The Genetic Architecture of Ecophysiological and Circadian Traits in *Brassica rapa*

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ABSTRACT Developmental mechanisms that enable perception of and response to the environment may enhance fitness. Ecophysiological traits typically vary depending on local conditions and contribute to resource acquisition and allocation, yet correlations may limit adaptive trait expression. Notably, photosynthesis and stomatal conductance vary diurnally, and the circadian clock, which is an internal estimate of time that anticipates diurnal light/dark cycles, may synchronize physiological behaviors with environmental conditions. Using recombinant inbred lines of *Brassica rapa*, we examined the quantitative-genetic architecture of ecophysiological and phenological traits and tested their association with the circadian clock. We also investigated how trait expression differed across treatments that simulated seasonal settings encountered by crops and naturalized populations. Many ecophysiological traits were correlated, and some correlations were consistent with expected biophysical constraints; for example, stomata jointly regulate photosynthesis and transpiration by affecting carbon dioxide and water vapor diffusion across leaf surfaces, and these traits were correlated. Interestingly, some genotypes had unusual combinations of ecophysiological traits, such as high photosynthesis in combination with low stomatal conductance or leaf nitrogen, and selection on these genotypes could provide a mechanism for crop improvement. At the genotypic and QTL level, circadian period was correlated with leaf nitrogen, instantaneous measures of photosynthesis, and stomatal conductance as well as with a long-term proxy (carbon isotope discrimination) for gas exchange, suggesting that gas exchange is partly regulated by the clock and thus synchronized with daily light cycles. The association between circadian rhythms and ecophysiological traits is relevant to crop improvement and adaptive evolution.

ECOPHYSIOLOGICAL traits are frequently correlated due to their common contribution to resource acquisition and assimilation (Poorter and Remkes 1990; Reich *et al.* 1999; Arntz and Delph 2001). Interspecific studies have shown that plant functional traits, including relative growth rate, photosynthesis, leaf nitrogen content, leaf conductance rate, leaf mass per area (LMA), and leaf life span, are correlated across diverse plant species (Poorter and Remkes 1990; Poorter *et al.* 1990; Cornelissen *et al.* 1997; Reich *et al.* 1997, 1999; Wright *et al.* 2004; Sterck *et al.* 2006; Freschet *et al.* 2010). These traits form a universal plant functional-trait spectrum; at one end of the spectrum are individuals that conserve and use nutrients slowly, have a slow growth rate, low photosynthetic rate, low leaf nitrogen, and low conductance rate, whereas at the other end of the spectrum are individuals with intensive resource use (Reich *et al.* 1997, 1999; Wright *et al.* 2004; Freschet *et al.* 2010).

Significant genetic correlations among ecophysiological traits have also been detected at the intraspecific level. For example, carbon isotope discrimination ($\Delta^{13}C$), an integrated measure of the ratio of the partial pressures of intercellular to ambient CO_2 concentrations (Farquhar *et al.* 1989), is strongly negatively correlated with intrinsic water-use efficiency (Seibt *et al.* 2008) in many taxa (Farquhar and Richards 1984; Hubick *et al.* 1986; Farquhar *et al.* 1988; Condon *et al.* 1990; Ehdaie *et al.* 1991), leading to its use as a long-term measure of water-use efficiency (WUE)

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(Donovan and Ehleringer 1994; McKay *et al.* 2003; Rebetzke *et al.* 2008; but see Seibt *et al.* 2008). In a double-haploid population of *Brassica oleracea* (Hall *et al.* 2005), positive genetic correlations were detected between leaf conductance (g_l) and $\Delta^{13}C$ (*i.e.*, higher leaf gas exchange rates were associated with higher $\Delta^{13}C$ and potentially lower WUE), between LMA, leaf *N*, and photosynthesis (*A*) (*i.e.*, thicker leaves concentrated more nitrogen per unit leaf area and had higher photosynthesis), and between *A*, g_l, and transpiration rate (*E*) (*i.e.*, increased photosynthesis was associated with higher rates of water loss). These significant correlations indicate that common genetic factors affect multiple traits. It is noteworthy that common biophysical processes underlie many ecophysiological traits (for example, *A*, g_s, *E*, and $\Delta^{13}C$ are all partly affected by the opening of the stomata and hence by laws of gas diffusion); the genetic basis of these traits should thus be similar, and pleiotropy is likely the cause of trait covariation. Covariation among ecophysiological traits within a species may alternatively reflect adaptation to environmental heterogeneity and may arise from selection for adaptive allelic combinations and ensuing linkage disequilibrium (LD) between causal loci (Armbruster and Schwaegerle 1996).

Traits traditionally viewed as having distinct developmental origins or functions may also be unexpectedly associated with plant ecophysiological traits. For example, phenology (*i.e.*, traits that measure the timing of periodic life events such as flowering) was negatively correlated with $\Delta^{13}C$ in cowpea (Menendez and Hall 1995), common bean (White 1993), and wheat (Ehdaie *et al.* 1993) and was positively correlated with carbon isotope composition ($\delta^{13}C$) in segregating progenies and natural accessions of *Arabidopsis thaliana* (McKay *et al.* 2003; Hausmann *et al.* 2005; Juenger *et al.* 2005); this suggests that plants that delayed flowering may have increased WUE. QTL for $\delta^{13}C$ also affected flowering time (Hausmann *et al.* 2005; Juenger *et al.* 2005). Further characterization of the genetic relationship between physiology and traits with different or functional bases will strengthen our understanding of adaptive evolution in natural populations.

Physiological function often varies according to diurnal light/dark cycles (for example, photosynthetic enzyme activity fluctuates depending on light/dark cycles; Liu *et al.* 1996), hence ecophysiological traits may be partly regulated by the circadian clock. Circadian clocks are internal estimates of time that anticipate diurnal light/dark cycles and synchronize biological events to match environmental conditions (Michael *et al.* 2003; Webb 2003). Circadian rhythms continue in the absence of environmental cues but may be entrained by environmental cues and generally have an endogenous period around 24 hr (although natural variation exists among individuals in period length; Michael *et al.* 2003). Genes involved in photosynthesis, carbon allocation, stress response, or wood formation are expressed on diurnal cycles in *Arabidopsis*, *Populus*, and *Eucalyptus* (Liu *et al.* 1996; Hoffmann *et al.* 2005; Solomon *et al.* 2010). Aberrant circadian clock function is also associated with reduced biomass accumulation, possibly attributable to reduced photosynthetic rate or suboptimal starch utilization (Dodd *et al.* 2005; Graf *et al.* 2010). However, the connection between naturally segregating genetic variation in the circadian clock and in physiology remains unknown. Aside from enhancing our mechanistic understanding of clock outputs, understanding the association between the clock and plant ecophysiology has important evolutionary implications because photosynthetic rate and water relations are significant determinants of fitness.

In this study, we used *B. rapa* L. to investigate ecophysiological, phenological, and circadian trait covariation. We quantified ecophysiological and phenological traits in a population of 150 recombinant inbred lines resulting from a cross between two inbred genotypes of *B. rapa*, R500 and IMB211 (Iniguez-Luy *et al.* 2009). The IMB211 genotype is derived from the Wisconsin Fast Plant population, and artificial selection for rapid generation time in IMB211 resembles that experienced by naturalized populations and agricultural weeds of this species (Dorn and Mitchell-Olds 1991; Mitchell-Olds 1996). The R500 genotype is a seed-oil cultivar planted in India for at least 3000 years (Prakash and Hinata 1980). Given their divergent selection histories, genetic variation segregating in the RILs may resemble that segregating in crop × wild hybrids found commonly in nature (Adler *et al.* 1993). Moreover, these parental genotypes have contrasting life-history strategies; relative to the IMB211 parent, the R500 parent flowers later, reaches a larger size at flowering and accumulates more biomass (Edwards *et al.* 2009; Edwards and Weinig 2011). The parents also differ in *A*, g_s, *E*, intrinsic WUE, and $\Delta^{13}C$ (Edwards *et al.* 2009). Because the parents of these RILs have contrasting life-history strategies and ecophysiological characteristics, this is a relevant population in which to investigate the genetic architecture of (co)variation in ecophysiological, phenological, and circadian traits.

Here, our specific goals were to investigate: (1) the patterns of (co)variation among ecophysiological traits and between ecophysiological and phenological traits at the quantitative-genetic and QTL levels, (2) the extent to which genotypic and QTL associations observed between pairs of ecophysiological traits agree with biophysical expectations, (3) whether specific genotypes possess combinations of ecophysiological traits that may be targeted for crop improvement (or by natural selection), (4) the quantitative-genetic and QTL association between circadian (as measured by Lou *et al.* 2011) and the ecophysiological traits measured here, and (5) whether the genetic architecture of a subset of these traits is affected by seasonally variable abiotic conditions typically encountered by crops and naturalized populations of *B. rapa*.

Materials and Methods

Study species, plant material, and experimental design

The genotypes used in this study were 150 recombinant inbred lines (RILs) derived from a cross between the R500 ×

IMB211 genotypes of *B. rapa* and previously described by Iniguez-Luy *et al.* (2009). *B. rapa* is an oilseed and vegetable crop species whose native range extends from the western Mediterranean to Central Asia (Gomez Campo 1999). Oil as well as leaf and root vegetable crops of *B. rapa* are cultivated worldwide, and the species also occurs commonly in naturalized populations in association with crop fields (Dorn and Mitchell-Olds 1991). In addition to the environmental variability imposed by the large geographic range that *B. rapa* inhabits, further environmental variation arises from life-history variation: wild individuals are frequently spring annuals, most leafy vegetables and oilseed varieties are cultivated as spring or fall annuals, while root vegetables such as turnips are cultivated as fall annuals or winter biennials.

Temperature and photoperiod are two environmental factors that vary across the geographic range and seasonal environments in which *B. rapa* grows. In this study, we raised plants under three different combinations of temperature and photoperiod characteristic of the locations and seasonal environments that *B. rapa* occupies, including: (1) long days and warm temperatures, which are experienced by spring or summer annuals (such as pak choi or wild genotypes that grow in the spring), (2) long days and cool temperatures, which are experienced by overwintering biennial crops that flower in the spring (such as turnips planted in fall in Europe), and (3) short days and cool temperatures, which are experienced by fall annuals (such as leafy vegetables planted in the fall). We tested how these three different combinations of temperature and photoperiod conditions affected (co)variation in $\Delta^{13}C$, LMA, leaf nitrogen, and phenology. We did not examine short photoperiods and warm temperatures, as this combination of conditions is unlikely to exist in any of the locations where *B. rapa* grows.

Six growth-chamber compartments (PGC-9/2 with Percival Advanced Intellus Environmental Controller, Percival Scientific, Perry, IN) were used to simulate the three seasonal settings. Because previous research using the same growth chambers revealed that photosynthesis by experimental plants reduced the concentration of CO_2, thereby affecting the expression of ecophysiological traits (Edwards *et al.* 2009), compartments were modified by adding additional ventilation to ensure that CO_2 concentrations were maintained at $\sim$375 μmol mol^{-1}, that $\delta^{13}C_{air}$ was maintained at $>-8‰$, and that both were unaffected by plant photosynthesis during the experiment.

One replicate of each of the 150 RILs and the two parental genotypes were grown in each of six growth-chamber compartments. Each growth-chamber compartment was set to one of three conditions: (1) WL, warm temperature (24°), long photoperiod (14 hr/10 hr light/dark cycles); (2) CL, cool temperature (12°), long photoperiod (14 hr/10 hr light/dark cycles); and (3) CS, cool temperature (12°), short photoperiod (10 hr/14 hr light/dark cycles). Each treatment occupied two of the six growth-chamber compartments at one time. The full planting design was temporally replicated three times, with treatments rotated among growth-chamber compartments so that each treatment occupied each compartment once. This experimental design resulted in a total of six replicates of each of 152 genotypes in three treatments (2736 individuals total). Irradiance in all growth-chamber compartments during light cycles was maintained at $\sim$500 μmol m^{-2} sec^{-1}, and the vapor pressure deficit (VPD) was maintained below 1.7 kPa. Details of growth conditions are as described in Edwards and Weinig (2011).

Trait measurements

All germinated plants were checked daily for bolting (*i.e.*, when buds differentiated from the apical meristem). In the WL treatment, leaf gas-exchange traits were measured on all bolting plants each day; we did not perform gas-exchange measurements on plants in the CL and CS treatments because their leaves were too small for the gas-exchange measurement chamber. Gas exchange was measured on a young, fully expanded leaf using a steady-state gas-exchange system equipped with a leaf chamber fluorimeter (LICOR-6400; LI-COR Biosciences Inc., Lincoln, NE). We measured photosynthethesis (A), chlorophyll fluorescence in light (F_v'/F_m', or maximum photosystem II efficiency in light), stomatal conductance (g_s), and transpiration rate (E) using standard techniques (Long and Bernacchi 2003). These measurements were also used to calculate intrinsic WUE (W_g) by dividing A by g_s (Seibt *et al.* 2008). Measurements were taken at least 1 hr after subjective dawn in the chamber. Leaf cuvette conditions were set to a photosynthetic irradiance of 2000 μmol m^{-2} sec^{-1} to measure the maximum photosynthetic rate of the plants (preliminary light response curves showed no decrease in photosynthesis at this irradiance compared with measurements taken at 500 μmol m^{-2} sec^{-1}), with air CO_2 concentration at 400 μmol mol^{-1}, leaf temperature at 24°, and the leaf-to-air VPD between 1.3 and 1.7 kPa. Measurements were taken when the readings stabilized after approximately 5–10 min.

Because preliminary analyses of the gas-exchange traits using six replicates per genotype demonstrated large within-genotype variance, we carried out one additional temporal replicate of the WL treatment, in which each of the 152 genotypes were grown in two growth-chamber compartments, increasing replication of gas-exchange traits to eight replicates per genotype in the WL treatment.

Each day after leaf gas-exchange measurements were taken in the WL treatment, and for all bolting plants in the CL and CS treatments, one young, fully expanded leaf was removed and scanned; leaf area was measured from the images using ImageJ (Rasband 1997–2007). Leaves were oven dried, weighed, and used to calculate leaf mass per area (LMA; g m^{-2}). After bolting, plants were checked daily for flowering, which was scored when the sepals opened and petals became visible or when a flower bud senesced prior to opening. To estimate phenology, days to flowering was

recorded as the number of days that elapsed from planting to flowering.

$\delta^{13}C$ and mass-based leaf nitrogen content were analyzed on four individuals of each genotype in each of the three treatments. The oven-dried leaves collected at bolting were ground and analyzed using an elemental analyzer (ECS 4010, Costech Analytical Technologies, Inc., Valencia, CA) coupled to a continuous-flow inlet isotope ratio mass spectrometer (CF-IRMS; Delta-plus XP, Thermo Scientific, Waltham, MA). $\delta^{13}C$ values were reported in parts per thousand relative to Vienna Peedee Belemnite (VPDB). Estimates of the stable carbon isotope ratio value of the chamber air CO_2 ($\delta^{13}C_{air}$) in growth chambers were taken from a previous experiment (Edwards *et al.* 2009) that used the same growth chamber compartments, experimental setup, and growth conditions. All stable isotope analyses were performed at the University of Wyoming Stable Isotope Facility. The precision of repeated measurements of laboratory standards was <0.1‰. $\Delta^{13}C$ was calculated for each individual as (Farquhar *et al.* 1989)

$$\Delta^{13}C\ (‰) = (\delta^{13}C_{air} - \delta^{13}C_{leaf})/(1 + \delta^{13}C_{leaf}/1000).$$

Leaf nitrogen concentration per unit of leaf area (N_{area}) was calculated using the equation

$$N_{area} = N_{mass} \times LMA.$$

Statistical analyses

In each treatment in which a trait was sampled, we used restricted maximum likelihood (PROC MIXED, SAS v. 9.2) to estimate the random effects of genotype and temporal block on each phenotypic trait. 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. For the gas-exchange traits sampled only in WL (*e.g.*, A, F_v'/F_m', g_s, E, and W_g), this analysis was used to estimate the genotypic values of each trait as best linear unbiased predictors (BLUPs) (Littell *et al.* 2006).

For the traits sampled in all three treatments ($\Delta^{13}C$, N_{area}, LMA, and days to flowering), we tested for genotypic differences in the response to treatment by carrying out a mixed-model nested ANOVA over all treatments (PROC MIXED, SAS v. 9.2). We evaluated the fixed effect of treatment and the random effects of genotype, chamber nested within treatment, and the genotype × treatment interaction (GEI) on each trait. For traits sampled in all three treatments, we used this analysis to estimate the genotypic values of each trait in each treatment as BLUPs.

To assess the relationship among traits within each treatment, we used a multivariate ANOVA approach (Messina and Fry 2003; Holland 2006; Dmitriew *et al.* 2010) to estimate correlations among traits using restricted maximum likelihood (SAS PROC mixed; SAS v. 9.2). Point estimates of the genotypic values (BLUPs) of each trait in each treatment were also used to quantify bivariate correlations (SAS PROC CORR, SAS v. 9.2). Additionally, to quantify the relationship with circadian period, we estimated correlations between the traits investigated in this study and genotypic measures of circadian period in constant light at 12°, 18°, and 24° (Lou *et al.* 2011). Specifically, correlations were estimated between traits measured in the same or similar temperature environment; for example, the bivariate correlation was estimated between ecophysiological traits measured in the WL treatment and circadian period as measured at 18° and 24°, or traits measured in the CL and CS treatments and circadian period at 12° and 18°. The significance values of all bivariate correlations were corrected for multiple comparisons by controlling the false discovery rate (FDR) (Benjamini and Hochberg 1995).

For traits measured in all three treatments, BLUPs were also used to estimate r_{GE}, the genotypic correlation of each trait across pairs of treatments (*e.g.*, WL/CL, WL/CS, CL/CS; SAS PROC CORR). Estimates of r_{GE} indicate the extent to which the same genetic loci are expressed and alleles have the same function across treatments; estimates of r_{GE} approaching one suggest that the same genetic loci are expressed and alleles have similar effects across treatments, whereas estimates approaching 0 suggest that different genetic loci are expressed or allelic function differs across treatments (Fry *et al.* 1996; Gurganus *et al.* 1998; Vieira *et al.* 2000). ANOVA of pairwise treatment comparisons were used to determine the significance of r_{GE}; r_{GE} is significantly different than 1 when the genotype × treatment interaction is significant and significantly different from 0 when the among-genotype variance is significant (Gurganus *et al.* 1998; Vieira *et al.* 2000).

QTL mapping

The linkage map used in this study was described previously by Iniguez-Luy *et al.* (2009) and consists of 224 RFLP and microsatellite markers covering 10 linkage groups, with an average marker density of 5.7 cM/marker. However, chromosomes 5, 8, 9, and 10 are presented in inverted north–south orientation relative to their orientation as described by Iniguez-Luy *et al.* (2009) and other studies using these RILs (Brock *et al.* 2010; Edwards and Weinig 2011) to be consistent with a previously accepted orientation (*i.e.*, that of Parkin *et al.* 2005). QTL mapping of each trait in each treatment was carried out using composite interval mapping as implemented in Windows QTL Cartographer v. 2.5 (Wang *et al.* 2007) following the methodology described in Edwards and Weinig (2011). The genome-wide significance threshold was determined for each trait using 1000 permutations (Churchill and Doerge 1994) with a type I error rate of 0.05. We carried out an additional analysis using a 0.075 type I error rate to detect any marginal QTL.

Because we were specifically interested in correlations between trait pairs and because many traits within an environment had QTL that colocalized, we carried out

multitrait composite interval mapping using QTL cartographer v. 2.5 (Wang *et al.* 2007), which takes the correlated structure of phenotypic traits into account to jointly map QTL affecting multiple traits (Jiang and Zeng 1995). We used multitrait analyses to test hypotheses of pleiotropy (*i.e.*, a single QTL/genomic region that affects multiple traits) *vs.* close linkage (*i.e.*, multiple neighboring QTL that affect the traits of interest). This hypothesis test addresses joint effects of a QTL, not genetic locus, on multiple traits. The multitrait CIM analysis was performed only for QTL identified using single-trait CIM that had overlapping 2-LOD support limits, and therefore suggested QTL that affected multiple traits. Significance thresholds were calculated using a 0.05 type I error rate and 1000 permutations, maintaining the correlations between traits. Although multitrait CIM has low power to test for pleiotropy (especially for QTL of small effect size), the hypothesis of QTL pleiotropy was supported for over 60% of QTL with overlapping 2-LOD support limits (*Appendix*, Table A1); QTL are generally referred to as "colocalizing" when multitrait CIM tests for pleiotropy are significant, and as "closely adjacent" when these tests are nonsignificant (see *Results*). Both "pleiotropic" and "adjacent" QTL may contribute to correlations observed at the genotypic level, given that physically linked QTL will rarely be disrupted by recombination.

For traits that were sampled in multiple treatments, we tested for significant differences in QTL effects across environments using multitrait composite interval mapping as implemented in QTL Cartographer v. 2.5 (Wang *et al.* 2007), using the G × E hypothesis test ("hypothesis four"). The results of these analyses were confirmed by single-marker analysis of variance (Lynch and Walsh 1998) (PROC GLM, SAS v. 9.2). To differentiate the effects of temperature and photoperiod on QTL expression, 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 CL, we evaluated QTL × treatment interactions across CL/CS and across CL/WL). In the single-marker analysis, the model included the fixed effects of treatment, the genotype at the marker nearest to each detected QTL, and the marker × treatment interaction on the genotypic values of each trait.

Finally, we carried out two-dimensional genome scans to detect pairs of QTL for each trait that interact epistatically using R/qtl (Broman *et al.* 2003). For this test, we scanned at 1-cM steps within each interval. Significance thresholds were determined with 1000 permutations and a 0.05 type I error rate.

Results

Quantitative genetics and correlations among ecophysiological and phenological traits within each treatment

All raw trait data is provided in supporting information, File S1. Within each treatment, all traits demonstrated highly significant effects of genotype ($P < 0.002$; Table 1), except for leaf mass per area (LMA) in CL. For the gas-exchange traits measured in the WL treatment, V_G/V_P was smallest for transpiration rate (E; 0.11), stomatal conductance (g_s; 0.12), and intrinsic WUE (W_g; 0.15) and was largest for photosynthesis (A; 0.35) and chlorophyll fluorescence in light (F_v'/F_m'; 0.56) (Table 1). For traits measured in all three treatments, the rank order of V_G/V_P across treatments varied by trait; V_G/V_P for carbon isotope discrimination ($\Delta^{13}C$) was smallest in WL (0.19) and largest in CS (0.63), V_G/V_P for N_{area} was smallest in CL (0.15) and largest in CS (0.33), and V_G/V_P for days to flowering and LMA were smallest in CL (0.16 and 0.04, respectively) and largest in WL (0.51 and 0.31, respectively) (Table 1).

To assess the relationship among traits, we estimated genotypic correlations between pairs of traits using both a multivariate ANOVA approach and a bivariate approach based on genotypic BLUPs. The multivariate approach accounts for experimental error and associations among multiple traits in estimating correlations between any two traits of interest; the bivariate approach does not account for estimation error (Hadfield *et al.* 2010). Correlation coefficients using the ANOVA approach were uniformly larger but proportional to results using the bivariate approach; we thus show only the results of the bivariate correlation analyses (Figure 1) because these correlation coefficients correspond directly to scatter plots, which we use to illustrate genotypes that have unusual trait combinations (see below). As expected, A was significantly correlated with most ecophysiological traits. A was positively correlated with F_v'/F_m' ($r = 0.506$, $P < 0.0001$; Figure 1), a measure of the efficiency of the light-harvesting reactions, indicating that efficient light-harvesting abilities were associated with higher photosynthesis. Most genotypes adhered closely to the slope of the $F_v'/F_m' \times A$ correlation, but several genotypes had unusually high values for both traits (see top right-hand quadrant of the scatter plot of this correlation; Figure 1). A was positively correlated with LMA ($r = 0.423$, $P < 0.0001$; Figure 1), a measure of leaf thickness, indicating that thicker leaves had higher photosynthesis. A was positively correlated with N_{area} ($r = 0.531$, $P < 0.0001$; Figure 1), which was expected given that nitrogen is an essential component of photosynthetic enzymes and chlorophyll. Several genotypes had the unusual combination of high A and low N_{area} (see bottom right-hand quadrant of the scatter plot of this correlation; Figure 1), indicative of high nitrogen-use efficiency. A was negatively correlated with $\Delta^{13}C$ ($r = -0.239$, $P = 0.0016$), and positively correlated with traits involved in water relations, such as g_s ($r = 0.342$, $P < 0.0001$; Figure 1) and E ($r = 0.337$, $P < 0.0001$; Figure 1) and W_g ($r = 0.320$, $P < 0.0001$; Figure 1), indicating that higher photosynthesis was associated with higher rates of water use and water-use efficiency. A few genotypes had the unusual combination of large A with small g_s and E (see bottom right-hand quadrants of the scatter plots of these correlations; Figure 1), indicative of high intrinsic WUE.

Table 1 Means, quantitative genetic partitioning, and significance of effects for ecophysiological and phenological traits for 150 RILs of *B. rapa* in each treatment in which the trait was measured

Trait	Treatment	Mean (SE)	V_G (SE)	V_G/V_P
A (μmol m^{-2} sec^{-1})	WL	18.95 (0.2445)	6.0043 (1.0076)****	0.3466
F_v'/F_m'	WL	0.4427 (0.0086)	0.0015 (0.0002)****	0.5564
g_s (mol m^{-2} sec^{-1})	WL	0.4513 (0.0496)	0.0035 (0.0008)****	0.1209
E (mmol m^{-2} sec^{-1})	WL	8.5765 (0.8372)	0.9133 (0.2262)****	0.1090
W_g (A g$_s^{-1}$)	WL	47.5810 (5.4830)	58.54 (13.0246)****	0.1489
Δ^{13}C	WL	24.2650 (0.1340)	0.2743 (0.0676)****	0.1889
	CL	23.2048 (0.1359)	0.4279 (0.0.1032)****	0.2747
	CS	23.9644 (0.1352)	0.6644 (0.09571)****	0.6261
N_{area} (g m^{-2})	WL	1.7496 (0.02994)	0.0324 (0.0093)***	0.1724
	CL	2.4315 (0.03169)	0.05839 (0.0202)**	0.1541
	CS	2.1822 (0.03112)	0.0771 (0.0156)****	0.3294
Days to flowering	WL	40.6675 (2.3888)	43.1109 (5.9667)****	0.5143
	CL	70.0479 (2.3961)	34.2299 (8.1085)****	0.1561
	CS	78.7036 (2.3925)	79.129 (12.4583)****	0.3388
LMA (g m^{-2})	WL	26.5130 (0.6258)	16.7614 (2.8080)****	0.3092
	CL	35.0710 (0.6458)	5.7805 (4.2830)	0.0398
	CS	31.9813 (0.6378)	18.9177 (4.3201)****	0.1974

Standard errors are indicated in parentheses. V_G, among-genotypic variance; V_G/V_P, among-genotypic variance divided by total phenotypic variance; A, photosynthetic rate; F_v'/F_m', chlorophyll fluorescence in light; g_s, stomatal conductance; E, transpiration rate; W_g, intrinsic water-use efficiency (A/g_s); Δ^{13}C, carbon isotope discrimination; N_{area}, nitrogen concentration on a leaf area basis; LMA, leaf mass per area. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

As expected, W_g was negatively correlated with Δ^{13}C ($r = -0.287$, $P < 0.0005$; Figure 1). g_s and E were negatively correlated with W_g ($r = -0.695$, $P < 0.0001$ and $r = -0.692$, $P < 0.0001$, respectively; Figure 1) and positively correlated with Δ^{13}C ($r = 0.538$, $P < 0.0001$ and $r = 0.550$, $P < 0.0001$ respectively; Figure 1), indicating that lower rates of water loss contributed to higher WUE. Interestingly, both W_g and Δ^{13}C were more strongly correlated with g_s and E than with A, suggesting that water relations may have a stronger affect on WUE than photosynthesis in this species (or at least in the genotypes used here). F_v'/F_m' was significantly positively correlated with W_g ($r = 0.418$, $P < 0.0001$; Figure 1) and negatively correlated with Δ^{13}C ($r = -0.262$, $P = 0.0016$; Figure 1), indicating that increased efficiency of the light-harvesting reactions is associated with higher WUE. W_g was not correlated with N_{area}, whereas Δ^{13}C and N_{area} were positively correlated in all three treatments ($r = 0.321$–0.439, $P < 0.0001$; Figure 1).

F_v'/F_m' was not correlated with E, g_s, N_{area}, or LMA, indicating that the genes involved in the light-harvesting reactions were not functionally associated with those that directly regulate leaf water, leaf nitrogen, or leaf biomass per unit area. As might be expected given their strong biophysical connection and the experimental regulation of vapor-pressure deficit, g_s and E were positively correlated with each other ($r = 0.972$, $P < 0.0001$; Figure 1). g_s and E were also significantly correlated with N_{area} ($r = 0.246$, $P < 0.0031$ and $r = 0.257$, $P < 0.0019$, respectively; Figure 1), but were not significantly correlated with LMA.

The phenological trait, days to flowering, was not significantly correlated with any trait except LMA in WL and CS ($r = 0.385$, $P < 0.0001$ and $r = 0.295$, $P < 0.001$ respectively; Figure 1), indicating that later-flowering plants had higher LMA values.

Patterns of main-effect QTL expression of ecophysiological and phenological traits within each treatment

The genome-wide scans for main-effect QTL detected 58 significant QTL using a 0.05 type I error rate and two additional QTL using a 0.075 type I error rate (Figure 2; Table A1). Of these 60 total QTL, 19 were detected for the gas-exchange traits measured only in WL (*e.g.*, F_v'/F_m', A, g_s, E, and W_g) and the remaining 41 QTL were detected for traits measured in all three treatments (*e.g.*, LMA, N_{area}, Δ^{13}C, and days to flowering); of these, 12 were detected in WL, 13 were detected in CL, and 16 were detected in CS (Figure 2; Table A1).

To assess the relationship among traits, we investigated patterns of colocalization of QTL and results of multitrait mapping within each treatment. In the WL treatment, colocalization of QTL was largely consistent with observed genotypic correlations (Figures 1 and 2 and Table A1). For example, A, F_v'/F_m', and W_g were significantly correlated and also had QTL that colocalized at the top of chromosome 1; QTL colocalization among many ecophysiological traits measured in WL also occurred in other locations on chromosomes 1, 2, 3, 6, 7, 9, and 10. In the CL treatment, most patterns of colocalization of QTL were likewise consistent with significant genotypic correlations among traits (Figures 1 and 2 and Table A1). For example, QTL colocalized (or were closely adjacent) for LMA and N_{area} on chromosomes 1, 3, and 8, and QTL for LMA and Δ^{13}C colocalized on chromosome 9. In the CS treatment, several instances of colocalization of QTL were consistent with observed genotypic correlations (Figures 1 and 2 and Table A1); for example, LMA, Δ^{13}C, N_{area} were correlated, and had QTL that colocalized on chromosomes 8 and 9.

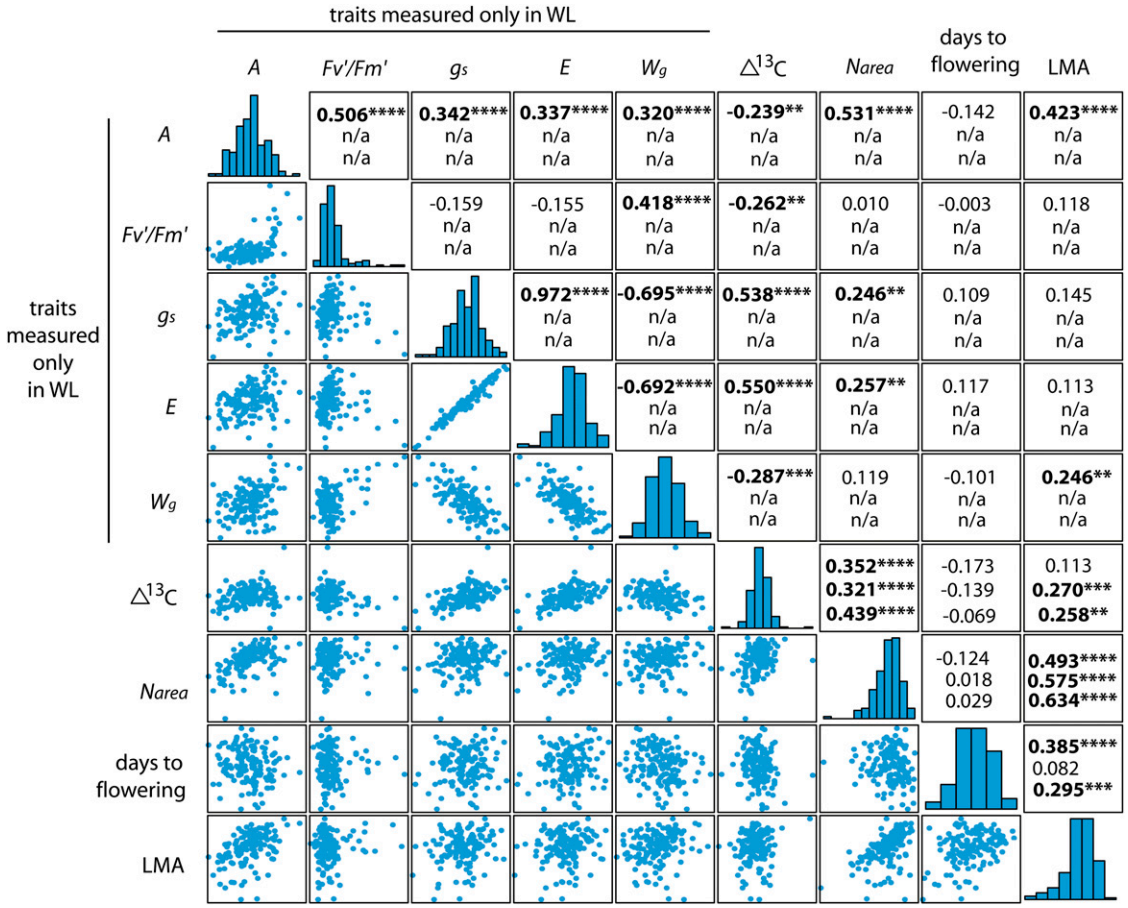

Figure 1 Correlations among traits within each treatment. Pearson correlation coefficients and significance of correlations among pairs of traits within the WL (top), CL (middle) and CS (bottom) treatments are indicated above the diagonal, with correlations that are significantly different than zero after false discovery rate correction indicated in boldface type. Histograms of each trait in the CL treatment are shown on the diagonal, and scatter plots of the bivariate correlation among pairs of traits in the WL treatment are shown below the diagonal; histograms and plots are shown only for the WL treatment because the largest number of traits was sampled in this treatment. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

Correlations and QTL colocalization between physiological and circadian traits

To understand the relationship between circadian parameters and physiology, we estimated bivariate genotypic correlations between the physiological traits from the present study and estimates of circadian period from the same RILs raised at similar temperatures (Lou *et al.* 2011). Many of the ecophysiological traits were significantly correlated with circadian period within each treatment (Table 2). In WL, *A* was significantly negatively correlated with circadian period at both 24° and 18°, while g_s, *E*, and $\Delta^{13}C$ were significantly negatively correlated with circadian period only at 18°; the greater number of associations at 18° relative to 24° likely arises from the greater robustness of circadian measures at the lower temperature (Lou *et al.* 2011). In CL, $\Delta^{13}C$ and N_{area} were significantly negatively correlated with period only at 12°, whereas in CS these two traits were significantly negatively correlated with period at both 12° and 18°.

We next investigated whether these correlated traits had QTL that colocalized. Consistent with the strong negative correlations between circadian period length and both N_{area} and $\Delta^{13}C$ in CS and CL (Table 2), five QTL jointly affected (or were closely adjacent for) period at 12° and N_{area} in the cold treatments (on chromosomes 1, 3, 8, 9, and 10; Table A1). Three QTL jointly affected period length at 12° and $\Delta^{13}C$ in the cold treatments (on chromosomes 7, 8, and 9). Consistent with the genotypic correlations between period and g_s, *E*, A, and $\Delta^{13}C$ in the WL treatment, a QTL for period length at 18° was closely adjacent to QTL for g_s and *E* on chromosome 2, and QTL for period length at 12° and 24° colocalized with QTL for *A* and $\Delta^{13}C$ on chromosome 9.

Quantitative genetics, correlations, and patterns of main-effect QTL expression across treatments

The four traits sampled in all three treatments demonstrated weakly to highly significant genotype by environment interactions (GEI; Table 3), indicating that genotypic sensitivity to the environment was trait-specific. Despite the significant GEI, the effect of genotype remained highly significant for all traits ($P < 0.0001$; Table 3). To measure the relative variance attributable to genotype and GEI, we

1
Fv′/Fm′-WL
A-WL
Wg-WL
E-WL
Period-12C
gs-WL
days to flowering-WL
LMA-CL
LMA-CS
Narea-CS
Narea-CL
Narea-WL
A-WL
Period-18C
2
Period-18C
E-WL
gs-WL
3
daystoflowering-CL
Period-12C
LMA-WL
LMA-CL
Narea-CS
Narea-CL
gs-WL
E-WL
Fv′/Fm′-WL
5
LMA-CS
Period-12C
6
LMA-CS
Fv′/Fm′-WL
gs-WL
E-WL
7
Period-12C
Wg-WL
Δ13C-WL
Δ13C-CS
Δ13C-CL
gs-WL
E-WL
Narea-CS
Δ13C-WL
8
Narea-WL
Narea-CL
Δ13C-CS
Δ13C-CL
days to flowering-CS
Period-24C
Period-18C
Period-12C
LMA-CL
LMA-CS
9
Period-24C
10
gs-WL
A-WL
Narea-CS
LMA-CL
LMA-WL
LMA-CS
Period-12C
Period-24C
Δ13C-WL
Δ13C-CL
Δ13C-CS
days to flowering-WL
LMA-WL
days to flowering-CL
Narea-CL
Narea-CS
Narea-WL
Period-12C
Period-24C
Period-18C

Table 2 Bivariate correlations between circadian and ecophysiological traits

Treatment	WL		CL		CS	
Circadian period:	18°	24°	12°	18°	12°	18°
A	*−0.294****	*−0.226**	N/A	N/A	N/A	N/A
F_v'/F_m'	0.111	0.081	N/A	N/A	N/A	N/A
g_s	*−0.340*****	−0.105	N/A	N/A	N/A	N/A
E	*−0.310****	−0.097	N/A	N/A	N/A	N/A
W_g	0.061	−0.144	N/A	N/A	N/A	N/A
$\Delta^{13}C$	*−0.308****	−0.014	*−0.377*****	−0.158	*−0.373*****	*−0.275***
N_{area}	0.069	−0.166	*−0.366*****	−0.155	*−0.381*****	*−0.198**
Days to flowering	0.029	−0.111	−0.012	0.158	−0.068	0.173
LMA	−0.051	−0.145	0.008	−0.047	−0.043	−0.066

Correlations that are significant after false discovery rate correction are indicated in italics. A, photosynthetic rate; F_v'/F_m', chlorophyll fluorescence in light; g_s, stomatal conductance; E, transpiration rate; W_g, intrinsic water-use efficiency; $\Delta^{13}C$, carbon isotope discrimination; N_{area}, nitrogen concentration on a leaf area basis; LMA, leaf mass per area. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

divided the GEI variance by the among-genotype variance (Gurganus *et al.* 1998). These values were 0.09 for N_{area}, 0.14 for $\Delta^{13}C$, 0.32 for days to flowering, and 0.67 for LMA; thus, the majority of the variance for most traits was attributable to among-genotype differences and less was attributable to differential sensitivity of genotypes to the environment.

To further assess whether the genetic architecture of traits changed across treatments, we estimated genotypic correlations (r_{GE}) and performed ANOVAs across pairs of treatments. Overall, values of r_{GE} were high (0.78–0.98; Table 4). For N_{area} and LMA, r_{GE} across CL/CS was significantly different than 0 and not significantly different from 1, indicating that the same loci affect trait variation and alleles have the same functional effects across treatment pairs, whereas r_{GE} estimates across WL/CL and WL/CS were significantly different than both 0 and 1 (Tables 3 and 4, significant genotype and GEI effects), indicating that some loci had variable effects across treatment pairs. The fact that r_{GE} for N_{area} and LMA were largest across the two treatments with the same temperature (CL/CS), and smaller between treatments with different temperatures (*e.g.*, WL/CL and WL/CS) suggests that the genetic basis of these two traits is more sensitive to temperature than photoperiod (Tables 3 and 4). However, QTL for each of these two traits colocalized in all three treatments (for example, for N_{area} on chromosomes 1, 8, and 10). For $\Delta^{13}C$, all r_{GE} estimates were significantly different than 0 and not or only moderately ($P > 0.01$) significantly different from 1 (Tables 3 and 4). Correspondingly, QTL typically affected $\Delta^{13}C$ in all three treatments (for example, on chromosomes 7 and 9; Figure 2, Table A1).

For days to flowering, all estimates of r_{GE} were significantly different than both 0 and 1 (Tables 3 and 4). Most QTL for flowering time were mapped in only one environment (Figure 2, Table A1), and flowering time was the only trait for which a significant QTL × E interaction was detected (*Appendix*).

Epistatically interacting QTL

In the two-dimensional genome scans to detect pairs of QTL that interact epistatically to affect trait variation, only five instances of loci with significant epistatic effects were detected (Table 5). One epistatic interaction was detected for N_{area} in WL, and four interactions were detected for F_v'/F_m' (Table 5).

Discussion

Patterns of trait covariation and QTL colocalization of circadian and physiological traits

The molecular genetic characterization of the clock is proceeding rapidly, but comparatively little is known about how quantitative clock variation affects plant performance (Harmer 2009; Más and Yanovsky 2009; Pruneda-Paz and Kay 2010; but see Yerushalmi *et al.* 2011). In this study, we provide some of the first evidence of an association between quantitative genetic variation in circadian period and ecophysiological traits. Specifically, circadian period was significantly genotypically correlated with a range of ecophysiological traits, and across most linkage groups, QTL regulating the clock also affected ecophysiological traits. These patterns suggest that either pleiotropic or physically linked genes contributed to covariation among circadian and ecophysiological traits. Although genetic correlations among traits may also be caused

Figure 2 QTL detected for physiological and phenological traits in *B. rapa*. The treatment in which QTL were detected is indicated after the trait name. The length of vertical bars designate the range of 2-LOD support limits for each QTL, with the peak of each QTL indicated. Note that chromosomes 5, 8, 9, and 10 are presented in inverted north–south orientation relative to their orientation as described by Iniguez-Luy *et al.* (2009) to be consistent with the accepted orientation (*i.e.*, that of Parkin *et al.* 2005). Solid bars indicate QTL that were significant using a 0.05 type I error rate and dashed bars indicate QTL that were significant using a 0.075 type I error rate. QTL trait names in gray are circadian period QTL taken from Lou *et al.* (2011) and are shown to illustrate patterns of circadian and physiological trait colocalization.

Table 3 Quantitative genetic partitioning of variation, and significance of effects for carbon isotope, elemental composition, phenological, and leaf traits across three treatments

	Random effects			Fixed effect Treatment			
Trait	Chamber (treatment)	Genotype	Genotype × treatment	Residual	df	F	Significant genotype × treatment contrasts
$\Delta^{13}C$	0.053 (0.028)**	0.385 (0.057)*****	0.0574 (0.0282)**	0.843 (0.0375)*****	$F_{2,\ 9.67}$	19.07****	WL/CS**, WL/CL*
N_{area}	0.001 (0.0010)**	0.038 (0.008)*****	0.018 (0.007)***	0.204 (0.009)*****	$F_{2,\ 12.3}$	175.56*****	WL/CS***, WL/CL***
Days to flowering	20.835 (7.790)****	33.542 (4.950)*****	10.726 (2.059)*****	62.1366 (2.097)*****	$F_{2,\ 15.7}$	84.96*****	CL/CS*****, WL/CS*****, WL/CL*****
LMA	1.191 (0.667)**	8.363 (1.982)*****	5.640 (1.903)****	80.071 (2.686)*****	$F_{2,\ 19.2}$	54.41*****	WL/CS*****, WL/CL*****

Standard error is indicated in parenthesis. $\Delta^{13}C$, carbon isotope discrimination; N_{area}, nitrogen concentration on a leaf area basis; LMA, leaf mass per area. $^{*}P < 0.1$; $^{**}P < 0.05$; $^{***}P < 0.01$; $^{****}P < 0.001$; $^{*****}P < 0.0001$.

by selection and ensuing LD among physically distant genes (*i.e.*, "adaptive character complexes"; Armbruster and Schwaegerle 1996), this is unlikely to be a significant factor contributing to trait covariation in this study because recombination during the formation of RILs would be expected to disrupt LD between unlinked genes. Furthermore, the consistent patterns of colocalization of QTL for circadian and ecophysiological traits suggest that pleiotropy may be a stronger factor contributing to trait covariation. Consistent with a hypothesis of pleiotropy, short- and long-period clock mutants in *A. thaliana* differed in physiology (Dodd *et al.* 2005; Graf *et al.* 2010).

The circadian clock provides an internal estimate of time that allows organisms to synchronize biological events with those of environmental day/night cycles (Michael *et al.* 2003; Dodd *et al.* 2005). The genetic link found between traits in this study suggests that ecophysiological traits may be outputs of the circadian clock and that the expression of photosynthetic and gas-exchange traits is regulated according to internal estimates of time to synchronize them with light cycles. Most RILs examined in this study had period lengths longer than 24 hr (Lou *et al.* 2011), and the RILs that had shorter period lengths (*i.e.*, closer to 24 hr) better coordinated production of photosynthetic proteins and stomatal behavior with the light cycle (Table 1), likely because their period length most closely matched that of the environment. However, the exact mechanistic link connecting circadian and physiological traits remains to be determined. The genetic link found in this study also suggests that naturally occurring quantitative variation in circadian period may be related to fitness; genotypes with periods closest to that of the environment have increased photosynthesis as well as stomatal conductance, which may lead to increased biomass accumulation and reproductive output.

Patterns of trait covariation and QTL colocalization among physiological traits

Results of this study also showed strong patterns of covariation among ecophysiological traits. Given that the effects of LD between physically unlinked genes are probably reduced in this population, either pleiotropy or physically linked genes likely underlie the genetic correlations observed among the ecophysiological traits measured here. Many of the correlations among ecophysiological traits were expected given the well-known mechanistic connections between these traits (Farquhar *et al.* 1980; Evans *et al.* 1986; Long and Bernacchi 2003). For example, the strong correlations found between A, g_s, and E were expected because the rate of photosynthesis must necessarily be related to the rate of gas exchange and transpiration through the stomates. Likewise, the correlation of $\Delta^{13}C$ with A, g_s, and W_g was expected because $\Delta^{13}C$ is a long-term proxy for photosynthesis and water-use efficiency. However, it is also worth noting that these trait pairs, such as A and g_s, were not perfectly correlated. Although A and g_s both partially depend on stomatal opening and gas diffusion rates, A is

Table 4 Pearson correlation coefficients of across-treatment genotypic correlations (r_{GE}) for each trait

Trait	rGE CL *vs.* CS	rGE WL *vs.* CL	rGE WL *vs.* CS
$\Delta^{13}C$	0.98283	0.96280	0.97078*
N_{area}	0.9011	0.8561**	0.8812*
Days to flowering	0.86436**	0.8615***	0.87657****
LMA	0.83332	0.78189**	0.81145****

P values indicated are from tests of significance of the genotype × treatment interaction term in ANOVA analyses across pairs of environments and indicate whether cross-environment correlations are significantly different from 1. Most comparisons were significantly different than 0 as indicated by a significant genotype effect in ANOVA analyses across pairs of environments, except N_{area} across WL/CL and WL/CS was not significantly different from 0. $\Delta^{13}C$, carbon isotope discrimination; N_{area}, nitrogen concentration on a leaf area basis; LMA, leaf mass per area. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

also determined by many other loci related to light harvesting and carbon fixation and utilization.

The relative strength of bivariate correlations may provide insight into the processes controlling specific ecophysiological traits. For example, both W_g and $\Delta^{13}C$ are at least partially controlled by A and g_s, and despite the fact that heritability for g_s was smaller than for A, both W_g and $\Delta^{13}C$ were more strongly correlated with g_s, suggesting that stomatal conductance is potentially a more important driver of both W_g and $\Delta^{13}C$. Furthermore, patterns of QTL colocalization among multiple traits may help to pinpoint the specific processes that underlie variation in an integrative trait. For example, QTL for W_g and A, both integrative traits, colocalized with a QTL for F_v'/F_m' on chromosome 1, suggesting that a gene (or genes) in this chromosomal region that affects efficiency of the light-harvesting reactions underlies the QTL detected for the integrative traits and supporting the hypothesis that photosynthesis, light harvesting, and water loss are linked mechanistically (Katul *et al.* 2003; Brodribb and Feild 2008; Skillman 2008) (although the hypothesis of linkage disequilibrium between multiple causal loci cannot be entirely discounted). On chromosome 7, a large-effect QTL (25% variance explained) for $\Delta^{13}C$ is closely adjacent to QTL for g_s and E, suggesting that the QTL in this region affects variation in $\Delta^{13}C$ by affecting stomatal traits. Along these lines, future research in *B. rapa* aims to clarify the processes that contribute to variation in ecophysiological traits in this species. Because of the tight connection between water transport and photosynthesis found here, we will quantify and QTL map traits such as cell-specific and whole-organ conductance in the roots, stems, and leaves in these RILs to determine if these hydraulic traits colocalize with QTL for (and presumably affect) A, g_s, E, $\Delta^{13}C$, and W_g.

There were, however, patterns of trait covariation that did not agree with expectations. A strong relationship exists between $\Delta^{13}C$ and days to flowering in *A. thaliana* (McKay *et al.* 2003; Hausmann *et al.* 2005; Juenger *et al.* 2005), common bean (White 1993), wheat (Ehdaie *et al.* 1993), and cowpea (Menendez and Hall 1995). Furthermore, crop and wild accessions of *B. rapa* grown in the field also demonstrated phenotypic correlations between physiology and flowering time; plants that delayed flowering had increased intrinsic WUE (Edwards and Weinig, unpublished data). Here, we did not find a significant relationship between days to flowering and $\Delta^{13}C$ or W_g at the genotypic level, which may reflect a lack of functional variation at the genes that jointly affect flowering time and $\Delta^{13}C$ in the parents of the RILs. McKay *et al.* (2003) attribute the flowering time–$\Delta^{13}C$ association to one gene of major effect, *FRIGIDA*, a vernalization response gene that is fixed in the *B. rapa* RIL parents (neither of which requires vernalization to flower) but may segregate in the crop/wild accessions we sampled (some of which do require vernalization to flower).

A more detailed examination of the correlations among ecophysiological traits has identified several genotypes that have desirable combinations of traits that could be targeted for crop improvement. For example, several genotypes (Figure 1) have large values of A in combination with low values of g_s or N_{area} and therefore are the most water- or nitrogen-use efficient. Additionally, most genotypes had parallel increases in A per unit change in F_v'/F_m', but several genotypes had unusually high values for both A and F_v'/F_m' and thus have the highest photosynthetic rate and most efficient light-harvesting reactions. Deviations from the standard A to F_v'/F_m' relationship are likely due to changes in allocation of resources to photoprotection mechanisms, which are especially prevalent at low temperatures and high light (Fryer *et al.* 1998). The genotypes that have the highest photosynthetic rate with the lowest water and nutrient demands could be used to increase crop productivity and efficiency, depending on the environmental conditions in which the crop is grown. Further, because the relationship between flowering time and ecophysiological traits was nonsignificant, genotypes that have favorable combinations of ecophysiological traits could produce lines that vary in flowering time, which would allow for physiological

Table 5 Traits and locations of pairs of loci that interact epistatically

Trait	Chromosome–locus 1	Marker–locus 1	Chromosome–locus 2	Marker–locus 2
N_{area}	2	fito529	2	pX142bH
F_v'/F_m'	3	pX142bH	4	fito066c
F_v'/F_m'	3	fito528	5	pX149cX
F_v'/F_m'	3	BRMS031a	6	fito423
F_v'/F_m'	5	pX101dH	6	fito423

improvement of the crops while maintaining the flowering time that is optimal for specific environments.

The effect of temperature and photoperiod on trait expression

For the four traits measured in all three combinations of temperature and photoperiod environments, the response of genotypes to the treatment varied by trait. For LMA and N_{area}, r_{GE} was greatest across treatments with the same temperature (but differing photoperiod). These results suggest that the genetic basis of these traits is similar across treatments with the same temperature (in CL *vs.* CS) but differs across treatments with different temperatures (in comparisons involving WL). Given that LMA and N_{area} are involved in important functions such as carbon allocation, light capture, and nutrient acquisition (Reich *et al.* 1999; Wright *et al.* 2004) variation in these traits may have effects on the photosynthetic rate, fitness, and overall physiological performance of genotypes. The GEI in response to temperature, along with the large natural differences in temperature environments that *B. rapa* experiences across its wide Eurasian distribution, indicates that genotypes may vary in physiological performance across temperature gradients.

Although $\Delta^{13}C$ has previously been shown to vary across temperature gradients (Körner *et al.* 1991; Loader and Hemming 2001), the genetic architecture of this trait was insensitive to the temperature and photoperiod treatments applied here, as evidenced by the consistently high values of r_{GE}, the consistent colocalization of QTL for $\Delta^{13}C$, and the lack of significant QTL × E across all treatments. However, the significant effect of environment (Table 3) indicates that $\Delta^{13}C$ exhibited acclimation to each treatment. In field studies, G × E interactions for $\Delta^{13}C$ were also nonsignificant, as were QTL × environment interactions in wheat (Rebetzke *et al.* 2008), and genotypes retained their rank orders of $\Delta^{13}C$ over time despite temporal environmental variation in a natural population of *Encelia farinosa* (Ehleringer 1993). However, other studies have found significant QTL × E for $\Delta^{13}C$ in response to field environments with different watering regimes (Ngugi *et al.* 1993; Teulat *et al.* 2002). The genotypic response of $\Delta^{13}C$ to environmental variation may depend either on species- or population-specific characteristics, or on the specific type of environmental variation that a population experiences.

Conclusions

Although the connection between the circadian clock and physiological processes has long been suggested, this is one of the first studies showing a link between quantitative genetic variation in the clock and in ecophysiological traits related to photosynthesis. This link suggests one means by which variation in the clock may affect fitness. The work also suggests new avenues by which to investigate the mechanistic controls over photosynthesis and to thus narrow the range of candidate genes underlying a QTL. To further investigate clock outputs and whether the clock–physiology relationships observed in the RILs are representative of the species, ongoing experiments are examining associations between circadian period, physiology, and fitness in natural and crop accessions of *B. rapa*.

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Appendix

Table A1 Results of composite interval QTL mapping and analyses of QTL × treatment interactions of physiological and phenological traits in *B. rapa* RILS

Trait treatment	Position, cM (2-LOD intervals)	LR	Additive effect	% variance explained	Closest marker	Significant contrasts for QTL × E
			Chromosome 1			
F_v'/F_m'	0.01 (0.01–5.86)	22.27	−0.02	10.42	pW190aH	N/A
A	0.67 (0.01–4.04)	18.95	−0.84	8.17	pW249dX	N/A
W_g	0.67 (0.01–13.89)	20.65	−1.72	9.56	pW249dX	N/A
E	24.99 (24.6–48.6)	16.14	0.17	6.09	fito433	N/A
g_s	38.25 (24.6–44.6)	18.05	0.02	6.83	fito019d	N/A
Days to flowering-WL	39.66 (24.99–50.77)	14.47	−1.34	5.16	fito019d	
LMA—CL	41.66 (32.56–44.6)[a+]	22.67	0.94	10.43	fito101a	
LMA—CS	43.22 (30.99–62.24)[b+]	16.01	0.75	6.36	pW108aE	
N_{area}—CS	48.6 (38.25–62.24)[b+]	23.47	0.058	8.92	pX136bE	
N_{area}—CL	52.77 (38.25–62.24)[a+]	27.46	0.074	13.08	pX136bE	
N_{area}—WL	52.77 (39.66–62.24)[c]	19.24	0.051	9.25	pX136bE	
A	62.24 (50.6–71.28)[c]	13.7	0.57	5.95	fito222	N/A
			Chromosome 2			
g_s	78.8 (67.3–85.28)[d+]	17.97	−0.01	6.37	pW208aH	N/A
E	78.8 (66.01–96.89)[d+]	13.69	−0.16	5.27	pW208aH	N/A
			Chromosome 3			
Days to flowering—CL	44.39 (31.83–55.73)[e+]	21.59	−1.9	10.89	pW174cX	
LMA—CL	68.06 (55.73–79.83)[f]	15.55	−0.75	7.12	pW147bH	
LMA—WL	66.06 (54.59–76.61)	13.19	−0.61	5.68	pW147bH	
N_{area}—CS	68.06 (56.25–76.61)	17.97	−0.05	7.09	fito492	
N_{area}—CL	82.49 (70.78–94.87)[f]	16.72	−0.05	6.58	fito378a	
E	130.2 (122.97–142.21)	16	−0.18	6.61	pW166aH	N/A
F_v'/F_m'	149.03 (140.21–157.05)	19.65	0.01	9.85	fito019c	N/A
g_s	130.2 (114.87–140.21)	13.3	−0.01	5.04	pW166aH	N/A
			Chromosome 5			
LMA—CS	19.285 (5.185–26.825)	13.45	0.78	5.28	fito130b	
			Chromosome 6			
g_s	108.37 (102.15–132.29)	18.62	−0.01	6.55	fito041	N/A
E	108.37 (100.15–132.29)	17.18	−0.18	6.58	fito041	N/A
LMA—CS	110.44 (72.54–132.29)	12.08	−0.64	4.65	fito100a	
### F_v'/F_m'	112.44 (90.15–120.29)	11.55	0.01	5.57	pX110aX	N/A
			Chromosome 7			
W_g	32.62 (26.62–42.96)[g]	31.45	2.11	15.26	fito057	N/A
Δ^{13}C—CS	33.4 (32.62–34.4)[h+]	62.69	−0.3	27.19	fito057	
Δ^{13}C—WL	33.4 (32.62–36.96)[g]	38.75	−0.23	16.68	fito057	
Δ^{13}C—CL	42.96 (35.08–51.23)[i+]	35.59	−0.24	13.52	fito348	
g_s	49.23 (32.62–56.99)[g]	20.91	−0.01	9.64	fito348	N/A
E	49.23 (33.79–56.99)[g]	26.06	−0.25	13.52	fito348	N/A
N_{area}—CS	76.23 (68.08–76.23)[j]	13.17	−0.05	4.98	pW202aX	
Δ^{13}C—WL	74.23 (62.99–84.82)	13.16	−0.13	5.05	pX144aH	
Δ^{13}C—CL	79.91 (70.08–84.82)	21.83	−0.16	7.5	pX101cH	
###Δ^{13}C—CS	86.82 (70.08–96.9)[j]	12.08	−0.13	4.55	fito101b	
Days to flowering—CS	96.88 (88.82–96.9)[j]	19.01	−2.05	9.32	pW150cH	WL/CS* CL/CS**
			Chromosome 8			
N_{area}—WL	27.063 (17.063–33.953)	17.8	0.05	8.28	pW138aX	
N_{area}—CL	27.063 (15.063–59.123)	14.07	0.05	5.54	pW138aX	
N_{area}—CS	46.463 (33.953–59.123)	17.8	0.07	11.10	pW177aE	
Δ^{13}C—CS	34.463 (27.063–58.463)	19.63	0.18	7.41	pW245aE	
LMA—CS	58.463 (33.953–59.123)	14.36	0.71	6.18	pW177aE	
LMA—CL	58.463 (33.953–59.123)	13.43	0.72	6.47	pW177aE	

(continued)

Table A1 Continued

Trait treatment	Position, cM (2-LOD intervals)	LR	Additive effect	% variance explained	Closest marker	Significant contrasts for QTL × E
			Chromosome 9			
Days to flowering—WL	46.331 (35.451–54.661)	13.93	−1.44	5.05	fito100b	
$\Delta^{13}C$—CS	56.391 (47.931–71.261)$^{k+}$	19.34	−0.16	7.05	pX147gE	
$\Delta^{13}C$—CL	62.591 (59.161–71.261)	30.1	−0.2	11.3	fito549	
$\Delta^{13}C$—WL	64.591 (50.201–71.261)	17.32	−0.15	7.29	fito549	
LMA—CS	56.391 (50.201–71.261)$^{k+}$	21.54	−0.86	8.56	pX147gE	
LMA—WL	70.591 (62.591–76.941)	30.13	−0.94	14.01	pW130cX	
LMA—CL	72.381 (59.161–80.441)	13.25	−0.68	5.85	BRMS016	
N_{area}—CS	70.591 (70.591–76.941)$^{k+}$	18.17	−0.05	6.73	pW130cX	
A	72.381 (61.471–76.941)	17.57	−0.6	7.77	BRMS016	N/A
g_s	85.441 (76.941–97.441)	32.91	−0.02	12.79	fito033b	N/A
			Chromosome 10			
N_{area}—WL	35.334 (24.824–46.124)$^{l+}$	16.92	−0.05	7.40	pW129dH	
N_{area}—CS	35.334 (35.334–46.124)$^{m+}$	13.96	−0.05	5.02	pW129dH	
N_{area}—CL	35.334 (26.834–44.274)$^{n+}$	25.98	−0.07	10.84	pW129dH	
Days to flowering—CL	26.834 (12.824–44.984)$^{n+}$	15.36	−1.55	7.3	pW240aE	
Days to flowering—WL	45.544 (44.274–58.434)$^{l+}$	32.07	−2.13	12.49	pW174aX	
LMA—WL	46.284 (35.334–54.584)$^{l+}$	15.51	−0.68	6.57	pW255aE	

The trait and treatment for which the QTL was detected, chromosomal position (in centimorgans), 2-lod support intervals (in centimorgans), likelihood ratio (LR) test statistic, additive effect of the IMB211 allele, and closest marker for each QTL are listed. QTL are organized by cM position of the QTL peak for each chromosome in accordance with their position in Figure 2. Note that chromosomes 5, 8, 9, and 10 are presented in inverted north–south orientation relative to their orientation as described by Iniguez-Luy *et al.* (2009) in order to be consistent with the accepted orientation (*i.e.*, that of Parkin *et al.* 2005). Superscript letters next to centimorgan positions indicate sets of QTL that colocalized within a treatment for which multitrait composite interval mapping identified a single, significant QTL, supporting a hypothesis of pleiotropy. Superscript plus symbols next to the letters indicate locations in which period QTL from the appropriate environment were included in multitrait composite interval mapping analyses and results identified a single, significant QTL. A, photosynthetic rate; F_v'/F_m', chlorophyll fluorescence in light; g_s, stomatal conductance; E, transpiration rate; W_g, intrinsic water-use efficiency (A/g_s); $\Delta^{13}C$, carbon isotope discrimination; N_{area}, nitrogen concentration on a leaf area basis; LMA, leaf mass per area. Also listed are pairs of treatments between which significant QTL × treatment interactions were detected. N/A indicates traits that were sampled in only the WL treatment. (###) QTL significant at 0.075 level. $^*P < 0.1$, $^{**}P < 0.05$.

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The Genetic Architecture of Ecophysiological and Circadian Traits in *Brassica rapa*

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File S1
Supporting Data

File S1 is available for download as an Excel file at
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