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ORIGINAL PAPER

Detection and resolution of genetic loci affecting circadian period in *Brassica oleracea*

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Abstract Circadian rhythms regulate many aspects of plant growth, fitness and vigour. The components and detailed mechanism of circadian regulation to date have been dissected in the reference species *Arabidopsis thaliana*. To determine the genetic basis and range of natural allelic variation for intrinsic circadian period in the closest crop relatives, we used an accurate and high throughput data capture system to record rhythmic cotyledon movement in two immortal segregating populations of *Brassica oleracea*, derived from parent lines representing different crop types. Periods varied between 24.4 and 26.1 h between the parent lines, with transgressive segregation between extreme recombinant lines in both populations of ~3.5 h. The additive effect of individual QTL identified in each population varied from 0.17 to 0.36 h. QTL detected in one doubled haploid population were verified and the mapping intervals further resolved by determining circadian period in genomic substitution lines derived from the parental lines. Comparative genomic analysis based on collinearity between *Brassica* and *Arabidopsis* also allowed identification of candidate orthologous genes known to regulate period in *Arabidopsis*, that may account for the additive circadian effects of specific QTL. The distinct QTL positions detected in the two populations, and the extent of transgressive segregation suggest that there is likely to be considerable scope for modulating the range of available circadian periods in natural populations and crop species of *Brassica*. This may provide adaptive advantage for optimising growth and development in different latitudes, seasons or climate conditions.

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Introduction

For most eukaryotes the circadian clock regulates 24-h rhythmicity of diverse processes such as metabolism, physiology and behaviour (Dunlap et al. 2003). This timing device enables an organism to maintain synchrony with the Earth's rotation around its axis and also facilitates the prediction of daily changes in the environment, such as sunrise and sunset (Eriksson and Millar 2003). The plant clock is thus "entrainable" to environmental cues and provides temporal information, such as time-of-day and some aspects of spatial information, such as proximity to other plants, based on light quality (Eriksson and Millar 2003; Salter et al. 2003). This information in turn either directly or indirectly regulates diverse developmental processes, ranging from rhythmic stomatal opening (Somers et al. 1998) to flowering time (Suarez-Lopez et al. 2001).

For plants, most detailed genetic analysis and research into the role and function of specific clock

genes has been conducted using the reference species *Arabidopsis thaliana* (Brassicaceae). Forward and reverse genetic approaches have been used to identify the genes that form the core circadian oscillator, together with numerous others that are regulated by the clock (reviewed in Southern and Millar 2005). Several studies have also identified additional allelic variation in clock function based on QTL analysis (Edwards et al. 2005; Swarup et al. 1999).

More recently, it has been demonstrated that the circadian clock is important for increasing plant biomass, photosynthetic capacity and enhancing survival (Dodd et al. 2005). An important aim of such research is to extend this knowledge to agriculturally important species, and determine the scope for adaptation in crop plants. Genetic variation associated with modulation of the circadian period might have played a major role in the domestication and adaptation of crops, particularly in their ability to optimise performance at different latitudes and seasons. In order to accumulate biomass and increase harvestable yield crops need to optimise their flowering time in relation to changes in day length and photosynthetic potential and thus maximise their growth period in the context of available thermal time. For example, Turner et al. (2005) have recently isolated *Ppd-H1* as a major determinant of the barley photoperiod response, which has been selected as highly advantageous in spring-sown varieties. *Ppd-H1* is a pseudo-response regulator involved in circadian clock function that appears to alter circadian expression of key photoperiod pathway regulator genes (Turner et al. 2005). Knowledge of the genetic constraints on adaptation of crops is likely to become more valuable in the context of rapid global climate changes.

Brassica species include the closest crop relatives to *Arabidopsis*, comprising major oil, vegetable, fodder and mustard crops grown worldwide. The *Brassica* C genome (*B. oleracea*, $n = 9$) is represented in diploid form by vegetables such as cabbages, kales, cauliflower, broccoli and Brussels-sprouts, and is retained intact in the amphidiploid AC genome (*B. napus*, $n = 19$), represented by oilseed rape, Canola and swede. *Arabidopsis* and *Brassica* diverged some 16–21 MYA (Koch et al. 2001), and subsequent chromosome segmental duplications have given rise to a more complex genome structure with an average of three loci in diploid *Brassica* genomes for each found in *Arabidopsis* (Lagercrantz 1998; Lagercrantz et al. 1996).

There is considerable conservation of gene order (collinearity and micro-synteny) between segmental blocks present within the *Brassica* and *Arabidopsis* genomes (Parkin et al. 2005; Rana et al. 2004). Gene sequences are highly conserved at the DNA and protein level, with an average of >85% nucleotide identity in coding regions (Cavell et al. 1998). Conservation of gene function has been shown to exist in a number of pathways, including those for fatty acid synthesis (Das et al. 2002), flowering time (Schranz et al. 2002) and floral identity (Schranz et al. 2002; Smith and King 2000). Comparative analysis at the genetic level between and *B. oleracea* and *A. thaliana* has also suggested at least partial conservation of the flowering time genes across the genera (Bohuon et al. 1998; O'Neill and Bancroft 2000).

The centre of diversity for the *Brassica* C genome appears to be the eastern Mediterranean, with some crops such as cauliflower and broccoli radiating from southern Italy and surrounding regions. The ability to accumulate and recombine alleles that contribute to the circadian clock is likely to have played a significant role in the domestication and widespread adoption of *Brassica* crops throughout most latitudes of global temperate regions. For crops such as cauliflower, there has been strong selection for sets of landrace and modern varieties that are exquisitely optimised for harvestable maturity for each season throughout the year. Moreover, scheduling of such crops to provide continuous production has in many cases given rise to genotypes that can be matched to at least 4 week periods in any given latitude or growing area (Massie 1998). Optimisation of flowering time and accumulation of tissue-specific biomass is also of increasing importance for the major rapeseed crops where the ability of agronomic models optimised for growth of particular genotypes is unlikely to match rapid changes in climate.

In order to assess the range of natural allelic variation for intrinsic circadian period present within *Brassica* crop species, and to determine the scope for ecological or domesticated adaptation, we undertook a genetic analysis amongst reference lines representing different crop types. The circadian clock is expected to be an intrinsic property of a plant at the cellular level, and therefore cotyledon and early leaf assays have routinely been used as a reliable indicator of circadian period (for example, see Edwards et al. 2005; Engelmann et al. 1992; Tseng et al. 2004).

We carried out QTL analysis using lines selected to represent maximal recombination from two existing immortal segregating populations of *B. oleracea*. This enabled us to determine the overall and specific contribution of genetic loci to variation in period. Following the behaviour of plants arising from genetic recombination in an unselected population provides information on the additive effect of specific alleles, and thus allows an assessment of the scope for extending the range of available circadian periods in natural or crop

genotypes. By accounting for significant contributions to period variation, and further resolving these loci using substitution lines of introgressed genomic segments that conferred decreasing effect on period, we were able to carry out comparative genomic analysis with *Arabidopsis*, and demonstrate the potential for identifying existing or novel candidate clock genes.

Materials and methods

Mapping populations

The AGDH *Brassica oleracea* reference doubled haploid (DH) mapping population and associated genetic map has been described previously (Bohuon et al. 1996; Sebastian et al. 2000), and derives from an F_1 of a cross between the doubled haploids A12DHd *B. oleracea* var. *alboglabra* (Chinese white kale) as the maternal parent and GDDH33 *B. oleracea* var. *italica* (Calabrese broccoli) as the paternal parent. A subset of 87 lines was selected, based on maximal levels of recombination across the genome and seed availability.

The AGSL substitution line population (Rae et al. 1999) derives from the same parental lines, with segments of the GDDH33 genome (paternal parent) introgressed into the A12DHd recurrent maternal parent. Seed were kindly provided by M. Kearsey, University of Birmingham, UK.

The NGDH *B. oleracea* population and linkage map integrated with that of AGDH has been described previously (Sebastian et al. 2000). This was derived from a cross between the *B. oleracea* var. *botrytis* cauliflower DH line CA25 as the maternal parent and the *B. oleracea* var. *gemmifera* Brussels sprout DH line AC498 as the paternal parent. Eighty-three lines were selected, based on maximal levels of recombination across the genome and seed availability.

Growth conditions

B. oleracea seed were sown at a depth of 0.5–1.0 cm in medium nutrient (Levingtons M2) compost and grown in glasshouses at Warwick HRI (Wellesbourne, UK) in individual modules of P40 MultiPack Trays (Avoncrop Ltd., Avon, UK). A 16:8 h light/dark cycle was maintained by provision of supplementary illumination with Camplex 400 Watt Sont-T lamps) calibrated to provide 5000 lx m^{-2} at a plant height of 2–3 cm.

Plants were watered daily, and temperature maintained between 16 and 18 °C until they reached the growth stage of fully emerged cotyledons, but prior to development of visible true leaves. This typically required between 7 and 14 days, depending largely upon the month of year.

Entrainment and leaf movement assays

Seedlings at the cotyledon stage were transferred to controlled environment growth conditions at the University of Warwick. Entrainment to a 12:12 LD cycle was established over 5 days in a Fitotron cabinet (Sanyo Gallenkamp), under 35–40 μmol m^{-2} s^{-1} fluorescent cool white light. To reinforce the entrainment signal, seedlings were simultaneously subjected to temperature cycles of 15 °C during the light period and 12 °C during the dark period. This ensured that the plants grew with short and stout stems and were stable during the subsequent image capture period.

Individual plant modules containing single seedlings were sealed from the top with black PVC tape to reduce evaporation from the compost during the time over which the plant was being imaged. Growth conditions were standardised at 17–20 μmol m^{-2} s^{-1} cool fluorescent white light, with air temperature maintained at 22 °C (as in Edwards et al. 2005). In order to track the rhythmic movement of the cotyledons, white polystyrene balls were glued to the end of each cotyledon blade with araldite epoxy adhesive (see Fig. 1). A fully randomised design was adopted at the level of individual plants and lines, in order to avoid positional bias in the imaging arrays. Seven seedlings were imaged by a monochrome video camera (Ultrak KC4300E, Preston, UK). An array of 15 such cameras under computer control (KujaMorph system developed by K. Edwards and AJM; http://www.amillar.org/Kujamorph.htm) was used to record 8-bit TIFF images at 20 min intervals over 7 days in constant light.

For the AGDH and NGDH populations, data were collected from 12 and 14 occasions, respectively, with at least one seedling per line being assayed on each occasion, apart from when a line failed to germinate, thus giving approximately equal numbers of replicates of each line over the set of occasions.

For the AGSL population, data were collected from three independent occasions, with all substitution lines being used in each experiment.

Period determination and statistical analysis

The sequential frame similarity matching software of the "track objects" add-in function of Metamorph version 4.5 (Universal Imaging Corporation, USA) was used to track the rhythmic movements of cotyledons. The vertical pixel position of cotyledons was plotted against elapsed time and data were exported to a fast

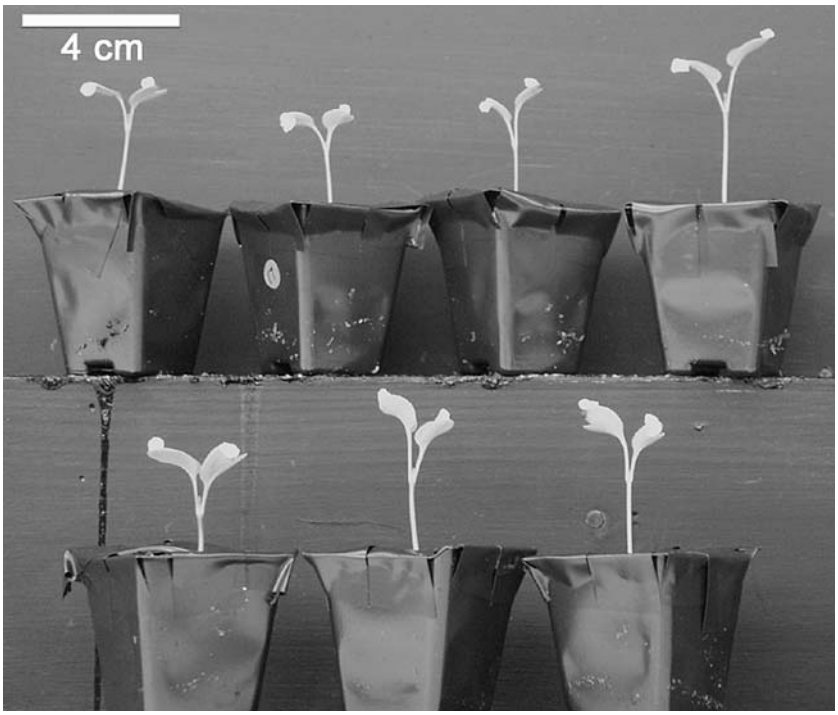

Fig. 1 Single camera set-up for video imaging of *B. oleracea* seedling circadian rhythms. *White polystyrene balls* are glued to the cotyledon blade against a *dark background* enabling tracking of rhythmic movement by computer software.

Fourier transform nonlinear least-squares program (FFT-NLLS) (Plautz et al. 1997) to estimate the period of individual cotyledon traces. To remove any effects not associated with endogenous clock function that may have arisen during plant transfer from entrainment, the first 24 h of each leaf trace were excluded from the period estimation. Eighty-hour windows of data were used for period analysis.

The period data were analysed using REML which is suitable for unbalanced datasets (Paterson and Thompson 1971) in the statistical package GENSTAT 5 (Payne et al. 1993). Experiment, camera within experiment, plant within camera, and cotyledon within plant were taken as random factors, with genotype (line) as a fixed factor. The analysis was weighted to allow for the inherent variabilities of estimation of period from the different traces. The period estimate for each cotyledon recording was weighted for analysis by the reciprocal of the error associated with the period, as estimated by FFT-NLLS. Comparisons of individual introgressed lines to A12DHd were performed using t-tests following the REML analysis.

QTL mapping

For each population, QTL analysis was carried out with a subset of 75 marker loci at approximately 10 cM intervals evenly spaced over the nine linkage groups for which the most complete genotype information was available. QTL detection and location was carried out using the multiple marker regression approach (Kearsey and Hyne 1994) based on line means, implemented in the "QTL Café" software package (http://www.qtl.cap.ed.ac.uk/cafe/index.html). This method derives a linear relationship between the additive effect of the marker means and the recombination frequency between individual markers to identify putative QTL (Kearsey and Hyne 1994). The presence of one or more QTL was tested by ANOVA. A single QTL model on a given linkage group was accepted when the residual mean square in the ANOVA was not significant ($P > 0.05$) and the regression mean square was significant ($P < 0.05$). The parental contribution to each QTL was assigned by the direction of the QTL effect, such that positive values indicate a contribution deriving from the female parent and negative values indicate from male parent. To assist in comparison of QTL between populations, QTL were also calculated using marker locus coordinates for each population derived from an updated integrated linkage map calculated in JoinMap 3.0 (Van Ooijen and Voorrips 2001).

Analysis of the AGSL substitution lines

To better define the location of the circadian period QTL identified, the same statistical methods outlined above were employed to estimate the circadian period of selected AGSL lines. Lines were chosen based on introgression events of GDDH33 genome in a A12DHd background only in the genomic region of a circadian period QTL (see Rae et al. 1999 for map details).

Comparative genomics

Based on *Brassica* C genome genetic map locations defined by sequence-tagged RFLP and SSR markers (Parkin et al. 2005; Sebastian et al. 2000), collinear regions of the *Arabidopsis* genome were identified that corresponded to the position of *B. oleracea* circadian period QTL. This was followed by an inspection of the set of *Arabidopsis* gene models to identify those affecting circadian period. A systematic search was based on a keyword search for "circadian" within the complete TAIR database, as well as in the bibliographic database available on the TAIR website. This was complemented by searching the KEGG annotations for genes involved in the *Arabidopsis* clock, as well as prior knowledge of genes affecting the *Arabidopsis* clock. This provided a set of orthologous candidate genes that are likely to be present in the relevant QTL regions (Table 3), and which we would prioritise for map-based characterisation.

Results

We have developed an assay system that is capable of automatically capturing and analysing cotyledon movement data from sets of *Brassica* seedlings in order to generate reliable data relating to intrinsic circadian period. This required modifications to existing experimental approaches previously developed for studying much smaller *Arabidopsis* seedlings. The entrainment, assay and data capture were successful in obtaining reproducible estimates of circadian period under continuous light conditions. The analysis of variance indicated that the majority of the variance was attributed to the interaction of genotype with experiment, and slightly exceeded that of the main effect of genotype.

Period variation in crop-adapted subtaxa

We observed significant variation in period between the different homozygous parent lines that represented different subtaxa and crop types of *B. oleracea*. Periods varied between 24.36 h (SEM = 0.4) for the AC498 line to 26.1 h (SEM = 0.5) for A12DHd, with CA25 and GDDH33 having similar periods of 25.67 h (SEM = 0.7) and 24.97 h (SEM = 0.6), respectively.

Period variation resulting from recombination of alleles

Within the two reference segregating DH populations, we observed a surprising level of variation that arose through recombination of alleles from homozygous parents, which themselves differed by only 1.13 (AG) and 1.31 h (NG). The range of transgressive segregation observed across 84 lines of the AGDH population encompassed periods which varied by 3.55 h (23.65–27.20 h), with an almost identical range of 3.54 h across the 83 lines of the NGDH population (23.24–26.78 h) (Fig. 2).

Identification of circadian period QTL

Significant circadian period QTL ($P < 0.02$) were identified in both reference mapping populations (Table 1). For the NG population these were located on linkage groups linkage groups O4 (50 cM) and O5 (38 cM), with individual additive effects of 0.27 and 0.36 h, respectively, and both loci conferring positive additive effects (longer periods) contributed by alleles from the female (CA25) parent. In the case of the AG population, a significant circadian period QTL was identified on linkage group O4 (0 cm), accounting for an additive effect of 0.25 h. In this population, additional indicative QTL ($P < 0.1$) were also identified on linkage groups O2 (100 cM), O7 (76 cM) and O9 (106 cM), each having smaller additive effects of between 0.17 and 0.21 h.

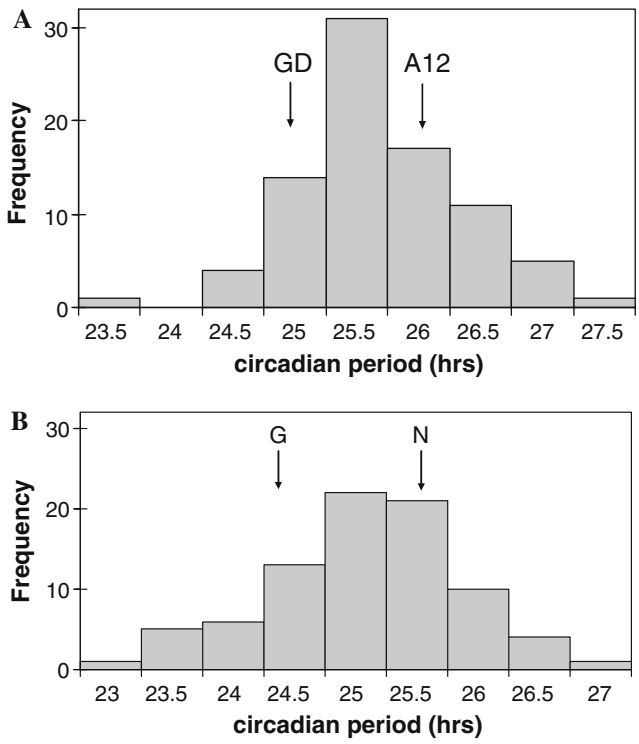

Fig. 2 Distribution of mean circadian periods for DH lines of *B. oleracea* plants in the two mapping populations. Period bins on the *x*-axis are labelled with the middle bound. Parental line (GD, A12 and G, N) periods are indicated with *arrows*

Table 1 QTL detected for cotyledon period movement in *B. oleracea* doubled haploid mapping populations

Population	Linkage group	Position[a] (cM)	Integrated map position[b] (cM)	Confidence interval (cM)	Additive effect (h)	Regression *P*	Residual *P*	V_A	% of genetic variance
NG	O4	48	50.0	36.4–62.1	0.27	0.007	0.67	0.07	24.2
NG	O5	42	38.0	29.9–48.2	0.36	<0.001	0.49	0.13	43.9
AG	O2	96	100.0	67.3–115.3	0.17	0.088	0.44	0.03	9.6
AG	O4	2	0.0	0.0–30.0	0.25	0.015	0.84	0.06	20.4
AG	O7	70	76.0	48.9–86.7	0.21	0.074	0.66	0.04	14.4
AG	O9	106	106.0	59.4–115.5	−0.21	0.092	0.23	0.04	14.2

[a] cM positions are given for the individual linkage maps for the AG and NG populations

[b] Positions and 95% confidence intervals are also given for the integrated map

QTL detected in this population accounted for total additive effects of 0.84 h, with all QTL apart from that on LGO9 conferring positive additive effects (longer periods) contributed by alleles from the female parent line (A12DHd).

Verification and resolution of chromosomal regions affecting circadian period

In order to verify the presence of significant QTL detected in the AGDH segregating population, we analysed a series of lines from the AGSL substitution line population that consisted of defined regions of the GDDH33 parental genome introgressed into the A12DHd parental genome. Lines used in our analysis segregate in a single linkage group containing, or proximal to the region of a circadian QTL, but are otherwise isogenic.

The region of linkage group O2 for which a QTL was detected in the AGDH population was between 67.3 and 115.3 cM, with decreasing period additive effect conferred from the GDDH33 parent. Substitution line SL120 has a segment of GDDH33 on this linkage group from 91.0 to 119.3 cM and SL123 a segment between 67.2 and 82.7 cM, introgressed into the A12DHd genomic background. For SL120 we identified a highly significant reduced period of 25.4 h (SEM = 0.3) compared to 27.5 h (SEM = 0.7) for A12DHd ($P < 0.001$). For SL123, we detected only a marginally significant reduced circadian period of 26.1 h (SEM = 0.4) compared to A12DHd ($P < 0.05$). As shown in Table 2, GDDH33 had a period of 24.3 h (SEM = 0.6). This suggests that the introgressed region does contribute an effect of decreasing period consistent with the QTL analysis, and that the relevant alleles are more likely to be located in the 91.0–119.3 cM interval, which further resolves this locus by approximately 24 cM.

For the QTL detected on linkage group O4, between 0 and 30 cM, with decreasing period additive effect conferred from the GDDH33 parent, we analysed period variation in the substitution lines SL143 (0–15.3 cM) and SL144 (27.1–100.3 cM) and SL148 (31.7–100.3 cM). For SL143 we detected a highly significantly shortened circadian period of 25.7 h (SEM = 0.2) compared to A12DHd ($P < 0.001$). SL144 period of 26.4 h (SEM = 0.5) was not significantly different to A12DHd ($P > 0.05$), while SL148 period of 26.4 (SEM = 0.2) was only marginally significantly reduced compared to A12DHd ($P < 0.05$) (Table 2). We therefore suggest that the introgressed region between 0 and 15.3 cM confers decreasing period, consistent with the detection of the QTL, and that the QTL region has been further resolved by approximately 14.7 cM.

Table 2 Circadian period associated with substitution lines

Line	Linkage group	Region (cM)	*n*	Period (h)	SEM (h)
A12DHd			15	27.5	0.7
SL123	O2	67.2–82.7	13	26.1*	0.4
SL148	O4	31.7–100.3	17	26.4*	0.2
SL144	O4	27.1–100.3	2	26.4	0.5
GDDH33			8	24.3	0.6
SL120	O2	91.0–119.3	15	25.4**	0.3
SL143	O4	0.0–15.3	22	25.7**	0.2

cM distances for the substitution lines were recalculated using a linear regression of mapping intervals referred to by Rae et al. (1999) and those used for the QTL analysis in this paper, where common sets of markers had been scored and compared

* Significant difference of circadian period between SL line and A12DHd at the $P < 0.05$ level

** Significant difference at the $P < 0.001$ level

Identifying candidate genes associated with period loci in *Brassica*

To date, approximately forty genes have been identified in *Arabidopsis* that are associated with control of circadian period (see "Materials and methods" section). We compared the regions of the *B. oleracea* genome contributing additive effects on circadian period (based on QTL and AGSL period data) with

Table 3 Candidate *Brassica* orthologues of *Arabidopsis* genes regulating the circadian clock, inferred from collinearity of period *Brassica* period QTL regions and the corresponding regions of the *Arabidopsis* genome

Brassica oleraea			*Arabidopsis*						Description
Linkage group	Population	Period QTL (cM)	Anchor gene position		Anchor gene	Anchor gene position (*Arabidopsis*, kbp)	Circadian gene position (*Arabidopsis*, kbp)	Circadian gene/ gene model	
			cM	Probe ID					
O2	AGSL	91.0–119.3	110.3	AtFULcDNA-PCR-b-1	At5g60910	24,522	24,694	TOC1/At5g61380	Short period mutant. Involved in the generation of circadian rhythms. Forms a regulatory feedback loop closely associated with the circadian oscillator
							24,217	PRR3/At5g60100	Short period mutant
							24,018	SRR1/At5g59560	Short period mutant
							23,260	ZTL/At5g57360	Long period mutant. Implicated in regulated protein degradation of TOC1
O4	AGSL	0.0–15.3	4.9	pR94E2	At2g47510	19,507	19,254	CCA1/At2g46830	Over-expression causes arrhythmia in numerous clock regulated pathways. Forms a regulatory feedback loop closely associated with the circadian oscillator
O9	AGSL	59.4–115.5	74.8	BoSEP1-cDNA-PCR-1	At5g15800	5,153	5,013	ZGT-like/At5g15440	Similar to circadian clock coupling factor ZGT (*Nicotiana tabacum*) GI: 14210079
			95.0	BoFLC-BAC-PCR-2	BoFLC	3,176	3,176	FLC/At5g10140	Short period mutant. MADS-box protein

the gene content of collinear regions of the *Arabidopsis* genome. The seven most significant *Arabidopsis* candidates located in regions collinear with those in the *B. oleracea* genome regulating circadian period are summarised in Table 3. These data indicate that orthologues of a number of key genes crucial for *Arabidopsis* circadian function may also regulate circadian period in *Brassica*. For example, of the three genes proposed to form the core oscillator in *Arabidopsis* (Alabadi et al. 2001), our collinearity data suggest that orthologues of two (*TOC1* and *CCA1*) may regulate period in *Brassica*.

Discussion

We have successfully adapted an automated data capture system to obtain reliable comparative data for cotyledon movement period from replicated plants of genetically characterised *Brassica* lines. The ability to screen multiple plants in parallel is important in order to assign variance to genotypic and environmental effects.

Although from different genepools, each parent line had been selected in breeding and experimental programmes to be representative of its crop type and adapted to field growing conditions in the UK (52 N). The shortest period was found in the Brussels sprout line AC498. This crop type arose and is well adapted to Northern European latitude. The longest period was found in A12DHd, which derived from a Chinese white kale selected from lower latitudes in Asia. The other two parent lines arose from the broccoli/cauliflower genepool, which has centre of diversity of intermediate latitude in Italy, although the CA25 line had been selected for optimum performance under UK field conditions.

The transgressive variation in circadian period of approximately 3.5 h observed between extreme lines in both segregating populations is similar in magnitude to that found in *Arabidopsis* circadian mutants and segregating populations. Amongst *Arabidopsis* mutants such as *toc1* and *cca1*, differences of approximately 3 h have been observed (Millar et al. 1995; Green and Tobin 1999), whilst a range of approximately 3–5 h was observed in *Arabidopsis* recombinant inbred populations (Swarup et al. 1999).

The 0.25–0.36 h magnitude of the additive effects conferred by individual QTL are similar to those of 0.2–0.45 h identified at 22 °C in *Arabidopsis* (Edwards et al. 2005). Although such QTL can be reliably identified, the resolving power in terms of chromosomal location is limited when using doubled haploid populations. The populations of 87 and 83 lines used in this study were able to resolve period QTL to regions of the order 20–60 cM. However, we were able to confirm the additive effect and also improve the locus resolution by testing the involvement of specific regions. This required testing replicate individual plants from only a very limited number of lines that were selected from the *B. oleracea* AG substitution library (Rae et al. 1999). The utility of these lines in the process of 'Mendelising' individual QTL has previously been demonstrated for QTL controlling *Agrobacterium*-mediated transformation in *B. oleracea* (Cogan et al. 2004). Further resolution of such QTL regions is possible by developing additional recurrent recombinant lines in the regions of interest.

The ability to resolve individual QTL can greatly enhance the ability to identify likely candidate genes. In the case of *Brassica*, the use of comparative genetic and genomic data is increasingly able to contribute to this process through a detailed understanding of the collinear relationships with the *Arabidopsis* genome (Parkin et al. 2005; Rana et al. 2004). Our analysis was based on sequence-tagged loci that had been mapped in consensus *Brassica* C genome (*B. napus* and *B. oleracea*) linkage maps and aligned with corresponding *Arabidopsis* gene models. Where conserved blocks of genes were well described (Parkin et al. 2005), we were able to infer the likely position of candidate circadian gene orthologues in the QTL regions we had identified. Such inferences have previously proved useful in identifying causal genes for circadian QTL in *Arabidopsis* (Edwards et al. 2005; Swarup et al. 1999) and we propose that this can be extended across closely related species as has been done for flowering time traits between *Arabidopsis* and rice (for example, see Takahashi et al. 2001; Yano et al. 2000). Furthermore, we expect this process to become considerably more informative in the near future. The recent completion of end-sequencing of at least 95,000 BACs from *B. rapa* as the first stage of the Multinational *Brassica* Genome Sequencing project now allows rapid identification of candidate BACs and contigs that span regions collinear with *Arabidopsis*.

The involvement of the circadian clock in key developmental processes such as flowering via integrating regulators such as CONSTANS, and effects on biomass (Dodd et al. 2005; Suarez-Lopez et al. 2001) suggest that allelic variation in component clock genes can contribute to adaptive selection that is important for survival in natural populations, or changes in crop growth requirements. Further investigation of the range of variation present within naturally adapted and crop selected genepools would provide valuable

information on the relative contribution of clock alleles to plant performance in different latitudes and other environmental growth conditions. Likewise, determining the range of transgressive segregation possible in populations from a wider range of crosses may uncover the full extent to which variation at specific gene loci can contribute to modulation of clock period.

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