS; 12°C, 10 h light/14 h dark), cool long days (CL; 12°C, 14 h light/10 h dark) and warm long days (WL; 24°C, 14 h light/10 h dark). A total of six QTL were mapped in the RIL population for flowering time under different temperature and day length settings, with some of the QTL showing temperature or day length specificity (Table 2). Two flowering-time QTL, PtoFA2 and PtoFA3, were detected only in long days, BtoFA7 was detected only in short days, and PtoFA9 and PtoFA10 were detected only at high temperature. The major flowering-time QTL, BtoFA10, explained from 13 to 18% of the variation and was observed in all conditions for bolting to flowering. In all temperature and day length settings, the R500 alleles delayed flowering time, consistent with the relative flowering times of the parents (IMB211 is a rapid cyclus).

Correlations between circadian traits and flowering time

The magnitude of genotypic correlations between circadian period and flowering time was generally small, but

**Table 1** Summary of circadian period QTL detected in the *B. rapa* IRR1 population

QTL	CHR	12°C				18°C				24°C				QTL $\times$ E
		LOD	INT (POS)	VAR	ADD	LOD	INT (POS)	VAR	ADD	LOD	INT (POS)	VAR	ADD	
PERA1	A1	2.4	NA (35)	5.3	0.2									
PERA2a	A2					2.7	12–24 (15)	8.1	0.3					
PERA2b	A2					2.9	85–112 (95)	8.7	0.4					
PERA3	A3	2.3	NA (45)	4.6	0.2									
PERA5	A5	2	NA (55)	4	0.2									
PERA7	A7	4.3	8–42 (34)	10.6	–0.3									12°C/24°C *
PERA8	A8	2.2	NA (58)	6.3	0.2	2.1	NA (58)	6.4	0.3	2	NA (58)	6	0.3	
PERA9a	A9	3.8	66–74 (67)	10.8	–0.3					2.6	56–75 (61)	9.6	–0.5	18°C/24°C *
PERA9b	A9									3	31–40 (36)	11.2	–0.5	18°C/24°C *
PERA10	A10	5.1	25–45 (34)	14.2	–0.4	4	0–40 (11)	11.6	–0.5	3.4	0–17 (4)	12.1	–0.6	

QTL for circadian period (PER) of the cotyledon movement rhythm are numbered by chromosomal location. *CHR* chromosome in the *Brassica A* genome; *LOD* LOD score of the QTL. *INT/POS*  $\pm$  2-LOD QTL interval (peak position) (cM); *NA* not applicable, because the LOD score of the QTL was non-significant; *VAR* % variation explained by QTL; *ADD* additive effects of the QTL (in h). Positive values indicate that the IMB211 allele is associated with increased value of period. QTL values in italics are not statistically significant but are recorded because significant QTL were detected at similar positions in other conditions; *QTL  $\times$  E* QTL that demonstrate significant QTL  $\times$  E across treatment pairs. \* $p < 0.05$



**Table 2** Summary of flowering-time QTL detected in *B. rapa* IRR1 population

QTL	CHR	CL				CS				WL				QTL $\times$ E
		LOD	INT (POS)	VAR	ADD	LOD	INT (POS)	VAR	ADD	LOD	INT (POS)	VAR	ADD	
PtoFA2	A2	3.2	18–39 (31)	9.8	2.2					2.6	18–50 (31)	7.7	1.9	
PtoFA3	A3	2.5	29–52 (44)	7.6	1.8					3.0	29–79 (48)	9	2.0	
BtoFA7	A7					5.2	90–97 (97)	15.5	0.9					WL/CS ***
PtoFA9	A9									3.0	27–63 (46)	9.1	2.0	
PtoFA10	A10									3.1	19–65 (40)	9.2	2.1	
BtoFA10	A10	5.2	13–41 (28)	15.3	0.8	5.1	22–45 (31)	15.2	1.0	4.6	19–61 (48)	13.3	0.7	

QTL for planting to first mature flower (PtoF) or bolting to flowering (BtoF) are numbered by chromosomal location; *CHR* chromosome in the *Brassica* A genome, *CL* cool long days (12°C, 14-h light/10-h dark), *CS* cool short days (12°C, 10-h light/14-h dark), *WL* warm long days (24°C, 14-h light/10-h dark), *LOD* LOD score of the QTL, *INT (POS)*  $\pm$  2-LOD QTL interval (peak position) (cM), *VAR* % variation explained by QTL, *ADD* additive effects of the QTL (days). Positive values indicate the R500 allele is associated with increased value. *QTL  $\times$  E* QTL that demonstrate significant QTL  $\times$  E across treatment pairs.\*\*\*  $p < 0.005$

significant (Fig. S8). Period was negatively correlated with flowering time (BtoF) in cool, long days ( $r = -0.24$ ,  $p = 0.004$ ) as well as in cool, short days ( $r = -0.16$ ,  $p = 0.054$ ). This correlation was non-significant in warm long days (Fig. S9). Despite weak genotypic correlations, a number of circadian period QTL and flowering-time QTL co-localized (Fig. 4). For example, PERA10 overlapped with PtoFA10/BtoFA10, and the marginal QTL, PERA3, co-localized with PtoFA3. In addition, PERA2 overlapped with PtoFA2, although the peaks of these two QTL were separated by  $\sim 15$  cM, making it unlikely that they result from variation at a single locus.

## Discussion

Significant progress has been made in characterizing the molecular genetic basis of the circadian clock in model organisms such as *Arabidopsis*, *Neurospora crassa*, *Drosophila melanogaster* and *Mus musculus*. Much less is known about the genetic basis of quantitative variation in clock function in natural populations and non-model systems. In this study, we investigated natural variation in circadian clock function and flowering time in *Brassica rapa*. We observed wide variation (5–6 h) in circadian period length among cultivated accessions and among RILs at all three temperatures (Fig. 1, 2). This is greater than the range seen among *Brassica oleracea* populations (Salathia et al. 2007), but may simply reflect larger sample sizes permitting the identification of more extreme variants. There was more variation in period length among the RILs than among the accessions, and there was little variation among natural populations. This is consistent with natural and artificial selection reducing the frequency of extreme phenotypes, although it is important to acknowledge that we sampled only a small number of natural populations from a restricted geographic region, and broader sampling

might reveal considerably more variation in circadian phenotype. Among the RILs, there are several extreme short-period lines. For example, RIL18 showed a period of less than 22 h, which is considerably shorter than the extremes among the accessions and wild populations. Consistent with the importance of wild-type circadian function for fitness in a diurnal environment (Dodd et al. 2005; Graf et al. 2010; Green et al. 2002; Yerushalmi et al. 2011), this extreme short-period line shows reduced growth vigor, slow development, reduced seed set and low survival rate in greenhouse environments under natural photoperiods, although it must be emphasized that we have no direct evidence establishing a causal relationship between short circadian period and the growth, survival and reproductive phenotypes.

We observed no correlation between circadian parameters and geographic origin, unlike the case in *Arabidopsis* (Michael et al. 2003). This may reflect the difference in evolutionary histories, as *Arabidopsis* is an inbreeding wild species, whereas *B. rapa* is an outcrossing crop that has been subject to strong human manipulation for a long time, which may have obscured any relationship between period and geography. It is also worth noting that a latitudinal cline was found in flowering time among the subset of *Arabidopsis* accessions carrying the *FRI*<sup>+</sup> allele, but not among the subset carrying *fri* loss of function alleles (Stinchcombe et al. 2004). So, it is possible that relationships between clock parameters or flowering time and geographic origin may exist within restricted genotypic subsets.

We observed a correlation between period and temperature compensation/sensitivity. In each population, period and  $Q_{10}$  were inversely related (Fig. 3). Nonetheless, in every case the  $Q_{10}$  fell within a narrow range between 0.8 and 1.2, indicating that the *B. rapa* clock is very well buffered against temperature changes, at least across this range of temperatures. However, we observed that fewer

lines exhibited robust rhythmicity at higher temperature. This could suggest that clock function is compromised at higher temperatures in this cool-weather crop, but it could also simply indicate that the overt rhythm of cotyledon movement is compromised even when clock function persists relatively unperturbed. Distinguishing between these possibilities will require the development of alternate assays for rhythmicity, such as gene expression. For example, robust circadian rhythmicity was detected at 24°C with a transcriptional *CCA1 promoter:LUCIFERASE* fusion expressed in tissue culture derived from *B. rapa* hypocotyl explants taken from R500, IMB211 and a number of the RILs (Xu et al. 2010), suggesting that the *B. rapa* clock is functional at 24°C, at least in tissue culture.

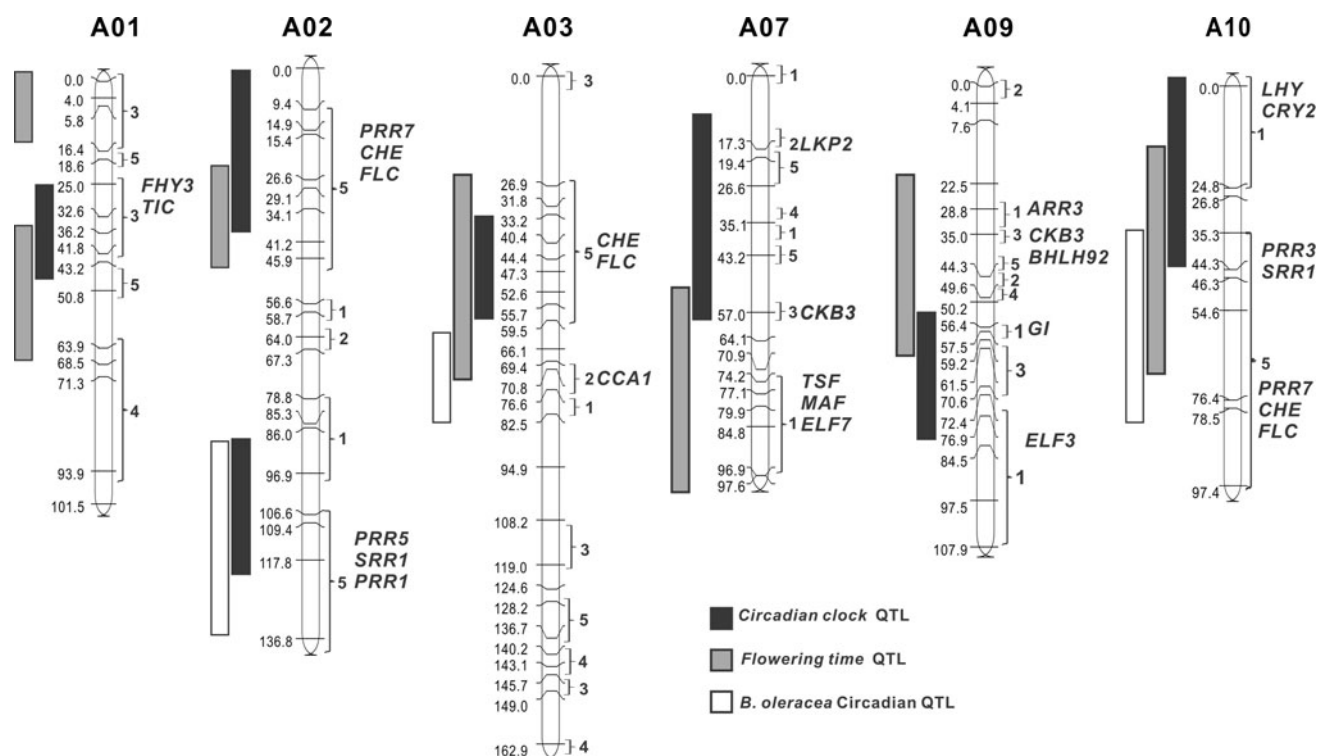
Among the RILs, we identified five major QTL controlling circadian period (Fig. 4, Table 1). Consistent with significant genotype  $\times$  environment interaction effects, only one of the period QTL, PerA10, was detected at all three temperatures, which suggests that QTL effects are environment-specific. Such QTL  $\times$  environment interactions could also account for the constant Q<sub>10</sub> because different QTL apparently regulate period in different temperatures. This is consistent with the observations in *Arabidopsis* that the specific loci responsible for temperature compensation are only evident at specific temperatures. For example, *LHY* is more important than *CCA1* for period length determination at high temperatures and vice versa (Gould et al. 2006), and *PRR7* and *PRR9* are important for period determination at high temperature but are dispensable at low temperature (Salomé et al. 2010). For flowering time, only one major QTL, BtoFA10, was detected at all temperature and day length combinations, again consistent with strong QTL  $\times$  environment effects. Interestingly, these two QTL, PerA10 and BtoFA10, partially overlap at higher temperatures and completely overlap at 12°C (cool) in long days (Fig. 4).

The genotypic correlation observed between circadian period and flowering time ( $r = -0.24$ ,  $p = 0.004$ ) and the co-localization of period and flowering-time QTL (Fig. 4) could indicate a common locus underlying the two phenotypes, but could also result from tight linkage of two distinct loci or from epistasis between flowering-time genes and circadian-rhythm genes. Consistent with a hypothesis of pleiotropy in our quantitative-genetic analyses, molecular genetic studies have shown that the circadian clock is an important regulator of flowering via its role in the photoperiodic pathway (Imaizumi 2010; Turck et al. 2008). Considerable evidence indicates that flowering time is under selection in wild populations and is an important trait selected during crop domestication and improvement (e.g., Blackman et al. 2010; Buckler et al. 2009; Stinchcombe et al. 2004; Wilczek et al. 2009). With regard to fitness consequences of the clock, studies to date have

emphasized on the role of the clock in the regulation of photosynthesis and starch utilization in experimental genetic material such as mutants carrying loss-of-function alleles of clock genes as well as transgenic lines over-expressing clock genes (Dodd et al. 2005; Graf et al. 2010). Regulation of flowering time by quantitative variation in the clock may also contribute to the adaptive significance of the clock in natural populations and crops.

Since its divergence from *Arabidopsis*, the *B. rapa* genome has undergone triplication and segmental rearrangement, so typically genes are present in up to three homeologous copies in the *B. rapa* genome (Park et al. 2005; Schranz et al. 2002; Trick et al. 2009). However, because of gene loss following duplication, the *B. rapa* genome contains at most only approximately twice the number of genes as in *Arabidopsis* (Mun et al. 2009). The pattern of segmental duplications of *Arabidopsis* segments within the *B. rapa* genome has been described (Cheung et al. 2009; Parkin et al. 2005; Schranz et al. 2006) and permits the identification of candidate genes for most of the QTL we have identified (Fig. 5). At least three of the period QTL identified in this study co-localize with period QTL identified in *B. oleracea* (Salathia et al. 2007): PERA2b co-localizes with the *B. oleracea* O2 period QTL, PERA3 with *B. oleracea* O4 and PERA10 with *B. oleracea* O9. In each case, one or more candidate genes, based on synteny with *Arabidopsis*, can be proposed (Fig. 5), although the low resolution of our QTLs compromises our confidence in these proposals.

With the exception of BtoFA7, all the flowering-time QTL at least partially overlap with a circadian period QTL, which generally supports the observed phenotypic correlations between circadian clock function and photoperiodic flowering time. PerA2a and PtoFA2 partially overlap near the top of A2. Two strong candidate genes map to this region. One, *FLC2*, has also been observed in other flowering-time QTL studies in *B. rapa* (Li et al. 2009; Lou et al. 2007; Schranz et al. 2002; Zhao et al. 2007) and is a strong candidate for the PtoFA2 QTL. In *Arabidopsis*, *FLC* was also identified as a period QTL and has been shown to contribute to period length (Edwards et al. 2005, 2006; Salathia et al. 2006; Swarup et al. 1999), making *FLC2* a candidate for PerA2a as well. However, a second strong candidate, *PRR7*, also maps to this region. *PRR7* is known to affect period length and has been suggested to underlie a period QTL on *Arabidopsis* chromosome 5 (Edwards et al. 2005; Michael et al. 2003). In cereals, *PRR7* orthologs have been shown to be important determinants of photoperiodic flowering (Beales et al. 2007; Turner et al. 2005). In *Arabidopsis*, *PRR7* together with *PRR5* and *PRR9* encode partially redundant positive regulators of flowering time (Nakamichi et al. 2007), making *PRR7* a candidate for PtoFA2 as well. Of course, different genetic loci may also



**Fig. 5** Candidates for period and phenological (flowering-time) quantitative trait loci. Overview of *Brassica rapa* QTL for circadian rhythms and flowering time, with candidate *B. rapa* loci based on

underlie QTL that affect clock period and flowering time, and a more detailed analysis will be required to identify the causal loci. Efforts to identify genes responsible for the QTL are ongoing, using both classical genetics (development of near isogenic lines and heterogeneous inbred families) as well as molecular methods. Toward this latter approach, it is exciting that an excellent TILLING population of mutants in the transformable accession R-o-18 has been recently described (Stephenson et al. 2010), which will permit transgenic analysis of candidates for specific QTL by phenotypic rescue.

Of relevance to plant fitness and the role of the circadian clock in adaptive evolution, we have also observed that period is strongly correlated with many ecophysiological traits at both the genotypic and QTL levels in *B. rapa* (data not shown). For instance, quantitative variation in period is correlated with estimates of net carbon assimilation (A), stomatal conductance ( $g_s$ ), transpiration (E), nitrogen concentration (per unit leaf area) and  $\Delta^{13}\text{C}$  a long-term proxy for water-use efficiency (Donovan and Ehleringer 1994; McKay et al. 2003; Rebetzke et al. 2008; Seibt et al. 2008). Period QTL co-localize with QTL for ecophysiological traits on all linkage groups. These results provide some of the first evidence for a link between quantitative variation in the clock and plant ecophysiology. Further, the observations support the functional hypothesis that the

synteny with the *Arabidopsis* genome (Parkin et al. 2005; Schranz et al. 2006). *B. oleracea* circadian QTL (Salathia et al. 2007) co-localizing with *B. rapa* circadian QTL are indicated for comparison

adaptive significance of the clock lies in part in its regulation of ecophysiological traits. With regard to agronomy, as the genetic basis of circadian rhythms and flowering time becomes clearer, we hope to identify loci and markers useful in breeding *Brassica* vegetables with improved growth and development in different latitudes, growing seasons and climates, thereby improving production and yield of these important food crops.

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**TABLE S1**  
**List of accessions**

CGN <sup>a</sup> code	Lab code	Type <sup>b</sup>	Country <sup>c</sup>	Common Name
CGN06825	BRO-027	BRO	ITA	Quarantina
CGN15158	BRO-103	BRO	IDN	Tsja Sin
CGN17278	BRO-127	BRO	JPN	Edible Flowers
CGN06867	CC-048	CC	SUN	
CGN07182	CC-057	CC	CHN	
CGN07183	CC-058	CC	CZE	
CGN07189	CC-062	CC	DEU	
CGN07196	CC-068	CC	BGR	
CGN07200	CC-071	CC	JPN	
CGN07201	CC-072	CC	CHN	
CGN15222	CC-125	CC	KOR	
CGN06673	FT-002	FT	GBR	Grote Ronde Witte
CGN06675	FT-003	FT	NLD	Lange Witte Roodkop
CGN06678	FT-004	FT	DNK	Lang Gele Bortfelder
CGN06866	FT-047	FT	RUS	Moskovskij
CGN07164	FT-051	FT	SUN	Krasnaja
CGN07179	FT-056	FT	FRA	Daisy
CGN07223	FT-086	FT	PAK	
CGN10985	FT-088	FT	NLD	Blauwkop Heelblad-Oliekannetjes
CGN11010	FT-097	FT	DEU	Buko
CGN06843	KOM-041	KOM	JPN	Perviridis
CGN15202	KOM-118	KOM	JPN	Komatsuna
CGN07213	MIZ-079	MIZ	JPN	
CGN17279	MIZ-128	MIZ	JPN	Round leaved Mibuna
CGN15211	MIZ-071	MIZ	DEU	
CGN13924	PC-099	PC	CHN	Chinese White Cabbage
CGN15184	PC-107	PC	CHN	Dwarf
CGN06834	SO-032	SO	IND	Pusa Kalyani
CGN06836	SO-034	SO	BGD	Australian RARS
CGN06840	SO-038	SO	DEU	Toria
CGN06841	SO-039	SO	BGD	Sampad
CGN17280	TG-129	TG	JPN	Vitamin Na
CGN17282	TG-131	TG	JPN	Maruba Santo Sai
CGN06709	VT-006	VT	IND	Pusa Chandrina
CGN06710	VT-007	VT	SUN	Maiskaja
CGN06711	VT-008	VT	IND	Pusa Chandrina
CGN06718	VT-010	VT	HUN	Blad Lila Ker
CGN06722	VT-014	VT	ITA	Heelblad-Milan
CGN06859	VT-044	VT	RUS	Soloveckaja
CGN07166	VT-052	VT	NLD	Hilversumse
CGN10995	VT-089	VT	FRA	D'Auvergne Hative
CGN10999	VT-091	VT	GBR	Snow Ball
CGN15199	VT-115	VT	JPN	Kairyou Hakata
CGN15200	VT-116	VT	JPN	Nagasaki Aka
CGN15210	VT-120	VT	NLD	Platte Gele Boterknol
CGN20735	VT-137	VT	UZB	
CGN06818	WO-024	WO	SWE	Svalof 0308
CGN07217	WO-081	WO	PAK	
CGN07226	WO-087	WO	PAK	
CGN06835	YS-033	YS	DEU	Dys 1

<sup>a</sup> CGN: Centre for Genetic Resources, The Netherlands.

<sup>b</sup> Type: BRO, Broccoletto; CC, Chinese cabbage; FT, fodder turnip; KOM, Komatsuna; Miz, Mizuna; PC, Pak choi, Caixin and Wutacai; SO, Spring turnip rape; TG, turnip green; VT, Vegetable turnip; WO, Winter turnip rape; YS, Yellow sarson.

<sup>c</sup> Country code: ITA: Italy; IDN: Indonesia; JPN: Japan; SUN: former Soviet Union; CHN: China; CZE: Czech Republic; DEU: Germany; BGR: Bulgaria; KOR: Republic of Korea; GBR: United Kingdom; NLD: Netherlands; DNK: Denmark; RUS: Russian Federation; FRA: France; PAK: Pakistan; HUN: Hungary; UZB: Uzbekistan; SWE: Sweden; BGD: Bangladesh; IND: India.

**TABLE S2**  
**Means, quantitative genetic partitioning, and significance of effects for circadian parameters and phenological traits in *B. rapa***

Population	Trait	Treatment	Mean (SE)	V <sub>G</sub> (SE)	V <sub>G</sub> /V <sub>P</sub>
RILs	Circadian Period	12°C	24.83 (0.08)	0.81 (0.11)***	0.36
		18°C	25.19 (0.08)	0.91 (0.12)***	0.28
		24°C	25.52 (0.10)	1.18 (0.24)***	0.29
	Circadian Phase	12°C	11.61 (0.16)	2.10 (0.36)***	0.14
		18°C	11.61 (0.17)	1.50 (0.33)***	0.09
		24°C	13.75 (0.24)	6.49 (2.22)**	0.14
	Amplitude	12°C	2.54 (0.07)	0.51 (0.07)***	0.29
		18°C	3.03 (0.07)	0.92 (0.12)***	0.34
		24°C	2.73 (0.09)	0.36 (0.08)***	0.25
	BtoF	CL	27.38 (2.19)	4.09 (1.11)***	0.10
		CS	35.61 (2.19)	6.80 (1.45)***	0.06
		WL	12.29 (2.19)	3.61 (0.65)***	0.27
	PtoF	CL	70.05 (2.40)	34.23 (8.10)***	0.16
		CS	78.70 (2.40)	79.13 (12.46)***	0.34
		WL	40.67 (2.39)	43.11 (5.97)***	0.51
Accessions	Circadian Period	12°C	25.55 (0.15)	0.82 (0.29)**	0.13
		18°C	25.74 (0.16)	0.65 (0.21)**	0.20
		24°C	24.55 (0.22)	0.83 (0.29)**	0.45
	Circadian Phase	12°C	14.34 (0.48)	5.75 (2.05)**	0.13
		18°C	13.71 (0.52)	2.83 (1.60)*	0.11
		24°C	16.28 (0.68)	17.46 (6.03)**	0.41
	Amplitude	12°C	2.50 (0.21)	0.61 (0.21)*	0.22
		18°C	2.76 (0.22)	0.69 (0.19)**	0.34
		24°C	6.73 (0.28)	5.28 (1.76)**	0.51
Wild genotypes	Circadian Period	12°C	25.39 (0.14)	0.12 (0.07)*	0.20
		18°C	26.15 (0.17)	0.25 (0.18) <sup>†</sup>	0.28
		24°C	25.76(0.17)	0 (0) <sup>NS</sup>	0
	Circadian Phase	12°C	15.84 (0.46)	1.16 (0.71) <sup>†</sup>	0.12
		18°C	16.54 (0.46)	0.99 (0.81) <sup>NS</sup>	0.02
		24°C	17.41 (0.55)	2.89 (2.13) <sup>†</sup>	0.16
	Amplitude	12°C	2.75 (0.21)	0.02 (0.03) <sup>NS</sup>	0.02
		18°C	3.75 (0.21)	0.30 (0.20) <sup>†</sup>	0.10
		24°C	2.45 (0.24)	0.78 (0.46)*	0.42

Trait nomenclature: PtoF, planting to first mature flower; BtoF, bolting to flowering; WL, warm (24°C) long (14 hr light) days; CL, cool (12°C) long (14 hr light) days; CS, cool (12°C) short (10 hr) days. Standard errors are indicated in parenthesis. V<sub>G</sub>, among-genotypic variance; V<sub>G</sub>/V<sub>P</sub>, among-genotypic variance divided by total phenotypic variance; <sup>NS</sup>, not significant; <sup>†</sup>, P<0.1; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.0001.

TABLE S3

**Quantitative genetic partitioning of variation and significance of effects for traits across treatments**

Population	Trait	Genotype	Genotype ×Treatment	Residual	Treatment (df)	Treatment (F)	Significant Genotype × treatment contrasts
RILs	Period	0.60 (0.09)***	0.33 (0.05)***	2.03 (0.04)***	F <sub>2, 240</sub>	26.74***	12°C/18°C***12°C/24°C***18°C/24°C***
	Phase	0 (0) <sup>NS</sup>	2.98 (0.38)***	16.79 (0.36)***	F <sub>2, 330</sub>	31.75***	12°C/18°C***12°C/24°C***18°C/24°C***
	Amplitude	0.36 (0.06)***	0.28 (0.04)***	1.46 (0.03)***	F <sub>2, 265</sub>	24.57***	12°C/18°C***12°C/24°C***18°C/24°C***
	BtoF	3.65 (0.68)***	1.93 (0.44)***	14.23 (0.48)***	F <sub>2, 15.1</sub>	29.26***	WL/CL**WL/CS***
	PtoF	42.92 (6.40)***	11.68 (2.56)***	84.45 (2.86)***	F <sub>2, 15.7</sub>	84.96***	WL/CL**WL/CS**
Accessions	Period	0.39 (0.15)**	0.27 (0.13)*	3.81(0.18)***	F <sub>2, 81.1</sub>	13.10***	12°C/24°C <sup>†</sup>
	Phase	0.68 (1.23)*	6.04 (1.91)**	31.30(1.59)***	F <sub>2, 77.9</sub>	4.79*	
	Amplitude	0 (0) <sup>NS</sup>	1.71 (0.29)***	2.38(0.12)***	F <sub>2, 109</sub>	83.60***	12°C/18°C***12°C/24°C**18°C/24°C*
Wild genotypes	Period	0.09 (0.07) <sup>†</sup>	0.05 (0.04) <sup>NS</sup>	0.87 (0.06)***	F <sub>2, 14.1</sub>	11.25*	
	Phase	1.26 (0.77)*	0.08 (0.22)	14.66 (0.75)***	F <sub>2, 10.7</sub>	6.62*	12°C/18°C**12°C/24°C*18°C/24°C***
	Amplitude	0 (0) <sup>NS</sup>	0.29 (0.12)**	1.83 (0.09)***	F <sub>2, 16.7</sub>	9.96**	12°C/18°C*18°C/24°C*

Trait nomenclature: PtoF, planting to first mature flower; BtoF, bolting to flowering.

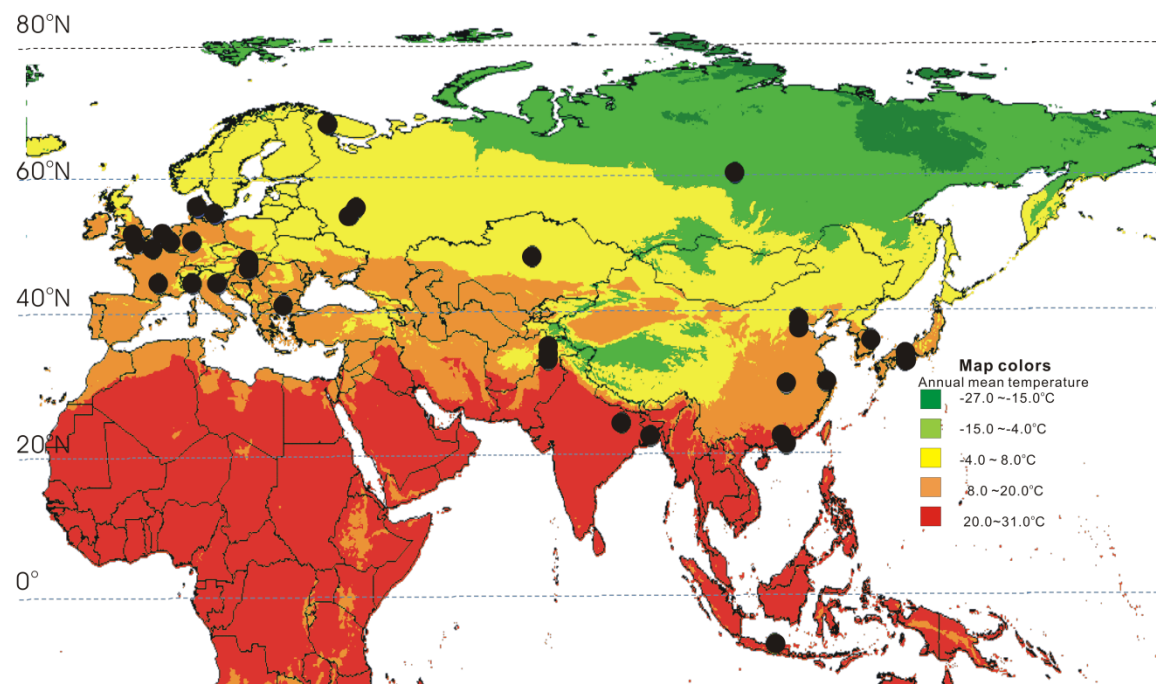
Standard error is indicated in parentheses. <sup>NS</sup>not significant, <sup>†</sup>P<0.1, \*P<0.05, \*\*P<0.01, \*\*\*P<0.0001

TABLE S4

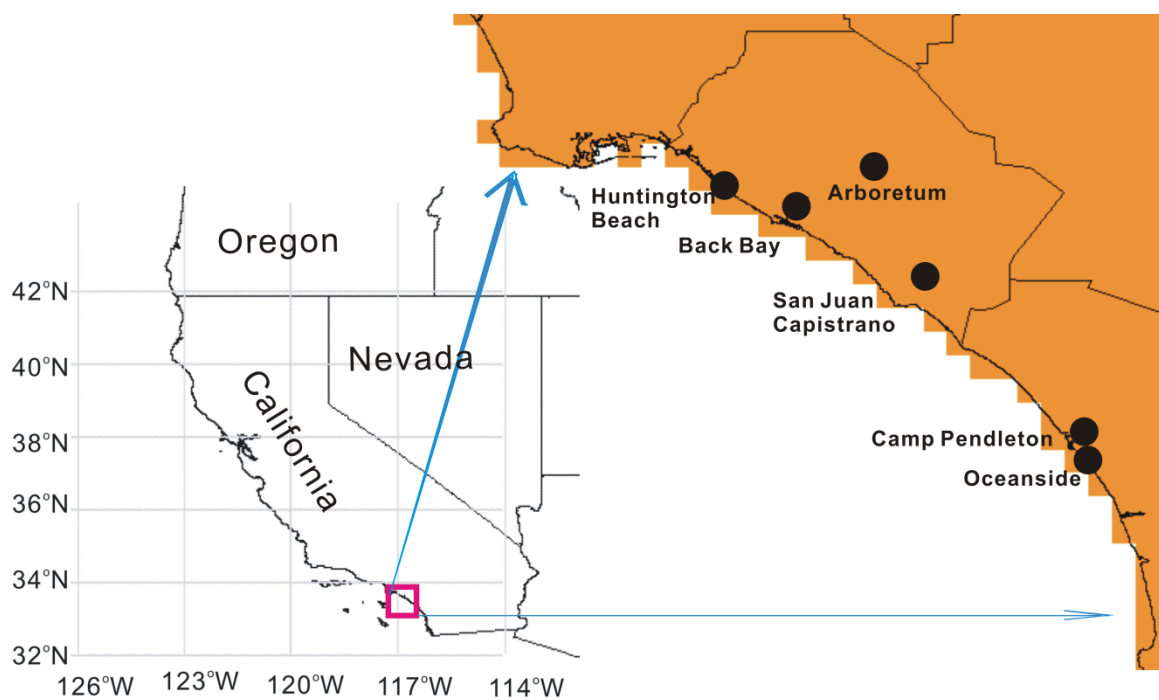
**Phenotypic value of circadian clock parameters for parental lines and RIL populations at three different temperatures**

Parameters	IMB211 R500		RIL (Mean)		RIL (Range)		IMB211 R500		RIL (Mean)		RIL (Range)	
	12°C						18°C				24°C	
Period (hr)	26.81	25.03	24.83	21.95-27.21	26.38	25.53	25.12	22.27-28.11	27.19	25.33	25.39	22.22-27.88
Amplitude	2.05	3.20	2.54	0.69-5.05	2.43	3.40	3.29	1.22-6.23	2.72	3.03	2.82	0.80-5.51
Phase (hr)	13.15	9.29	11.60	6.80-18	16.78	11.64	14.10	4.30-22.50	19.34	14.66	13.6	0.60-23

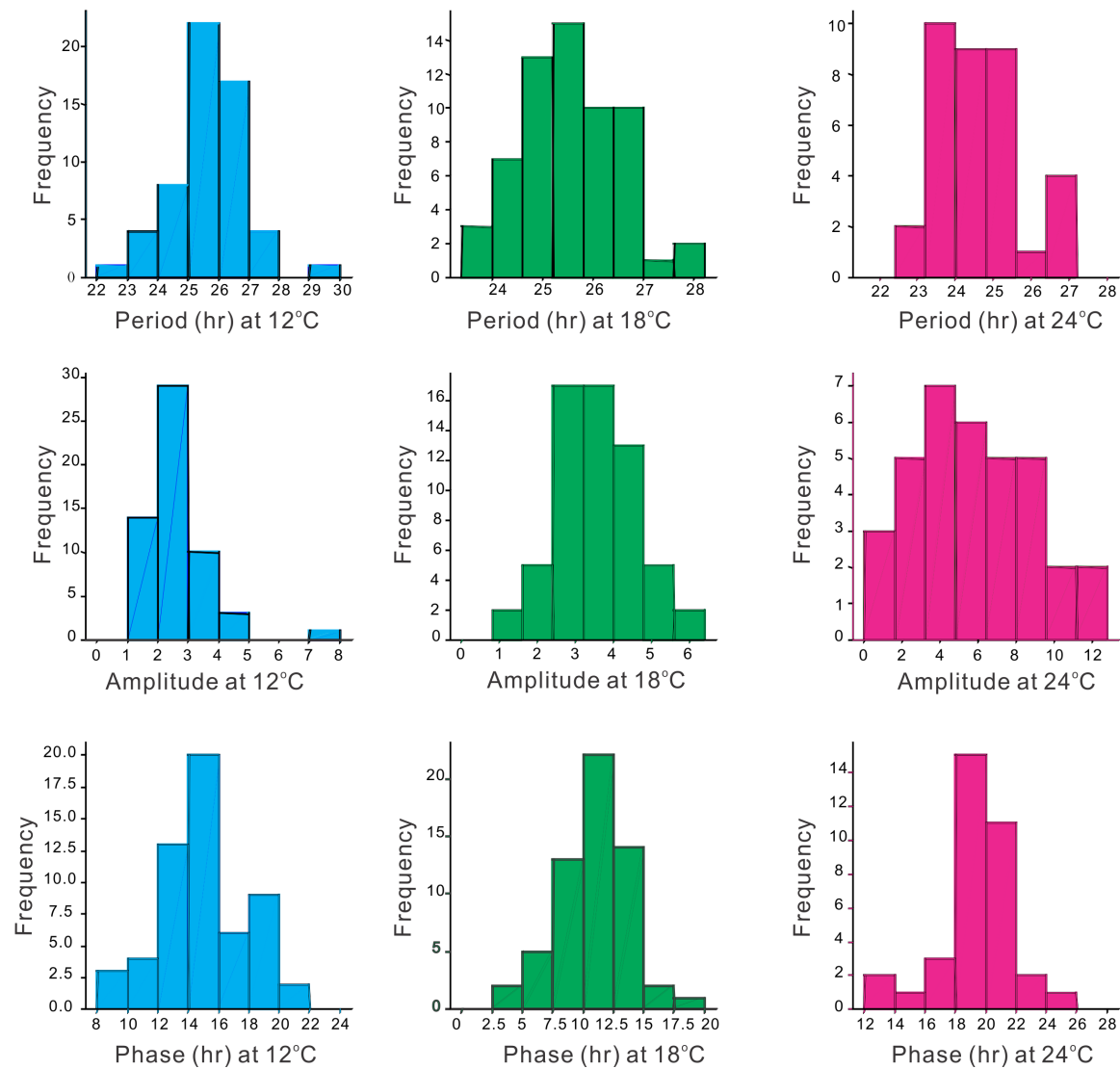




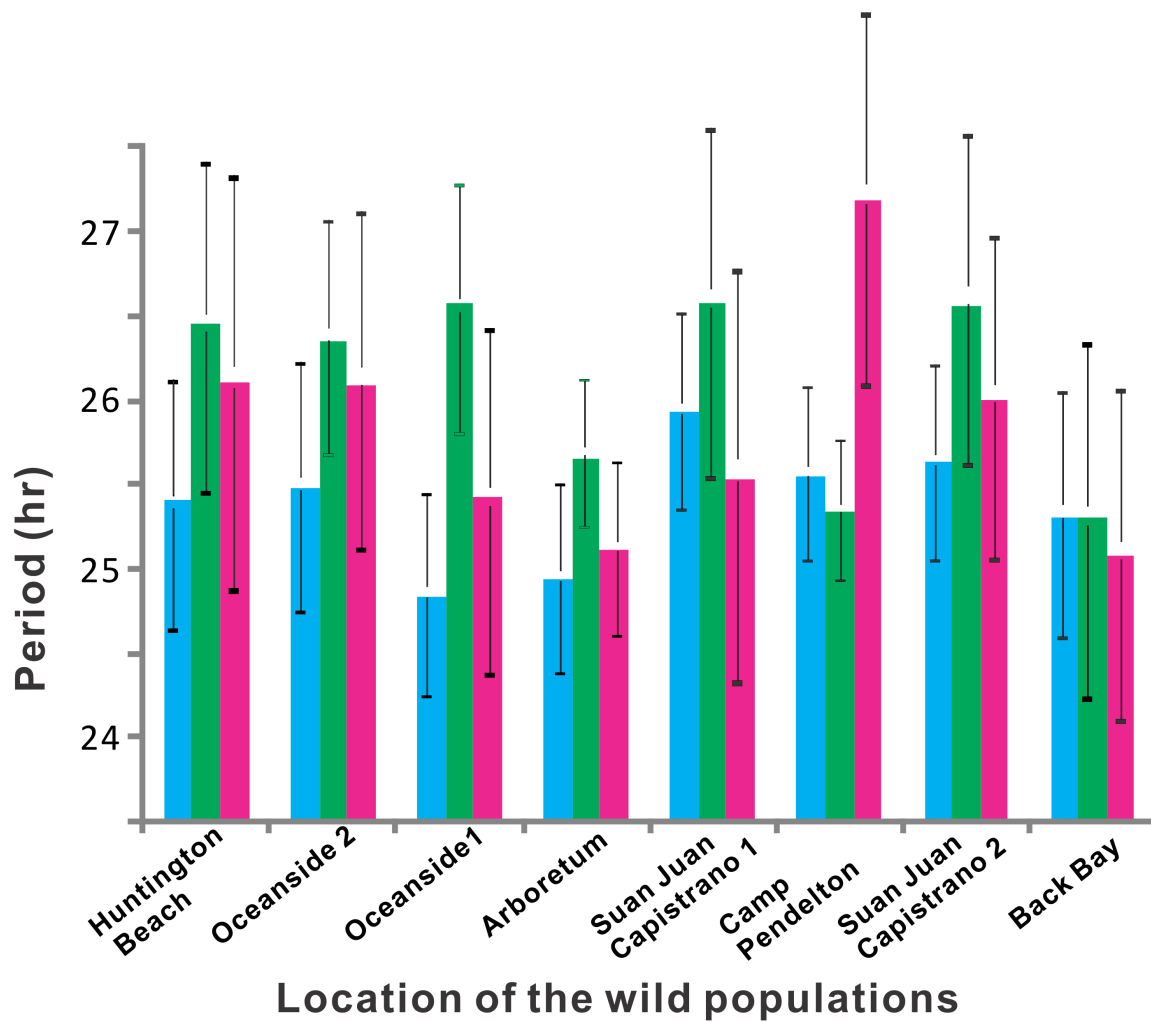
**Fig. S1** Geographic origins of *B. rapa* accessions.



**Fig. S2** Geographic origins of *B. rapa* wild populations.

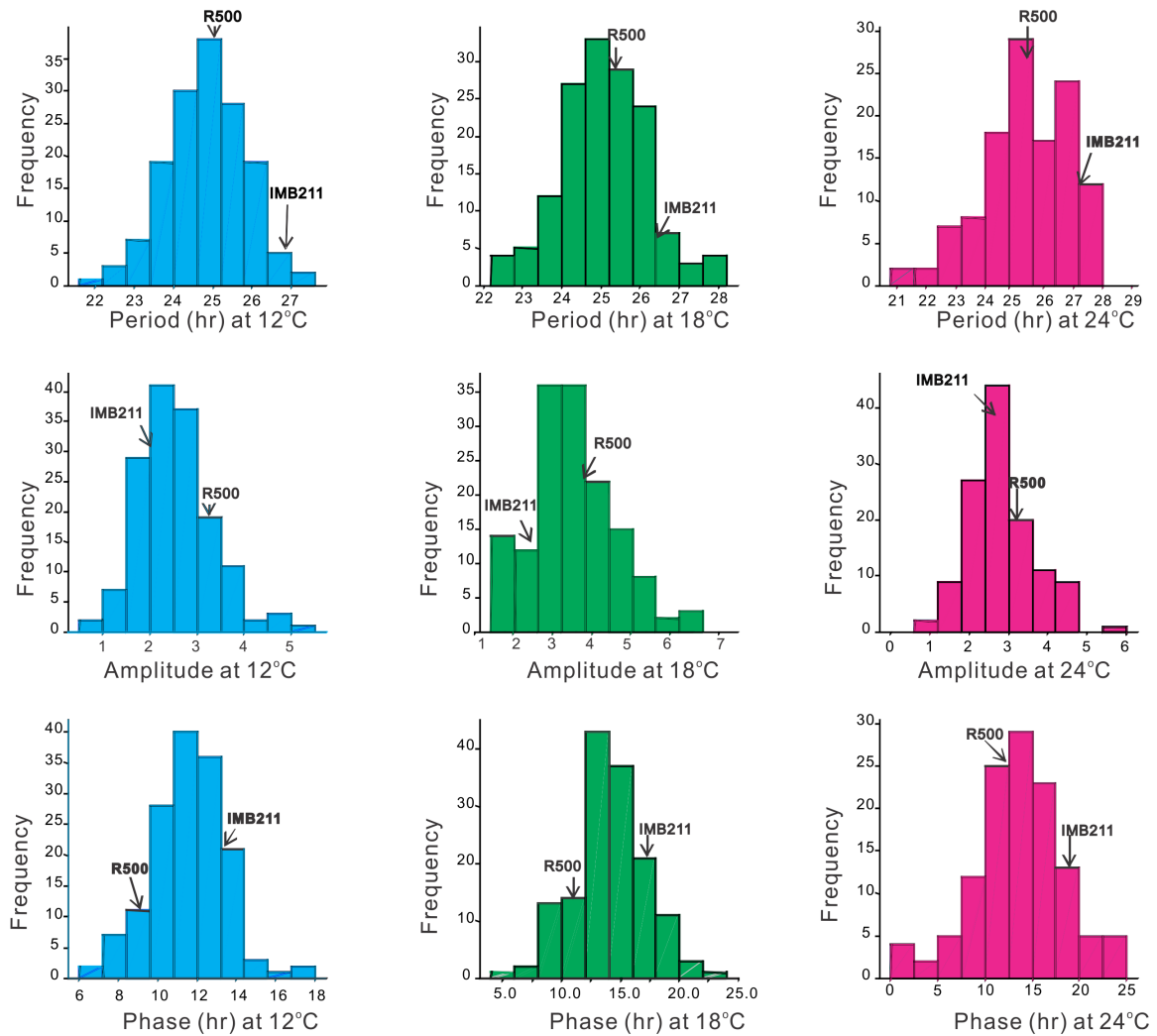


**Fig. S3** Distribution of clock parameters among accessions. Distributions of circadian parameters, period, amplitude and phase as determined by cotyledon movement, among natural accessions at three temperatures: 12°C (left), 18°C (middle), and 24°C (right).



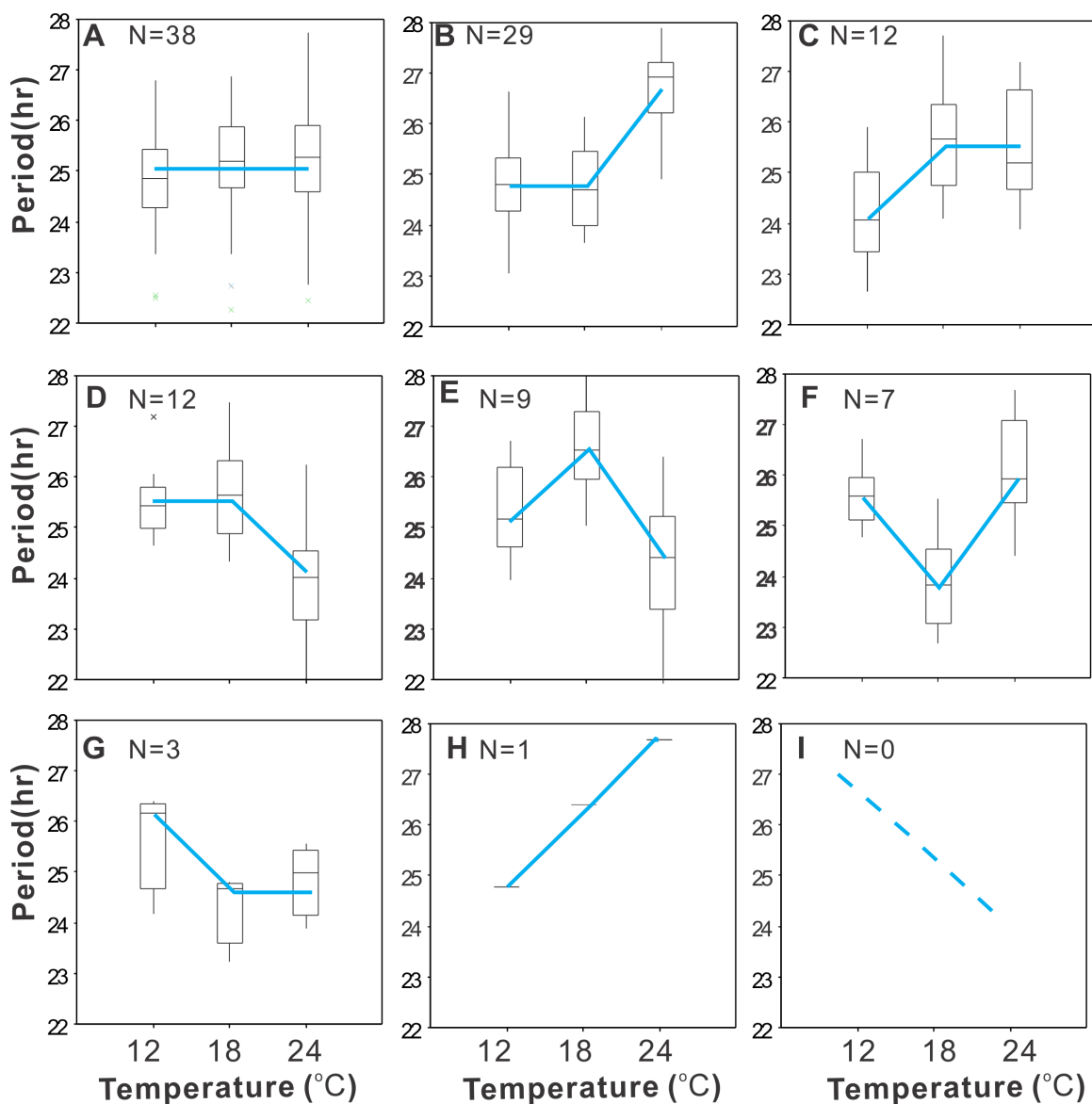
**Fig. S4** Response of circadian period to temperature among wild populations. Circadian period (mean  $\pm$  standard error) as determined by cotyledon movement among wild populations measured at three temperatures: 12°C (Cyan), 18°C (Green), and 24°C (Magenta).





**Fig. S5** Distribution of clock parameters among RILs.

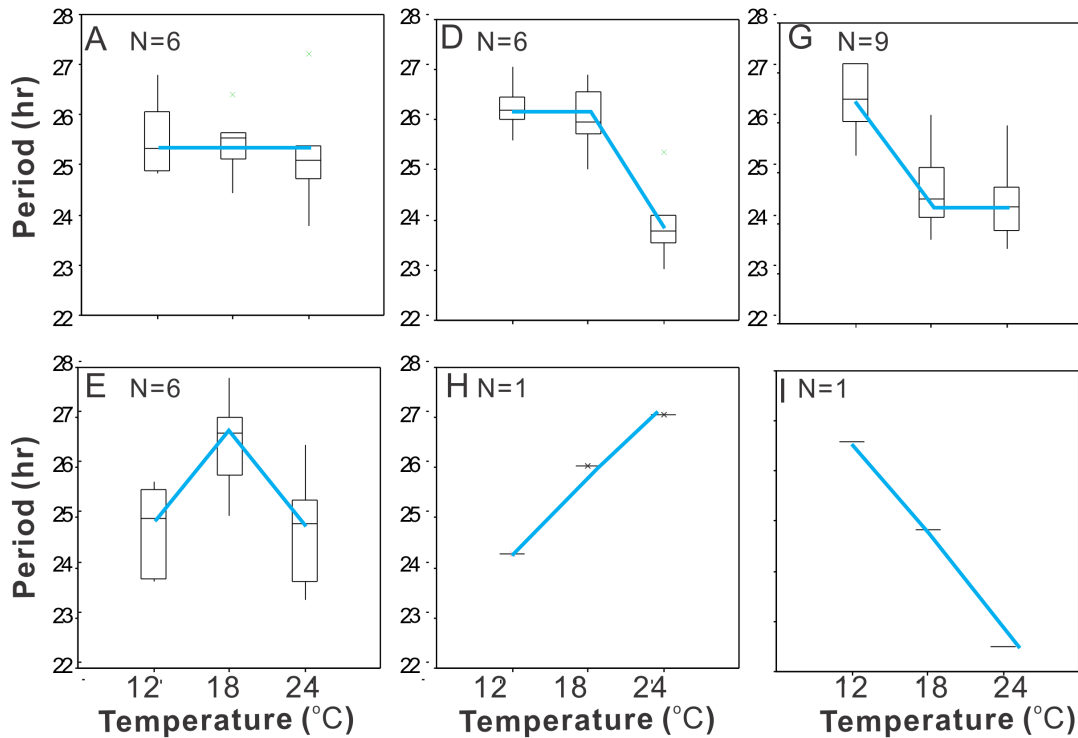
Distributions of circadian parameters, period, amplitude and phase as determined by cotyledon movement among RILs at three temperatures: 12 °C (left), 18°C (middle), and 24°C (right).



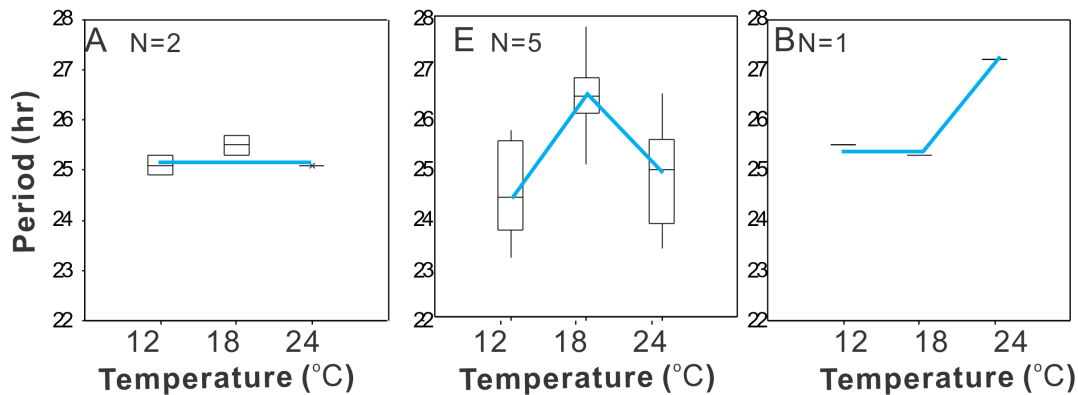
**Fig. S6** Effect of temperature on circadian period among RILs.

Circadian period in cotyledon movement among RILs was determined at three temperatures. Response in period of individual RILs to temperature was clustered using Cluster 3.0, visualized using treeview V2.11, and individual clusters are plotted as box plots. The box spans the interquartile range (25-75%) of the values in the variate so the middle 50% of the data lie within the box, with a horizontal line indicating the median. Whiskers extend beyond the ends of the box as far as the minimum and maximum values.

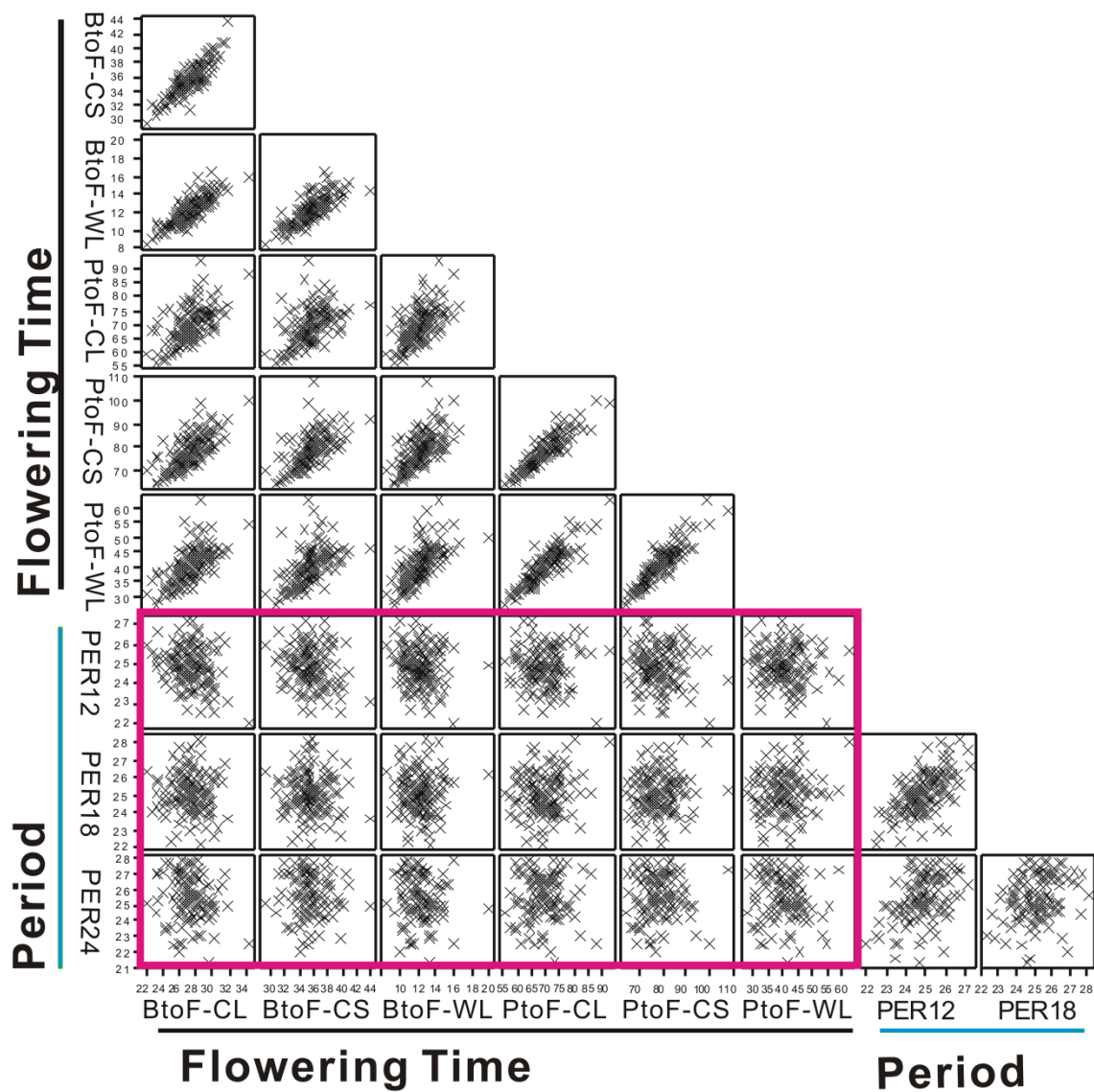
## Accessions



## Wild Populations

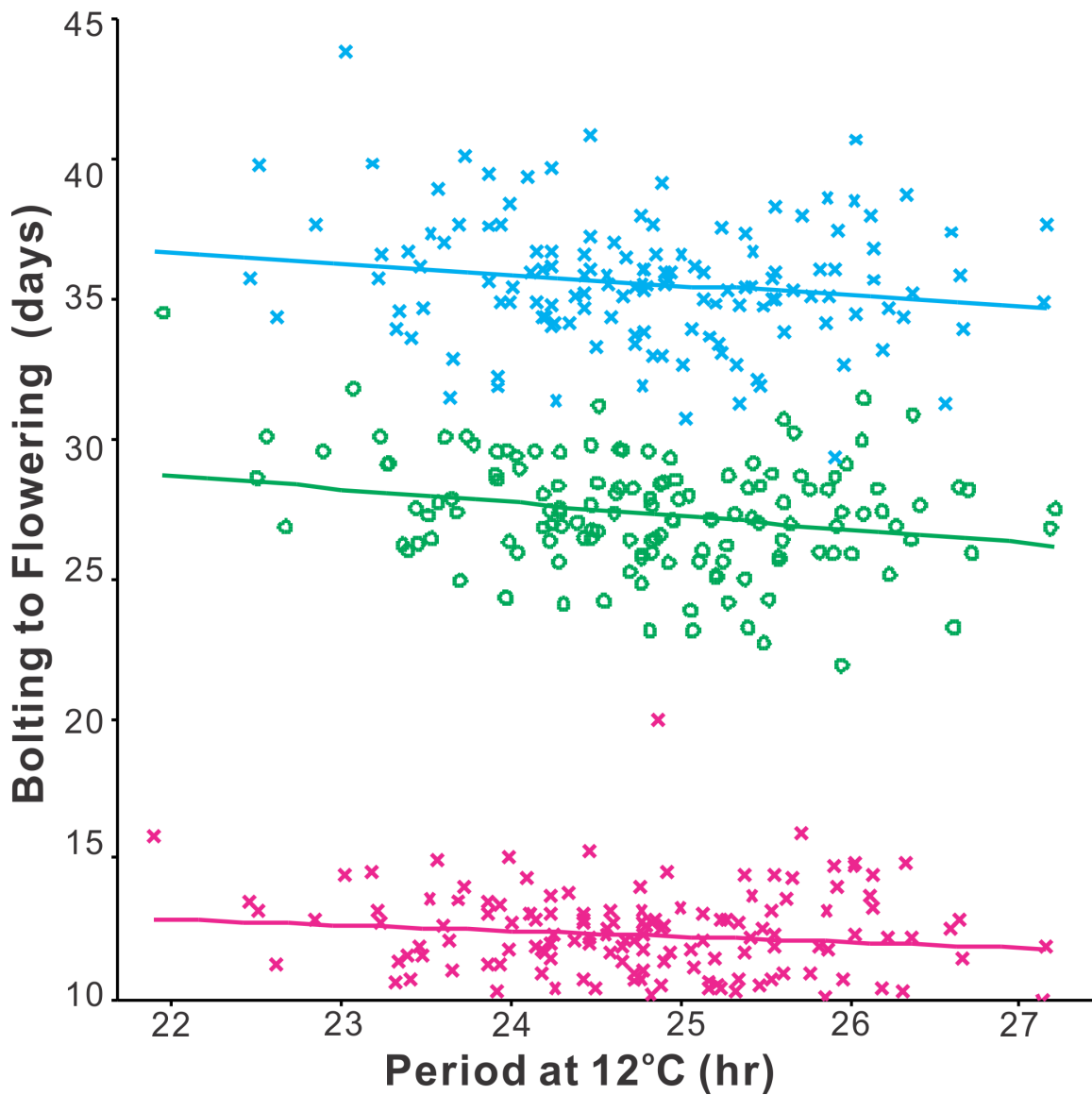


**Fig. S7** Effect of temperature on circadian period among accessions and wild populations. Circadian period in cotyledon movement was determined at three temperatures. Response in period of individual accessions and wild populations to temperature was clustered using Cluster 3.0, visualized using treeview V2.11, and individual clusters are plotted as box plots. The box spans the interquartile range (25-75%) of the values in the variate so the middle 50% of the data lie within the box, with a horizontal line indicating the median. Whiskers extend beyond the ends of the box as far as the minimum and maximum values.



**Fig. S8** Scatter plot matrix of circadian period and flowering time traits generated from RIL population. The off-diagonal scatter plots provide a visual representation of the correlation among the traits.





**Fig. S9** Days from bolting to flowering at different environmental settings plotted against period at 12°C.

CS (cold short days), cyan crosses; CL (cold long days), green circles; WL (warm long days), magenta crosses. Linear regression lines are shown for each treatment with the following probability values: CS (cyan),  $r = -0.16$ ,  $P = 0.054$ ; CL (green),  $r = -0.24$ ,  $P = 0.004$ ; WL (magenta),  $r = -0.12$ ,  $P = 0.134$