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1 **Circadian rhythms are associated with variation in photosystem II function and**
2 **photoprotective mechanisms**

3

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19 **Abstract.** The circadian clock regulates many aspects of leaf gas supply and biochemical
20 demand for CO₂, and is hypothesized to improve plant performance. Yet the extent to
21 which the clock may regulate the efficiency of photosystem II (PSII) and photoprotective
22 mechanisms such as heat dissipation remains largely unexplored. Based on measurements
23 of chlorophyll *a* fluorescence, we estimated the maximum efficiency of photosystem II in
24 light (F_v'/F_m') and heat dissipation by non-photochemical quenching (NPQ). We further
25 dissected total NPQ into its main components, qE (pH-dependent quenching), qT (state-
26 transition quenching) and qI (quenching related to photoinhibition), in clock mutant
27 genotypes of *Arabidopsis thaliana*, the cognate wild-type genotypes, and a panel of
28 recombinant inbred lines (RILs) expressing quantitative variation in clock period.
29 Compared to mutants with altered clock function, we observed that wild-type genotypes
30 with clock period lengths of approximately 24 hr had both higher levels of F_v'/F_m' ,
31 indicative of improved PSII function, and reduced NPQ , suggestive of lower stress on
32 PSII light harvesting complexes. In the RILs, genetic variances were significant for
33 F_v'/F_m' and all three components of NPQ , with qE explaining the greatest proportion of
34 NPQ . Bivariate tests of association and structural equation models of hierarchical trait
35 relationships showed that quantitative clock variation was empirically associated with
36 F_v'/F_m' and NPQ , with qE mediating the relationship with gas exchange. The results
37 demonstrate significant segregating variation for all photoprotective components, and
38 suggest the adaptive significance of the clock may partly derive from its regulation of the
39 light reactions of photosynthesis and of photoprotective mechanisms.

40 Key words: *Arabidopsis thaliana*, circadian rhythms, chlorophyll *a* fluorescence,
41 maximum efficiency of PSII, non-photochemical quenching

42 **Introduction**

43

44 The circadian clock is a time-keeping mechanism that enables organisms to adaptively
45 match many transcriptomic, physiological, developmental, and biochemical processes to
46 natural diurnal cycles (McClung *et al.*, 2013; Yerushalmi *et al.*, 2009; Sanchez *et al.*,
47 2016; Resco de Dios and Gessler, 2017). By comparing the phenotypes of wild-type
48 plants to mutant genotypes with altered clock function, several studies have demonstrated
49 that diverse ecophysiological traits (e.g., total CO₂ assimilation rates and sugar status) are
50 affected by the circadian clock (Dodd *et al.*, 2005; Graf *et al.*, 2010). More specifically,
51 circadian rhythms that are closer to 24 hours and resonate with environmental cycles
52 likely optimize the diurnal timing of gas exchange (Dodd *et al.*, 2005). Transcriptomic
53 studies on representative *Arabidopsis* genotypes also indicate that key gas-exchange
54 genes are regulated on a diel basis (Dodd *et al.*, 2014; Pilgrim & McClung, 1993).
55 Further, quantitative variation in the circadian clock is associated with gas-exchange in
56 segregating progenies (Edwards *et al.*, 2011; Lou *et al.*, 2011) and in crop types of
57 *Brassica rapa* (Yarkhunova *et al.*, 2016) as well as with timing of gas-exchange
58 responses to drought (Greenham *et al.*, 2017). Thus, the circadian clock emerges as an
59 important regulator of gas-exchange. Yet, its influence on the biophysical activity of both
60 photosystems remains poorly characterized, leaving unresolved the mechanistic
61 connection between the circadian clock and leaf level gas-exchange as well as
62 photoprotection (Greenham & McClung, 2015; Guadagno *et al.*, 2018).

63 Sunlight serves as the energy source for photosynthesis, and higher light
64 intensities typically correlate with increases in photosynthetic rates (A) (Björkman &

65 Demmig-Adams, 1995; McDonald, 2003). Further, the efficiency of photosystem II
66 (PSII) in utilizing light energy (F_v'/F_m') correlates with gas-exchange rates and plant
67 performance under various experimental conditions at a given light level (Maxwell &
68 Johnson, 2000). However, the absorbed light energy may exceed the demand for energy
69 and the reducing capacity of the light-independent reactions of photosynthesis,
70 potentially leading to photodamage through formation of reactive oxygen species (ROS).
71 In response to light stress, plants have evolved several photoprotective mechanisms. A
72 large number of enzymes take part in scavenging activities (Asada, 2006; Das &
73 Roychoudhury, 2014); some carotenoids have been shown to be highly efficient in
74 scrubbing excited chlorophyll molecules (Bassi & Caffarri, 2000), and ascorbate is also
75 an efficient antioxidant in various organisms (Fukumura *et al.*, 2012). However, when
76 excitation energy exceeds demand, the first line of defense to avoid damage to PSII is
77 heat dissipation. Thermal dissipation is a protective strategy to reduce photoinhibition,
78 and is ubiquitous to photosynthetic organisms (Müller *et al.*, 2001). This mechanism
79 competes with photochemistry and chlorophyll *a* fluorescence for the use of excitation
80 energy (Baker, 2008), and it is commonly referred to as non-photochemical quenching of
81 chlorophyll *a* fluorescence (*NPQ*).

82 *NPQ* comprises at least three major components: *qE* (pH-dependent quenching),
83 *qT* (state-transition quenching) and *qI* (quenching related to photoinhibition). The onset
84 of *qE* occurs quickly, within seconds to a few minutes, and is triggered through the
85 synergistic action of thylakoid lumen pH and the formation of an energy quenching
86 complex between the protein PsbS and the pool of xanthophyll and zeaxanthin (Horton *et*
87 *al.*, 2000; Li *et al.*, 2002). The *qT* component can occur following 2-15 minutes of

88 illumination and reflects the balance of excitation between the two photosystems, which
89 depends upon reversible photophosphorylation activity and ensuing relocation of light
90 harvesting complexes (Niyogi, 2000). *qi* has slow relaxation kinetics and is related
91 directly to photoinhibition, including down-regulation and complete deactivation of PSII
92 (Li *et al.*, 2002).

93 In the past two decades, the development of pulse amplitude modulated (PAM)
94 fluorometry has provided a sensitive and non-destructive method to estimate the
95 efficiency of PSII and the importance of *NPQ* and the variability of each component in
96 different environmental conditions (Baker, 2008; Schreiber, 2004). Among several
97 applications, the PAM method has made it possible to partition variance among
98 environmental and genetic sources. Prior studies have focused on partitioning sources of
99 variance in total *NPQ* (Fujiwara *et al.*, 2014; Jung & Niyogi, 2009; Kasajima *et al.*,
100 2011; van Rooijen *et al.*, 2015) and in PSII photoinhibition (Jansen *et al.*, 2010). Genetic
101 variances for total *NPQ* were highly significant in four *A. thaliana* accessions across an
102 extensive range of incident light (varying from 100 to 1800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$; (Jung &
103 Niyogi, 2009). However, the magnitude of genetic variances of all individual components
104 of *NPQ* have not been estimated, although such knowledge is important to understanding
105 possible regulatory paths and ultimately to breeding opportunities for crop improvement.

106 Light availability and light stress vary in predictable ways over the course of the
107 day. Quantitative clock variation is correspondingly associated with gas-exchange in
108 various species under field and controlled environmental conditions (Burstin *et al.*, 2007;
109 de Dios *et al.*, 2016; Edwards *et al.*, 2012; Edwards *et al.*, 2011; Yarkhunova *et al.*,
110 2016), and might contribute to the regulation of thermal dissipation of excess energy.

111 Further, although thermal dissipation is a photoprotective mechanism, it is metabolically
112 regulated and impacts the operational state of photosynthesis (Murchie & Harbinson,
113 2014), again consistent with the hypothesis that *NPQ* might be clock regulated.

114 Here, we first compared the maximum efficiency of PSII in light (F_v'/F_m') and
115 *NPQ* between wild-type genotypes of *Arabidopsis thaliana* and mutants with altered
116 clock function to empirically test for a possible role of the circadian clock in PSII
117 function and photoprotection. We then used recombinant inbred lines (RILs) that vary in
118 circadian periodicity to characterize the expression of genetic variation in leaf gas
119 exchange, chlorophyll *a* fluorescence traits, and *NPQ* across environments with high vs.
120 low light intensity. Finally, we used structural equation modeling to investigate
121 hypothesized causal relationships between quantitative variation in circadian rhythms,
122 leaf gas exchange, *NPQ*, and the components of *NPQ*.

123

124 **Materials and Methods**

125

126 *Plant material and growth*

127

128 We first compared F_v'/F_m' and total *NPQ* between mutant genotypes with altered
129 clock function and the cognate wild-type plants, in order to test the relationship between
130 clock (mis)function and efficiency of PSII function and photoprotection. We included
131 replicates harboring alleles of the clock mutant genotype, *zeitlupe* (*ztl-24*, *ztl-25*); (Kevei
132 *et al.*, 2006), that express a long clock period (28 hr) phenotype, the clock mutant, *timing*
133 *of cab expression 1* (*toc1-21*) (Ding *et al.*, 2007; Fujiwara *et al.*, 2008) that express a

134 short clock period (20 hr), and the cognate, Ws-2, wild-type genotype in which these
135 mutations reside.

136 Seeds of both mutant and wild type genotypes were placed in microcentrifuge
137 tubes stratified in water at 4°C for 1 week. Seeds were then planted into 6 × 6 × 9 cm
138 plastic pots filled with Sunshine #5 potting mix (Sunshine Redi-Earth Professional
139 Growing Mix, Sun Gro Horticulture, Bellevue, WA). Pots were placed in Percival PGC-
140 9/2 growth chambers (Percival Scientific, Perry, Indiana, USA) with the following
141 conditions: photoperiod 10/14 hours (light/dark), temperatures of 22 ± 1 °C during the
142 daytime and 19 ± 1 °C during nighttime, and PPFD = 350 μmol photons m⁻² s⁻¹. Short
143 days were used to allow for greater growth before the onset of flowering. Measurements
144 of *Fv'/Fm'* and *NPQ* were taken at the ambient light level of 350 μmol photons m⁻² s⁻¹ on
145 at least seven replicates per genotype using a portable PAR-FluorPen FP 100-MAX-LM
146 fluorometer (Photon System Instruments, Brno, Czech Republic).

147 To characterize genetic and environmental sources of variation in *Fv'/Fm'*,
148 components of *NPQ*, and associations between these two traits and clock period, we used
149 recombinant inbred lines (RILs) of *Arabidopsis thaliana* (L.) Heynh. (*Brassicaceae*). The
150 RILs were developed from a cross between Ler (Landsberg *erecta*, Germany) and Ws-2
151 (Wassilewskaja, Belarus), in which the Ws-2 parent harbors the reporter gene
152 *LUCIFERASE* (*LUC*) linked to the promoter of *COLD-CIRCADIAN RHYTHM-RNA*
153 *BINDING 2* (*CCR2*), allowing for quantification of circadian parameters (Millar, Short,
154 Chua & Kay, 1992). Details of the crossing design are provided in Boikoglou & Davis
155 (2009) and Rubin *et al* (2017). In brief, the two parents were crossed to create a

156 heterozygous F_1 . The F_1 was then backcrossed to the maternal parent, and the resulting
157 BC_1F_2 genotypes were selfed to the BC_1F_6 generation through single seed descent.

158 An initial experiment quantifying F_v'/F_m' associations with clock period was
159 conducted using 32 lines, following the same planting protocol and growth conditions as
160 the mutants. Due to the time-consuming nature of NPQ relaxation curve measurements
161 and limited space in the growth chambers, eleven RILs (8-10 replicates per RIL) were
162 chosen at random to conduct the leaf chlorophyll a fluorescence measurements and to
163 dissect the components of NPQ .

164

165 *Circadian measures*

166

167 For circadian measures, seeds of each RIL were surface-sterilized and cold-stratified. Six
168 to eight replicates of each RIL were planted into white 96-well microliter plates
169 containing Murashige and Skoog mineral plant growth media supplemented with 30g/L
170 sucrose. Plates were then moved to the growth chambers with the following conditions:
171 10/14 hours (light/dark) photoperiod, temperature of 22 ± 1 °C and relative humidity of
172 50 ± 1 % for five days, a period of time sufficient for clock entrainment. After
173 entrainment, 20 μ l of a 100 mM D-luciferin monopotassium salt and 0.01% Triton X-100
174 solution was added to each well, and plates were resealed and placed under an ORCA-II
175 ER digital camera (Hamamatsu Photonics C4742-98-24ER). Circadian parameters were
176 estimated from bioluminescence using fast Fourier transform nonlinear least-square
177 analysis (FFT-NLLS) (Hicks *et al.*, 1996).

178

179 *Leaf gas-exchange and chlorophyll fluorescence measurements*
180
181 Leaf gas-exchange measurements, including photosynthetic rate (A), stomatal
182 conductance (g_s), and chlorophyll a fluorescence emissions, were measured
183 simultaneously using a leaf chamber fluorometer LICOR LI-6400-40 (Open System
184 Vers. 4.0, Li-Cor, Inc., Lincoln, NE). Measurements were taken from a fully developed
185 rosette leaf at least 1 h after subjective dawn under the following chamber conditions:
186 PPFD= 500 (low light, LL) or 1500 (high light, HL) $\mu\text{mol m}^{-2} \text{s}^{-1}$, flow rate= 300 $\text{m}^2 \text{s}^{-1}$,
187 $\text{ref } [\text{CO}_2] = 400 \mu\text{mol m}^{-2} \text{s}^{-1}$, $T_{\text{leaf}} = 22^\circ\text{C}$ and VPD_L (Vapor pressure deficit based on leaf
188 temp, kPa) was kept between 1.3-1.7 kPa, fan mode set on FAST (Long & Bernacchi,
189 2003). After a dark acclimation period (30 min), the maximum fluorescence in darkness
190 (F_m) was determined by applying a saturating pulse (0.8 s) with intensity of $\sim 5000 \mu\text{mol}$
191 $\text{photons m}^{-2} \text{s}^{-1}$. The leaves were then exposed for 10 min to different actinic light levels
192 to obtain the maximum fluorescence in light conditions, F_m' . Calculations of F_o' used the
193 equation from Oxborough and Baker (1997), $F_o' = F_o / (F_v / F_m + F_o / F_m')$. After induction
194 of NPQ , recovery of the fluorescence signal was monitored in darkness for 40 min,
195 through the application of seven saturating pulses (0.8 s; intensity of $\sim 5000 \mu\text{mol photons}$
196 $\text{m}^{-2} \text{s}^{-1}$) at different times (2, 5, 10, 15, 20, 30, 40 min). NPQ data were expressed as
197 $NPQ = (F_m - F_m') / F_m'$ (Bilger & Schreiber, 1987), and the three NPQ components (qE ,
198 qT and qI) were quantified following a modified method of Walters and Horton (Walters
199 & Horton, 1990, Walters & Horton, 1991). For each recorded fluorescence curve and
200 each measured leaf, NPQ data were reported in a semi-logarithmic plot versus recovery
201 time. The components of NPQ were calculated by linear regression of three exponential

202 decays. The half-times for each component were reported as $qI = A$, $qT = (B - A)$, $qE =$
203 $(C - B)$, with A, B and C intercepts on the y axis (D'Ambrosio *et al.*, 2008).

204

205 ***Data analysis***

206

207 *Statistical approach and data treatments*

208

209 All analyses were conducted in R version 3.2.4 (Team, 2014), <http://www.r-project.org>.

210 Analysis of variance (ANOVA) was used to test for differences in Fv'/Fm' and total NPQ

211 between wild-type and clock mutant genotypes in the first experiment. ANOVA was also

212 used to test the influence of light treatments and genotypic effect on physiological traits

213 (including circadian period, Fv'/Fm' , total NPQ , A , g_s , qE , qT , qI) measured in the RILs

214 ('lm' and 'anova' functions of R). Further, we estimated the fold difference in NPQ or its

215 components by dividing the trait value in one light treatment by its value in the other

216 treatment (low light / high light treatment). Principal components analysis (PCA) was

217 performed using the 'prcomp' procedure in R, and scores were tested for the effect of

218 genotype.

219 We were further interested in testing the relative contribution of individual

220 physiological traits and circadian period to the expression of A_{max} . First, we determined

221 how clusters of traits related to genetic variation in the RILs using Principal Components

222 Analysis (PCA) as an approach to address collinearity between fluorescence variables.

223 Second, to quantify hypothesized causal relationships between traits, we used structural

224 equation modeling with observed variables. We developed an initial (saturated) model

225 based on observed bivariate correlations and known relationships among physiological
226 traits and between circadian and physiological traits. The fit of alternative structural
227 equation models to the observed data was tested with the `sem()` function of the ‘lavaan’
228 package (Rosseel, 2012) in R version 3.2.4 (Team, 2014). To identify a model with good
229 fit, a proposed model was evaluated through Confirmatory Factor Analysis within the
230 lavaan package and the fit indices that rank parsimony (Akaike’s Information Criterion;
231 AIC). If the fit criteria (described below) were not met for the proposed model, then
232 modification indices were used to adjust the model; specifically, variables were excluded
233 from the model with the highest AIC, and fit indices for the reduced model were again
234 evaluated. Model fit was assessed with a chi-square test, root mean square error of
235 approximation (RMSEA), and comparative fit index (CFI). Chi-square values associated
236 with a P-value > 0.05 and a RMSEA <0.05 and CFI > 0.95 indicate a good fit of the
237 model to the data (Kline, 2015).

238 Once the model with the best fit was identified, structural equation modeling was
239 used to partition variation in a response variable among multiple predictor variables.
240 Specifically, the multivariate regression model that is the basis for structural equation
241 modeling statistically accounts for variation in multiple predictor variables (in this case,
242 traits) simultaneously and tests their relationship to a response variable. We were
243 interested in the hierarchical relationships among measured traits (e.g., circadian period,
244 gas-exchange traits, *NPQ*). This approach reveals the extent to which a given trait
245 directly vs. indirectly affects the response variable (e.g., circadian period could affect A_{max}
246 directly or act indirectly through *NPQ*) (e.g., Fournier-Level *et al*, 2013).

247

248 **Results**

249

250 To test for a clock effect on chlorophyll fluorescence, we compared Fv'/Fm' and total
251 NPQ between wild-type plants that express a circadian period near 24 hrs to clock mutant
252 genotypes with short 20-hr (*toc1*) or long 28-hr (*ztl*) circadian cycles (Fig. 1). Analysis of
253 variance revealed a significant genotype effect on maximum efficiency of PSII in light
254 (Fv'/Fm') (Table 1a). Specifically, wild type Ws-2 plants had higher values of Fv'/Fm'
255 compared to short and long circadian period mutants, indicating that light absorbed by
256 PSII is converted more efficiently to photochemistry in the wild-type plants (Fig. 1a).
257 Furthermore, ANOVA showed that circadian clock mutants had higher values of NPQ
258 than the wild type (Fig. 1b), indicating potentially greater light stress and the need for
259 higher thermal dissipation in the mutant genotypes even under the comparatively low
260 light treatment conditions. In sum, the results suggest that significant deviations (± 4 h)
261 from a wild-type circadian period of approximately 24 hrs may lead to reduced PSII
262 efficiency and to a surplus of excitation energy for PSII.

263

264 *Genetic variation in RILs, light treatment effects, and bivariate correlations*

265

266 We first surveyed circadian period and other physiological parameters, including
267 photosynthetic rate (A), stomatal conductance (g_s) and maximum efficiency of PSII in
268 light (Fv'/Fm') in 32 RILs. Analysis of variance showed significant variation among RILs
269 in circadian period and all physiological traits (Table 1). Among the RILs, we observed a
270 significant association between Fv'/Fm' and circadian period, such that RILs with

271 circadian cycles closer to 24 hrs had higher quantum yield of PSII (Fig. 2a).

272 We then chose a subset of eleven genotypes to estimate genetic and
273 environmental variances in the underlying fluorescence and non-photochemical
274 quenching parameters under our two experimental light conditions (low light, LL, 500
275 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and high light, HL, 1500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and to further explore
276 the relationship between the circadian clock and chlorophyll *a* fluorescence. We observed
277 significant light treatment effects for *A*, *F_m'*, *F_v'/F_m'*, *NPQ*, *qE*, *qT*, and *qI* ($p < 0.001$;
278 Table 1). As expected, *A* decreased in low light conditions, while *F_v'/F_m'* decreased in
279 response to the high light conditions (Table 1c). *NPQ* typically rises with increasing light
280 intensity and light stress, and we correspondingly observed a significant increase in total
281 *NPQ* under the HL relative to LL treatment ($p < 0.0001$; Fig. 3a). The partitioning of
282 individual components of *NPQ* also varied across light treatments (Fig. 3b, c). Within
283 total *NPQ*, *qE* and *qT* were higher on average in the LL treatment, while *qI* was higher in
284 the HL treatment (Fig. 3b, c). Overall, in both treatments *qE* was the primary determinant
285 of total *NPQ* (Fig. 3b, c).

286 The subset of 11 RILs also differed significantly in the expression of all measured
287 physiological parameters (Table 1; Fig. 3). Specifically, *A*, *g_s*, *F_m'*, *F_v'/F_m'*, *NPQ*, *qE*, *qT*
288 and *qI* showed a significant genotype effect ($p < 0.001$; Table 1). Total *NPQ* differed by
289 60% between RILs with the highest vs. lowest values under HL and 59% under LL (Fig.
290 3a). Using LL for further comparison of the *NPQ* components, *qI* and *qT* differed by
291 more than 100% between RILs with the highest vs. lowest values of these two traits; in
292 particular, *qT* differed by 166% between RIL113 and Ws-2 under the LL treatment, while
293 *qI* differed by 175% between RIL36 and RIL136. Differences among RILs were less

294 pronounced for qE , which varied by at most 12% among RILs in LL (Fig. 3b).

295 To empirically assess relationships among physiological traits, we tested for
296 significant bivariate correlations. As expected, A was correlated positively with g_s . A was
297 also positively correlated with F_v'/F_m' and with other fluorescence parameters (Fm' ,
298 Fv/Fm , NPQ , qI) (Table 2). We observed that in both LL and HL conditions RILs with
299 circadian rhythms closer to 24 hours had higher values of F_v'/F_m' (Fig. 2b, c), consistent
300 with the experiment utilizing all 32 lines. The fold difference in NPQ under LL vs. HL
301 conditions was associated with circadian period length (Fig. 4a), such that RILs with
302 circadian periods longer than 24 hrs expressed fold differences closer to 1. Fold
303 differences near 1 reflect RILs with comparatively high NPQ values even under the LL
304 treatment suggesting those genotypes experienced surplus light energy that elicited a
305 quenching requirement even in low light, a result akin to that observed in the clock
306 mutants. We also observed an association between the fold difference in qT and circadian
307 period (Fig. 4b). Specifically, RILs with shorter period lengths closer to 24 hr showed a
308 ~1.5-fold increase in state-transition related quenching, qT , across the LL relative to HL
309 environment, whereas the plants with period lengths closer to 27 hr had lower values
310 across the two light treatments. Together, these findings suggest that there may be
311 coordinated circadian regulation of photochemical (F_v'/F_m') and non-photochemical
312 (NPQ) processes under two different levels of irradiance.

313

314 *Principal Component Analysis*

315

316 The PCA of data collected in the LL treatment revealed three major components that

317 describe genotypic variation (Table S1, Fig. S1a) and allow inference as to how different
318 traits (circadian period, A , g_s , chlorophyll fluorescence etc) are inter-related while
319 accounting for collinearity among multiple fluorescence measures. The first principal
320 component captured 43.95% of the total variance and was negatively related to F_o
321 (loading = -0.39), F_m (loading = -0.40), F_o' (loading = -0.41), and F_m' (loading = -0.40),
322 reflecting the well-known mathematical connection among fluorescence parameters. The
323 second principal component captured 28.47% of the variation and was positively related
324 to total NPQ (loading = 0.34), and negatively related to photosynthetic rates (loading = -
325 0.44), stomatal conductance (loading = -0.43), and F_v'/F_m' (loading = -0.42). The third
326 axis captured 10.77% of the variation and was positively related to circadian period
327 (loading = 0.52). Thus, PC2 and PC3 together account for variation that is independent of
328 fluorescence parameters F_o , F_m , F_o' , F_m' . The loading of circadian period (PCA2) was
329 opposite in sign to that with F_v'/F_m' (PCA3) (Fig. S1a), consistent with the observed
330 negative bivariate correlation between these two traits (Fig. 2a, Table S1). PCA of gas
331 exchange and fluorescence traits in the HL treatment had similar trait loadings but were
332 generally less structured (inter-correlated), and specifically the association of the clock
333 and fold difference in qT (Fig. S1b) was absent, an outcome that could reflect light stress.
334 For HL, PC1 explained 43% of the total variance and was positively related to
335 fluorescence parameters F_o , F_m , F_o' , F_m' . The second axes captured 20% and was
336 negatively related to parameters of gas-exchange (A , g_s) and F_v'/F_m' and positively
337 related to NPQ . The third and fourth axes both captured 11% of the variation were
338 positively related to F_v'/F_m' and circadian period. Overall, the PCA patterning is
339 consistent with univariate responses to the light treatments and observed bivariate

340 associations.

341 *Structural equation model*

342 To test the hierarchical relationships among measured circadian and physiological traits,
343 we used structural equation modeling. Based on AIC indices for all paths, we obtained a
344 model with good fit based on multiple metrics of Confirmatory Factor Analysis (Chi-
345 square p-value = 0.364, RMSEA = 0.026 ± 0.000 0.177 for the 90% CI, p-value = 0.466,
346 CFI = 0.999). The 'best fit' model is shown in Fig. 5a, and the standardized coefficients
347 for each of the modeled relationships are presented in Fig. 5b. The chi-square value of the
348 'best fit' model has a p-value > 0.05, which indicates that observed and expected
349 covariance matrices are not different and that the model has an adequate fit. The 90%
350 confidence interval (0.000-0.177) of the RMSEA indicates that the model has close
351 approximate fit to the data.

352 The SEM model revealed a network of connections between traits in the LL
353 treatment. As expected, photosynthetic rate (A) was regulated by stomatal conductance
354 (g_s) and Fv'/Fm' . Shorter circadian period (closer to 24 hrs) was associated with higher
355 Fv'/Fm' and lower values of NPQ (total non-photochemical quenching). NPQ was also
356 associated with stomatal conductance and qE . qE was the primary determinant of total
357 NPQ . The other two NPQ components, qT and qI , were removed during initial model
358 selection because they did not explain a significant proportion of the variance. Variation
359 in qE was also related to A and to NPQ . As expected from the traits' shared calculation
360 from fluorescence parameters, the decrease in NPQ was reflected in increased maximum
361 efficiency of PSII.

362

363 **Discussion**

364

365 Plants utilize the sun's energy as a source for photosynthesis. However, when plants
366 experience light intensities that exceed the needs of photochemistry, excess excitation
367 energy may be dissipated as heat or re-emitted as chlorophyll fluorescence. Excess
368 radiation may impose significant stress and damage PSII (Björkman & Demmig-Adams,
369 1995; McDonald, 2003). Light availability and light stress vary in predictable ways over
370 the course of the day such that quantitative clock variation is associated with gas-
371 exchange in various species under field and controlled environmental conditions (Burstin
372 *et al.*, 2007; de Dios *et al.*, 2016; Edwards *et al.*, 2012; Edwards *et al.*, 2011; Yarkhunova
373 *et al.*, 2016), and suggesting the circadian clock might contribute to regulation of thermal
374 dissipation of excess energy. Here, we first quantified chlorophyll fluorescence patterns
375 in mutant genotypes with disrupted clock function *vs.* genotypes with wild-type clock
376 function. Using a segregating population, we then estimated the quantitative-genetic
377 architecture of these traits, including estimation of genetic variances in gas-exchange
378 traits, *NPQ*, and components of *NPQ* as well as of genetic correlations between these
379 physiological traits and the circadian clock. We found significant connections between
380 clock period and both PSII efficiency and non-photochemical quenching.

381

382 *Wild-type clock function is associated with physiological parameters*

383 Circadian regulation of physiological traits has been documented in a large number of
384 studies and species (Dodd *et al.*, 2014; Faure *et al.*, 2012; Graf *et al.*, 2010; McClung,
385 2013), and delayed fluorescence expresses circadian oscillations and is a proposed proxy

386 for circadian rhythms (Gould *et al.*, 2009). Nevertheless, circadian regulation of the light
387 reactions of photosynthesis is not yet well-understood (Dodd *et al.*, 2014). We were
388 interested in ascertaining whether clock function is related to F_v'/F_m' and to NPQ and its
389 components. Our results show that disruption of clock function via large-effect mutation
390 leads to shifts in F_v'/F_m' and NPQ , such that wild-type plants have both higher F_v'/F_m'
391 and lower total NPQ , representing more efficient photosynthetic machinery.

392

393 *Quantitative (co)variation of physiological traits and clock period*

394

395 Chlorophyll *a* fluorescence is frequently utilized to investigate PSII function and to
396 estimate the response of photosynthetic machinery to environmental stress (Baker &
397 Bowyer, 1994; Baker & Rosenqvist, 2004; Maxwell & Johnson, 2000). The energy-
398 dependent non-photochemical quenching component, qE , was the greatest contributor to
399 total NPQ under both high and low light, consistent with its role in protecting against
400 short-term high light and light fluctuations such as those that occurred between the
401 growth ($350 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) and the measurement ($500 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ or 1500
402 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) conditions (Demmig-Adams *et al.*, 2014; Papageorgiou, 2014). On
403 average, the proportion of the qE component was higher among plants in the low light
404 treatment compared to high light treatment (although RILs also differed in the response
405 of this component to light treatment). The qI component of NPQ represents photodamage
406 to reaction centers of PSII (Demmig-Adams *et al.*, 2014; Krause, 1988); on average over
407 all genotypes, qI values were correspondingly greater in the HL conditions. The role of
408 the qT component may lie in maximizing photosynthetic efficiency under low light

409 conditions, and the percentage of qT may therefore increase when light is limited
410 (Coopman *et al.*, 2010, D'Ambrosio *et al.*, 2008), which is consistent with our
411 observation of higher values of qT under low light conditions (Fig. 3c).

412 While many studies have characterized the genetic architecture of A (Edwards *et*
413 *al.*, 2011, Fracheboud *et al.*, 2002, Hervé *et al.*, 2001, Teng *et al.*, 2004), fewer have
414 estimated genetic variances for NPQ and its component parameters (Jung & Niyogi,
415 2009, van Rooijen *et al.*, 2015). We find significant genetic variances for Fm' , Fv'/Fm' ,
416 NPQ and its individual components qE , qT , qI . Values of Fv'/Fm' ranged from 0.56 to
417 0.68 (Fig. 2a) among RILs, and NPQ values ranged from 1.1 to 1.8 in LL treatment. The
418 magnitude of NPQ variation among RILs is comparable to the magnitude of variation
419 observed among four accessions of *A. thaliana* (NPQ values = 1.5 to 2.0 at 600 μmol
420 photons $\text{m}^{-2}\text{s}^{-1}$) reported by Jung and Nigoyi (2009). We further observe variation among
421 RILs in qE (significant main effect of genotype on average across both treatments),
422 consistent with one prior study estimating genetic variances for qE among natural
423 accessions of *A. thaliana* (Niyogi *et al.*, 2005). Interestingly, these phenotypic differences
424 observed among a small sample of RILs (or accessions in Jung and Nigoyi, 2009 and
425 Niyogi *et al.* 2005) are comparable to interspecific differences for Fv'/Fm' and NPQ
426 (Demmig-Adams *et al.*, 2006; Guo & Trotter, 2004), indicating that segregating variation
427 in a within-species cross can reproduce phenotypic differences among species

428 Previous studies have found that circadian periods providing a match to
429 environmental conditions are beneficial for plant growth and performance under
430 controlled conditions (Barak *et al.*, 2000; Yerushalmi & Green, 2009) and in the field
431 (Rubin *et al.*, 2017), and can lead to higher gas-exchange values (Dodd *et al.* 2005;

432 Edwards et al. 2011; Yarkhunova et al. 2016). Further, many genes encoding proteins
433 associated with PSII functioning and *NPQ* (PsbS protein and other Psb subunits) are
434 circadian regulated (Covington *et al.*, 2008), suggesting the clock may regulate PSII
435 efficiency. We observe that circadian period lengths among a set of *A. thaliana* RILs
436 varies from 24 to 27 hours, and that this quantitative variation in circadian period
437 correlates with chlorophyll *a* fluorescence parameters. In addition, our data indicate that
438 this relationship is maintained under three different light conditions (Fig. 2a, b, c). This
439 association in the RILs together with the clock mutant results suggest that the adaptive
440 value of the circadian clock may arise in part from regulation of PSII function (Kreps &
441 Simon, 1997).

442 In addition to F_v'/F_m' , we observe that plants with high fold changes in *NPQ*
443 across low- to high-light conditions have period lengths that deviate from (are longer
444 than) 24 hrs. Genotypes with a circadian period closer to 27 hrs have higher initial rates
445 of *NPQ* under low light, indicating that the photoprotective mechanisms are induced at
446 lower light levels compared to the lines with shorter period lengths. These observations
447 demonstrate that there is a change in PSII excitation balance (Huner *et al.*, 1998) among
448 long-period genotypes such that even LL imposes stress, providing a further indication
449 that the clock is linked to PS II. We observed that genotypes with a circadian period
450 closer to 24 hr show comparatively greater values of qT under LL (500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$)
451 vs. HL (1500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) conditions (Fig. 4b), a pattern that is consistent
452 with the view that at least wild-type *A. thaliana* are generally not stressed at low light
453 levels of 500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ and may in fact be light limited (Bailey *et al.*, 2004).
454 Plants with normal clock function (expressing 24 hr periods) may experience light

455 limitation at 500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. Transcriptomic studies reveal that some genes that
456 code for enzymes that are required for state transitions (STN7 protein kinase,
457 AT1G68830, AT5G01920, AT4G27800) are circadian regulated (Covington *et al.*, 2008),
458 suggesting the clock plays an important role in synchronization of state transitions. It is
459 worth noting that neither *qE* nor *qI* showed correlations with circadian period in our
460 study, and neither the genes responsible for *qE* sites such as LHCII, CP29, and CP26
461 (AT1G19150, AT3G53460, AT4G10340), nor the genes associated with photoinhibition
462 (AT1G77510, AT2G30950, AT3G19570) are under circadian control (Covington *et al.*,
463 2008).

464

465 *PCA and Path analysis confirmed empirical relationships between physiological traits*

466

467 Three groups of traits that contribute to variation among the genotypes were identified
468 using the PCA analysis. The first group includes the fluorescence parameters *Fo*, *Fm*,
469 *Fo'*, and *Fm'*. All of these parameters are related and reflect physical properties of the
470 primary quinone acceptor of PSII, Q_A , or are partly influenced by PSII reaction center
471 redox activities (Roháček, 2002). The second group of traits contributes to variation in
472 *NPQ*, *Fv'/Fm'*, and gas-exchange traits; the third one is related to circadian period. PCA
473 and structural equation modeling revealed the correlation structure of complex traits and
474 potential mechanistic relationships, including how circadian period both directly and
475 indirectly interacts with and might influence physiological trait expression (Fig. 5; Fig.
476 S1).

477 Most of the paths in the SEM model were supported by bivariate correlations and
478 PC analysis, and specifically supported clock associations with chlorophyll fluorescence.
479 As noted, thermal dissipation, chlorophyll fluorescence and photochemistry (primarily
480 photosynthesis) are the three possible fates of light energy in the leaf, and all three occur
481 simultaneously (Baker, 2008), and therefore associations among components of *NPQ* as
482 well as between *A* and at least some chlorophyll fluorescence measures are anticipated.
483 Our SEM results are consistent with other studies, showing that *qE* is the primary
484 contributor to *NPQ* (Niyogi *et al.*, 2005). Further, *NPQ* does not directly affect *A*, but
485 instead acts indirectly through *Fv'/Fm'*. This indirect relationship likely reflects the fact
486 that *NPQ* (in contrast to PSII activity) does not result in ATP or NADPH production for
487 the Calvin Benson cycle, but instead dissipates excitation energy as heat (Ruban *et al.*,
488 2016). Although we do not observe a significant path between total *NPQ* and *A*, our
489 results show that the *qE* component of *NPQ* negatively affects *A*. *qE* regulates the
490 excitation rate of PSII reaction centers, which might contribute to energy utilization in the
491 photosynthetic apparatus and thereby affect values of *A* through the production of ATP
492 and NADPH. The SEM also reveal an association between circadian period and both
493 *Fv'/Fm'* and *NPQ*. In sum, our results from clock mutants and segregating lines are
494 consistent with the hypothesized importance of a functional circadian clock that resonates
495 with ambient conditions to plant growth, survival and reproduction (Dodd *et al.*, 2005,
496 Edwards *et al.*, 2011, Green *et al.*, 2002, Salmela *et al.*, 2015, Yarkhunova *et al.*, 2016).

497

498 **Conclusions**

499

500 The circadian clock has been implicated in plant performance in controlled settings, in
501 which alleles conferring a match between endogenous rhythms and diurnal cycles evolve
502 to higher frequency (Yerushalmi & Green, 2009) as well as in field settings, in which
503 discrete and quantitative clock phenotypes are associated with differences in allocation
504 (Salmela *et al.*, 2015) and in survival and fruit set (Rubin *et al.* 2017). The underlying
505 physiological reasons for these performance differences are unknown, although
506 quantitative clock variation correlates with gas-exchange traits (Edwards *et al.*, 2012,
507 Yarkhunova *et al.*, 2016). Recent studies also indicate that natural variation at the clock
508 gene, *GIGANTEA*, affects cold tolerance (Xie *et al.*, 2015) and growth patterns (de
509 Montaigu *et al.*, 2015) while in domesticated tomato delayed circadian clock was selected
510 during the process of domestication (Müller *et al.*, 2016). Our data suggest that circadian
511 rhythms might play an important role in regulation of plant photosynthetic machinery.
512 Specifically, the results of the present study suggest possible circadian regulation of
513 maximum efficiency of PSII, *NPQ* and the *qT* component of *NPQ*.

514

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518

519 **References**

520

521 Adams III W.W., Zarter C.R., Mueh K.E. & Demmig-Adams B. (2008) Energy
522 dissipation and photoinhibition: a continuum of photoprotection. In:
523 *Photoprotection, photoinhibition, gene regulation, and environment*, pp. 49-64.
524 Springer.

- 525 Asada K. (2006) Production and scavenging of reactive oxygen species in chloroplasts
526 and their functions. *Plant physiology*, **141**, 391-396.
- 527 Bailey S., Horton P. & Walters R.G. (2004) Acclimation of *Arabidopsis thaliana* to the
528 light environment: the relationship between photosynthetic function and
529 chloroplast composition. *Planta*, **218**, 793-802.
- 530 Bailey-Serres J. & Mittler R. (2006) The roles of reactive oxygen species in plant cells.
531 *Am Soc Plant Biol*.
- 532 Baker N.R. (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annu.*
533 *Rev. Plant Biol.*, **59**, 89-113.
- 534 Baker N.R. & Bowyer J.R. (1994) *Photoinhibition of photosynthesis: from molecular*
535 *mechanisms to the field*. Bios Scientific Publishers.
- 536 Baker N.R. & Rosenqvist E. (2004) Applications of chlorophyll fluorescence can
537 improve crop production strategies: an examination of future possibilities. *Journal*
538 *of Experimental Botany*, **55**, 1607-1621.
- 539 Barak S., Tobin E.M., Green R.M., Andronis C. & Sugano S. (2000) All in good time:
540 the *Arabidopsis* circadian clock. *Trends in plant science*, **5**, 517-522.
- 541 Bassi R. & Caffarri S. (2000) Lhc proteins and the regulation of photosynthetic light
542 harvesting function by xanthophylls. *Photosynthesis Research*, **64**, 243-256.
- 543 Bilger W. & Schreiber U. (1987) Energy-dependent quenching of dark-level chlorophyll
544 fluorescence in intact leaves. In: *Excitation Energy and Electron Transfer in*
545 *Photosynthesis*, pp. 157-162. Springer.
- 546 Björkman O. & Demmig-Adams B. (1995) Regulation of photosynthetic light energy
547 capture, conversion, and dissipation in leaves of higher plants. In: *Ecophysiology*
548 *of photosynthesis*, pp. 17-47. Springer.
- 549 Boikoglou E. & Davis S.J. (2009) Signaling in the circadian clock. In: *Signaling in*
550 *Plants*, pp. 261-285. Springer.
- 551 Burstin J., Marget P., Huart M., Moessner A., Mangin B., Duchene C., Desprez B.,
552 Munier-Jolain N. & Duc G. (2007) Developmental genes have pleiotropic effects
553 on plant morphology and source capacity, eventually impacting on seed protein
554 content and productivity in pea. *Plant physiology*, **144**, 768-781.
- 555 Coopman R.E., Fuentes-Neira F.P., Briceño V.F., Cabrera H.M., Corcuera L.J. & Bravo
556 L.A. (2010) Light energy partitioning in photosystems I and II during
557 development of *Nothofagus nitida* growing under different light environments in
558 the Chilean evergreen temperate rain forest. *Trees*, **24**, 247-259.
- 559 Covington M.F., Maloof J.N., Straume M., Kay S.A. & Harmer S.L. (2008) Global
560 transcriptome analysis reveals circadian regulation of key pathways in plant
561 growth and development. *Genome Biol*, **9**, R130.
- 562 D'Ambrosio N., Guadagno C. & De Santo A.V. (2008) Is qE always the major
563 component of non-photochemical quenching? In: *Photosynthesis. Energy from the*
564 *Sun*, pp. 1001-1004. Springer.
- 565 Das K. & Roychoudhury A. (2014) Reactive oxygen species (ROS) and response of
566 antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers*
567 *in Environmental Science*, **2**, 53.
- 568 de Dios V.R., Gessler A., Ferrio J.P., Alday J.G., Bahn M., del Castillo J., Devidal S.,
569 Garcia-Munoz S., Kayler Z. & Landais D. (2016) Circadian rhythms have

570 significant effects on leaf-to-canopy gas exchange under field conditions. *bioRxiv*,
571 054593.

572 de Dios V.R. & Gessler A. (2017) Circadian regulation of photosynthesis and
573 transpiration from genes to ecosystems. *Environmental and Experimental Botany*,
574 2017, vol. en premsa.

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

579 Demmig-Adams B. & Adams Iii W. (1992) Photoprotection and other responses of plants
580 to high light stress. *Annual review of plant biology*, **43**, 599-626.

581 Demmig-Adams B., Koh S.-C., Cohu C.M., Muller O., Stewart J.J. & Adams III W.W.
582 (2014) Non-photochemical fluorescence quenching in contrasting plant species
583 and environments. In: *Non-Photochemical Quenching and Energy Dissipation in*
584 *Plants, Algae and Cyanobacteria*, pp. 531-552. Springer.

585 Demmig-Adams B., Moeller D.L., Logan B.A. & Adams III W.W. (1998) Positive
586 correlation between levels of retained zeaxanthin+ antheraxanthin and degree of
587 photoinhibition in shade leaves of *Schefflera arboricola* (Hayata) Merrill. *Planta*,
588 **205**, 367-374.

589 Demmig - Adams B., Ebbert V., Mellman D.L., Mueh K.E., Schaffer L., Funk C., Zarter
590 C.R., Adamska I., Jansson S. & Adams W.W. (2006) Modulation of PsbS and
591 flexible vs sustained energy dissipation by light environment in different species.
592 *Physiologia Plantarum*, **127**, 670-680.

593 Derks A., Schaven K. & Bruce D. (2015) Diverse mechanisms for photoprotection in
594 photosynthesis. Dynamic regulation of photosystem II excitation in response to
595 rapid environmental change. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*,
596 **1847**, 468-485.

597 Ding Z., Doyle M.R., Amasino R.M. & Davis S.J. (2007) A complex genetic interaction
598 between *Arabidopsis thaliana* TOC1 and CCA1/LHY in driving the circadian
599 clock and in output regulation. *Genetics*, **176**, 1501-1510.

600 Dodd A.N., Kusakina J., Hall A., Gould P.D. & Hanaoka M. (2014) The circadian
601 regulation of photosynthesis. *Photosynthesis research*, **119**, 181-190.

602 Dodd A.N., Salathia N., Hall A., Kevei E., Toth R., Nagy F., Hibberd J.M., Millar A.J. &
603 Webb A.A. (2005) Plant circadian clocks increase photosynthesis, growth,
604 survival, and competitive advantage. *Science*, **309**, 630-633.

605 Edwards C.E., Ewers B.E., McClung C.R., Lou P. & Weinig C. (2012) Quantitative
606 variation in water-use efficiency across water regimes and its relationship with
607 circadian, vegetative, reproductive, and leaf gas-exchange traits. *Molecular Plant*,
608 **5**, 653-668.

609 Edwards C.E., Ewers B.E., Williams D.G., Xie Q., Lou P., Xu X., McClung C.R. &
610 Weinig C. (2011) The Genetic Architecture of Ecophysiological and Circadian
611 Traits in *Brassica rapa*. *Genetics*, **189**, 375-390.

612 Farquhar G.D. & Sharkey T.D. (1982) Stomatal conductance and photosynthesis. *Annual*
613 *review of plant physiology*, **33**, 317-345.

614 Faure S., Turner A.S., Gruszka D., Christodoulou V., Davis S.J., von Korff M. & Laurie
615 D.A. (2012) Mutation at the circadian clock gene EARLY MATURITY 8 adapts

616 domesticated barley (*Hordeum vulgare*) to short growing seasons. *Proceedings of*
617 *the National Academy of Sciences*, **109**, 8328-8333.

618 Fournier-Level A., Wilczek A.M., Cooper M.D., Roe J.L., Anderson J., Eaton D.,
619 Moyers B.T., Petipas R.H., Schaeffer R.N. & Pieper B. (2013) Paths to selection
620 on life history loci in different natural environments across the native range of
621 *Arabidopsis thaliana*. *Molecular ecology*, **22**, 3552-3566.

622 Fracheboud Y., Ribaut J.M., Vargas M., Messmer R. & Stamp P. (2002) Identification of
623 quantitative trait loci for cold-tolerance of photosynthesis in maize (*Zea mays L.*).
624 *Journal of experimental botany*, **53**, 1967-1977.

625 Fujiwara S., Sakamoto S., Kigoshi K., Suzuki K. & Ohme-Takagi M. (2014) VP16 fusion
626 induces the multiple-knockout phenotype of redundant transcriptional repressors
627 partly by Med25-independent mechanisms in *Arabidopsis*. *FEBS letters*, **588**,
628 3665-3672.

629 Fujiwara S., Wang L., Han L., Suh S.-S., Salomé P.A., McClung C.R. & Somers D.E.
630 (2008) Post-translational regulation of the *Arabidopsis* circadian clock through
631 selective proteolysis and phosphorylation of pseudo-response regulator proteins.
632 *Journal of Biological Chemistry*, **283**, 23073-23083.

633 Fukumura H., Sato M., Kezuka K., Sato I., Feng X., Okumura S., Fujita T., Yokoyama
634 U., Eguchi H. & Ishikawa Y. (2012) Effect of ascorbic acid on reactive oxygen
635 species production in chemotherapy and hyperthermia in prostate cancer cells.
636 *The Journal of Physiological Sciences*, **62**, 251-257.

637 Gilroy S., Suzuki N., Miller G., Choi W.-G., Toyota M., Devireddy A.R. & Mittler R.
638 (2014) A tidal wave of signals: calcium and ROS at the forefront of rapid
639 systemic signaling. *Trends in Plant Science*, **19**, 623-630.

640 Gould P.D., Diaz P., Hogben C., Kusakina J., Salem R., Hartwell J. & Hall A. (2009)
641 Delayed fluorescence as a universal tool for the measurement of circadian
642 rhythms in higher plants. *The Plant Journal*, **58**, 893-901.

643 Graf A., Schlereth A., Stitt M. & Smith A.M. (2010) Circadian control of carbohydrate
644 availability for growth in *Arabidopsis* plants at night. *Proc Natl Acad Sci U S A*,
645 **107**, 9458-9463.

646 Green R.M., Tingay S., Wang Z.-Y. & Tobin E.M. (2002) Circadian rhythms confer a
647 higher level of fitness to *Arabidopsis* plants. *Plant Physiology*, **129**, 576-584.

648 Greenham K. & McClung C.R. (2015) Integrating circadian dynamics with physiological
649 processes in plants. *Nature Reviews Genetics*, **16**, 598-610.

650 Guadagno C.R., Ewers B.E., & Weinig C. (2018) Circadian rhythms and redox state in
651 plants: till stress do us part. *Frontiers in Plant Science*.

652 Guo J. & Trotter C.M. (2004) Estimating photosynthetic light-use efficiency using the
653 photochemical reflectance index: variations among species. *Functional Plant*
654 *Biology*, **31**, 255-265.

655 Hervé D., Fabre F., Berrios E.F., Leroux N., Al Chaarani G., Planchon C., Sarrafi A. &
656 Gentzbittel L. (2001) QTL analysis of photosynthesis and water status traits in
657 sunflower (*Helianthus annuus L.*) under greenhouse conditions. *Journal of*
658 *experimental botany*, **52**, 1857-1864.

659 Hicks K.A., Millar A.J., Carré I.A. & Somers D.E. (1996) Conditional circadian
660 dysfunction of the *Arabidopsis* early-flowering 3 mutant. *Science*, **274**, 790.

661 Horton P., Ruban A.V. & Wentworth M. (2000) Allosteric regulation of the light-
662 harvesting system of photosystem II. *Philosophical Transactions of the Royal*
663 *Society of London B: Biological Sciences*, **355**, 1361-1370.

664 Huner N.P., Öquist G. & Sarhan F. (1998) Energy balance and acclimation to light and
665 cold. *Trends in Plant Science*, **3**, 224-230.

666 Jansen M.A., Martret B.L. & Koornneef M. (2010) Variations in constitutive and
667 inducible UV - B tolerance; dissecting photosystem II protection in *Arabidopsis*
668 *thaliana* accessions. *Physiologia plantarum*, **138**, 22-34.

669 Jung H.-S. & Niyogi K.K. (2009) Quantitative genetic analysis of thermal dissipation in
670 *Arabidopsis*. *Plant physiology*, **150**, 977-986.

671 Kangasjärvi S. & Kangasjärvi J. (2014) Towards understanding extracellular ROS
672 sensory and signaling systems in plants. *Advances in Botany*, **2014**.

673 Kasajima I., Ebana K., Yamamoto T., Takahara K., Yano M., Kawai-Yamada M. &
674 Uchimiya H. (2011) Molecular distinction in genetic regulation of
675 nonphotochemical quenching in rice. *Proceedings of the National Academy of*
676 *Sciences*, **108**, 13835-13840.

677 Kevei E., Gyula P., Hall A., Kozma-Bognár L., Kim W.-Y., Eriksson M.E., Tóth R.,
678 Hanano S., Fehér B. & Southern M.M. (2006) Forward genetic analysis of the
679 circadian clock separates the multiple functions of ZEITLUPE. *Plant physiology*,
680 **140**, 933-945.

681 Kline R.B. (2015) *Principles and practice of structural equation modeling*. Guilford
682 publications.

683 Krause G.H. (1988) Photoinhibition of photosynthesis. An evaluation of damaging and
684 protective mechanisms. *Physiologia Plantarum*, **74**, 566-574.

685 Kreps J.A. & Simon A.E. (1997) Environmental and genetic effects on circadian clock-
686 regulated gene expression in *Arabidopsis*. *The Plant Cell*, **9**, 297-304.

687 Lai A.G., Doherty C.J., Mueller-Roeber B., Kay S.A., Schippers J.H.M. & Dijkwel P.P.
688 (2012) CIRCADIAN CLOCK-ASSOCIATED 1 regulates ROS homeostasis and
689 oxidative stress responses. *Proceedings of the National Academy of Sciences*, **109**,
690 17129-17134.

691 Ledford H.K. & Niyogi K.K. (2005) Singlet oxygen and photo-oxidative stress
692 management in plants and algae. *Plant, Cell & Environment*, **28**, 1037-1045.

693 Li X.-P., Müller-Moulé P., Gilmore A.M. & Niyogi K.K. (2002) PsbS-dependent
694 enhancement of feedback de-excitation protects photosystem II from
695 photoinhibition. *Proceedings of the National Academy of Sciences*, **99**, 15222-
696 15227.

697 Long S. & Bernacchi C. (2003) Gas exchange measurements, what can they tell us about
698 the underlying limitations to photosynthesis? Procedures and sources of error.
699 *Journal of experimental botany*, **54**, 2393-2401.

700 Long S., Humphries S. & Falkowski P.G. (1994) Photoinhibition of photosynthesis in
701 nature. *Annual review of plant biology*, **45**, 633-662.

702 Lou P., Xie Q., Xu X., Edwards C., Brock M., Weinig C. & McClung C. (2011) Genetic
703 architecture of the circadian clock and flowering time in *Brassica rapa*.
704 *Theoretical and applied genetics*, **123**, 397-409.

705 Maxwell K. & Johnson G.N. (2000) Chlorophyll fluorescence - a practical guide. *Journal*
706 *of experimental botany*, **51**, 659-668.

707 McClung C.R. (2013) *Beyond Arabidopsis: the circadian clock in non-model plant*
708 *species*. Paper presented at the Seminars in cell & developmental biology.

709 McDonald M.S. (2003) *Photobiology of higher plants*. John Wiley & Sons.

710 Millar A.J., Short S.R., Chua N.-H. & Kay S.A. (1992) A novel circadian phenotype
711 based on firefly luciferase expression in transgenic plants. *The Plant Cell*, **4**,
712 1075-1087.

713 Müller N.A., Wijnen C.L., Srinivasan A., Ryngajllo M., Ofner I., Lin T., Ranjan A., West
714 D., Maloof J.N. & Sinha N.R. (2016) Domestication selected for deceleration of
715 the circadian clock in cultivated tomato. *Nature genetics*, **48**, 89-93.

716 Müller P., Li X.-P. & Niyogi K.K. (2001) Non-photochemical quenching. A response to
717 excess light energy. *Plant physiology*, **125**, 1558-1566.

718 Mullineaux C.W. & Emlyn-Jones D. (2005) State transitions: an example of acclimation
719 to low-light stress. *Journal of experimental botany*, **56**, 389-393.

720 Murchie E.H. & Harbinson J. (2014) Non-photochemical fluorescence quenching across
721 scales: from chloroplasts to plants to communities. In: *Non-Photochemical*
722 *Quenching and Energy Dissipation in Plants, Algae and Cyanobacteria*, pp. 553-
723 582. Springer.

724 Nilkens M., Kress E., Lambrev P., Miloslavina Y., Müller M., Holzwarth A.R. & Jahns
725 P. (2010) Identification of a slowly inducible zeaxanthin-dependent component of
726 non-photochemical quenching of chlorophyll fluorescence generated under
727 steady-state conditions in Arabidopsis. *Biochimica et Biophysica Acta (BBA)-*
728 *Bioenergetics*, **1797**, 466-475.

729 Niyogi K.K. (1999) Photoprotection revisited: genetic and molecular approaches. *Annual*
730 *review of plant biology*, **50**, 333-359.

731 Niyogi K.K. (2000) Safety valves for photosynthesis. *Current opinion in plant biology*, **3**,
732 455-460.

733 Niyogi K.K., Grossman A.R. & Björkman O. (1998) Arabidopsis mutants define a central
734 role for the xanthophyll cycle in the regulation of photosynthetic energy
735 conversion. *The Plant Cell*, **10**, 1121-1134.

736 Niyogi K.K., Li X.-P., Rosenberg V. & Jung H.-S. (2005) Is PsbS the site of non-
737 photochemical quenching in photosynthesis? *Journal of Experimental Botany*, **56**,
738 375-382.

739 Oxborough K. & Baker N.R. (1997) Resolving chlorophyll a fluorescence images of
740 photosynthetic efficiency into photochemical and non-photochemical
741 components—calculation of qP and Fv/Fm-; without measuring Fo.
742 *Photosynthesis research*, **54**, 135-142.

743 Papageorgiou G.C. (2014) The non-photochemical quenching of the electronically
744 excited state of chlorophyll a in plants: definitions, timelines, viewpoints, open
745 questions. In: *Non-Photochemical Quenching and Energy Dissipation in Plants,*
746 *Algae and Cyanobacteria*, pp. 1-44. Springer.

747 Pilgrim M.L. & McClung C.R. (1993) Differential involvement of the circadian clock in
748 the expression of genes required for ribulose-1, 5-bisphosphate
749 carboxylase/oxygenase synthesis, assembly, and activation in Arabidopsis
750 thaliana. *Plant Physiology*, **103**, 553-564.

751 Pospíšil P. (2009) Production of reactive oxygen species by photosystem II. *Biochimica*
752 *et Biophysica Acta (BBA)-Bioenergetics*, **1787**, 1151-1160.

753 Powles S.B. (1984) Photoinhibition of photosynthesis induced by visible light. *Annual*
754 *Review of Plant Physiology*, **35**, 0066-4294.

755 Roháček K. (2002) Chlorophyll fluorescence parameters: the definitions, photosynthetic
756 meaning, and mutual relationships. *Photosynthetica*, **40**, 13-29.

757 Rosseel Y. (2012) lavaan: An R package for structural equation modeling. *Journal of*
758 *Statistical Software*, **48**, 1-36.

759 Rubin, M.J., M.T. Brock, A.M. Davis, Z.M. German, M. Knapp, S.M. Welch, S.L.
760 Harmer, J.N. Maloof, S.J. Davis, and C. Weinig. Circadian rhythms vary over the
761 growing season and correlate with fitness components. In press, *Molecular*
762 *Ecology* 2017.

763 Salmela M.J., Greenham K., Lou P., McClung C.R., Ewers B.E. & Weinig C. (2015)
764 Variation in circadian rhythms is maintained among and within populations in
765 *Boechera stricta*. *Plant, cell & environment*.

766 Sanchez S.E. & Kay S.A. (2016) The Plant Circadian Clock: From a Simple Timekeeper
767 to a Complex Developmental Manager. *Cold Spring Harbor Perspectives in*
768 *Biology*, **8**, a027748.

769 Schreiber U. (2004) Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse
770 method: an overview. In: *Chlorophyll a Fluorescence*, pp. 279-319. Springer.

771 Sharma P., Jha A.B., Dubey R.S. & Pessarakli M. (2012) Reactive oxygen species,
772 oxidative damage, and antioxidative defense mechanism in plants under stressful
773 conditions. *Journal of Botany*, 2090-0120.

774 Szabó I., Bergantino E. & Giacometti G.M. (2005) Light and oxygenic photosynthesis:
775 energy dissipation as a protection mechanism against photo - oxidation. *EMBO*
776 *reports*, **6**, 629-634.

777 Takahashi S. & Badger M.R. (2011) Photoprotection in plants: a new light on
778 photosystem II damage. *Trends in plant science*, **16**, 53-60.

779 Team R.C. (2014) R: A language and environment for statistical computing. R
780 Foundation for Statistical Computing, Vienna, Austria. 2013. ISBN 3-900051-07-
781 0.

782 Teng S., Qian Q., Zeng D., Kunihiro Y., Fujimoto K., Huang D. & Zhu L. (2004) QTL
783 analysis of leaf photosynthetic rate and related physiological traits in rice (*Oryza*
784 *sativa* L.). *Euphytica*, **135**, 1-7.

785 van Rooijen R., Aarts M.G. & Harbinson J. (2015) Natural genetic variation for
786 acclimation of photosynthetic light use efficiency to growth irradiance in
787 *Arabidopsis*. *Plant physiology*, **167**, 1412-1429.

788 Walters R.G. & Horton P. (1990) The use of light pulses to investigate the relaxation in
789 the dark of chlorophyll fluorescence quenching in barley leaves. In: *Current*
790 *Research in Photosynthesis*, pp. 631-634. Springer.

791 Walters R.G. & Horton P. (1991) Resolution of components of non-photochemical
792 chlorophyll fluorescence quenching in barley leaves. *Photosynthesis Research*,
793 **27**, 121-133.

794 Wrzaczek M., Brosché M. & Kangasjärvi J. (2013) ROS signaling loops—production,
795 perception, regulation. *Current Opinion in Plant Biology*, **16**, 575-582.

796 Xie Q., Lou P., Hermand V., Aman R., Park H.J., Yun D.-J., Kim W.Y., Salmela M.J.,
797 Ewers B.E. & Weinig C. (2015) Allelic polymorphism of GIGANTEA is

798 responsible for naturally occurring variation in circadian period in *Brassica rapa*.
799 *Proceedings of the National Academy of Sciences*, **112**, 3829-3834.
800 Yarkhunova Y., Edwards C.E., Ewers B.E., Baker R.L., Aston T.L., McClung C.R., Lou
801 P. & Weinig C. (2016) Selection during crop diversification involves correlated
802 evolution of the circadian clock and ecophysiological traits in *Brassica rapa*. *New*
803 *Phytologist*, **210**, 133-144.
804 Yerushalmi S. & Green R.M. (2009) Evidence for the adaptive significance of circadian
805 rhythms. *Ecology letters*, **12**, 970-981.
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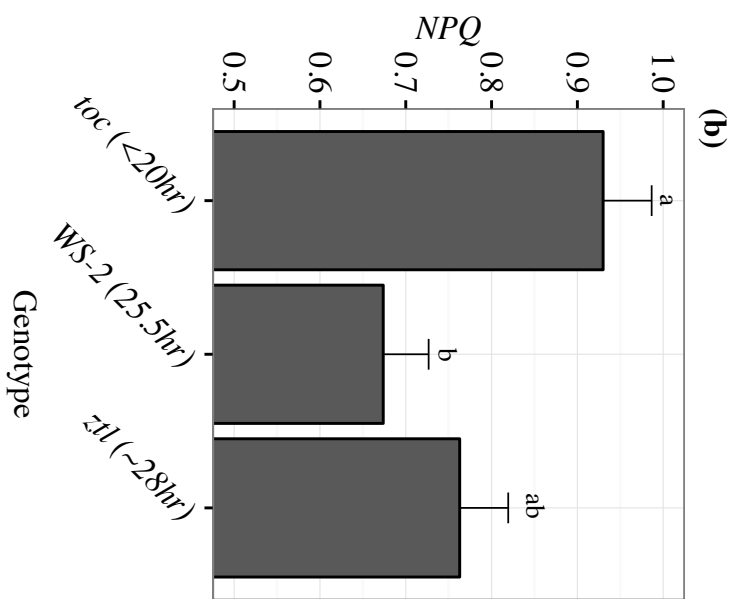
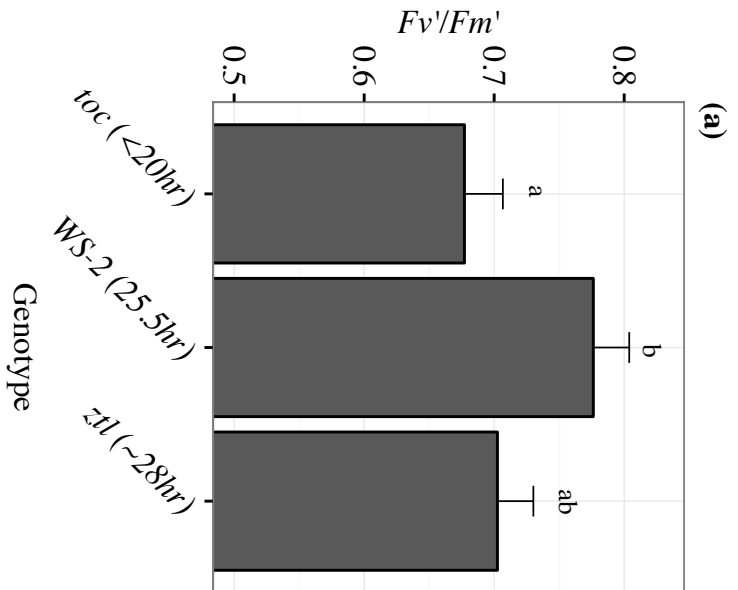


Figure 1. Differences in quantum yield of PSII (Fv'/Fm') (**a**) and total non-photochemical quenching NPQ (**b**) among circadian clock mutant and wild type genotypes of *Arabidopsis thaliana* growing at 350 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, $22 \pm 1^\circ\text{C}$. Error bars indicate \pm SE. Different letters indicate statistically significant differences among ($p < 0.05$).

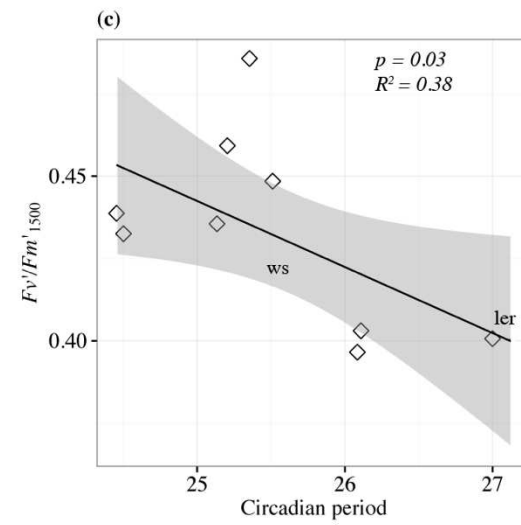
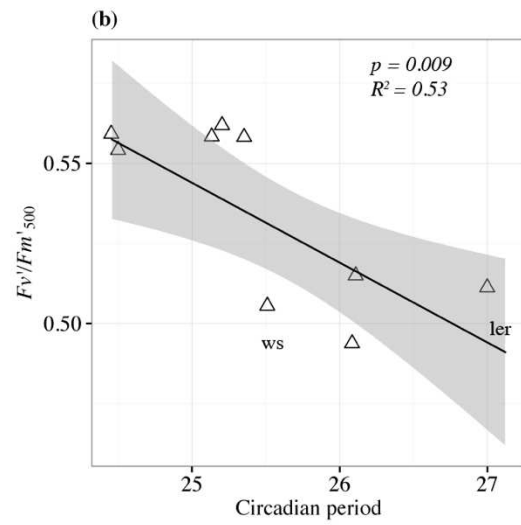
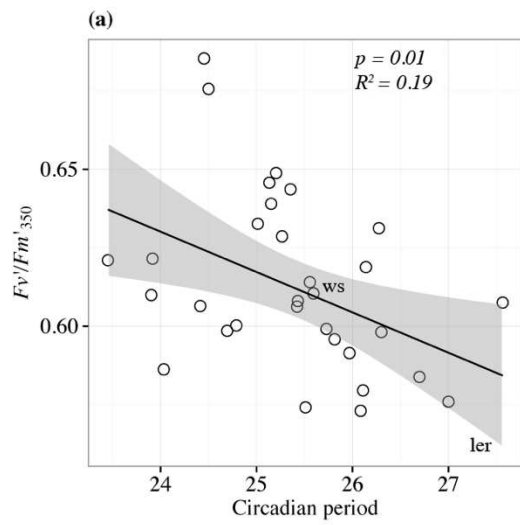


Figure 2. Association between circadian period and quantum yield of photosystem II (F_v'/F_m') at different light levels.

(a) Association between circadian period and F_v'/F_m' for thirty-two *Arabidopsis thaliana* genotypes at 350 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Each circle represents a genotype while *ws* and *ler* represents the parental genotypes. The line represents the following relationship: $R^2=0.19, p=0.01$

(b) Association between circadian period and F_v'/F_m' in low light condition (500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; LL) for eleven *Arabidopsis thaliana* genotypes. Each triangle represents a genotype. The line represents the following relationship: $R^2=0.53, p=0.0099$

(c) Association between circadian period and F_v'/F_m' in high light conditions (1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; HL) for eleven *Arabidopsis thaliana* genotypes. Each diamond represents a genotype. The line represents the following relationship: $R^2=0.38, p=0.03$

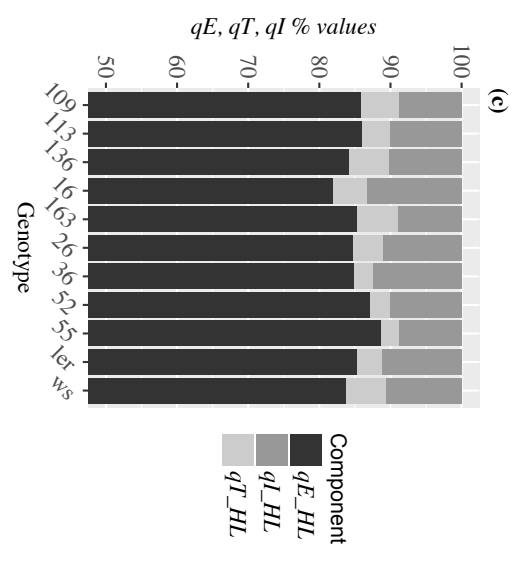
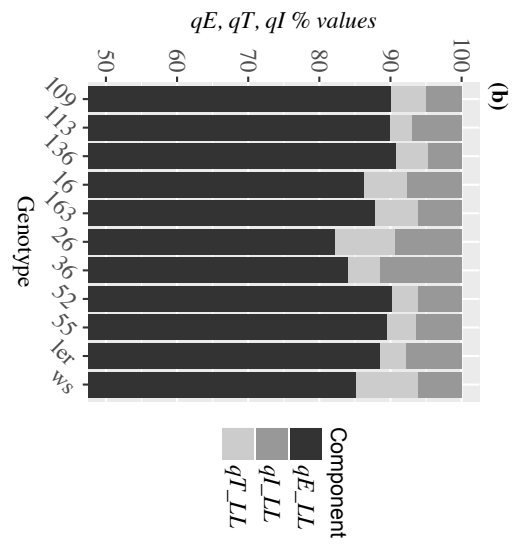
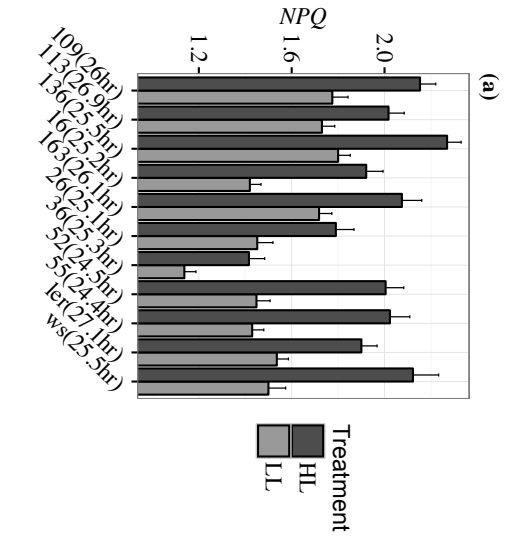


Figure 3. (a) Differences in total *NPQ* among RILs of *Arabidopsis thaliana* under different light conditions (500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, LL and 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, HL). (b) Individual *NPQ* components (*qE*, *qT* and *qI*) expressed as percentage values in leaves of *A. thaliana* RIL genotypes measured at 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, LL and (c) at 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, HL

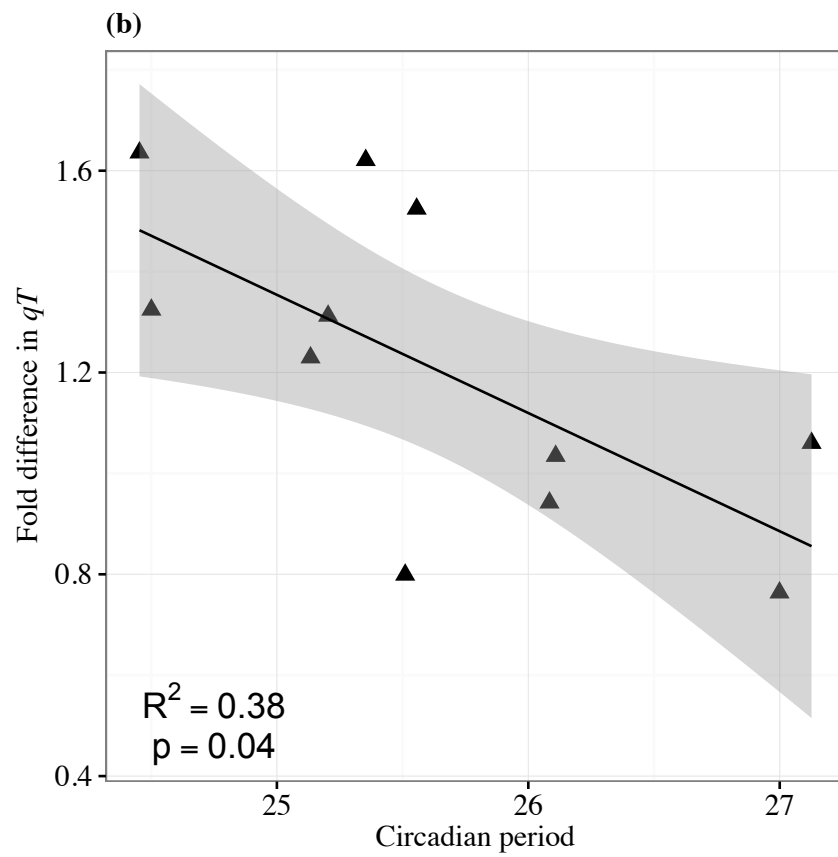
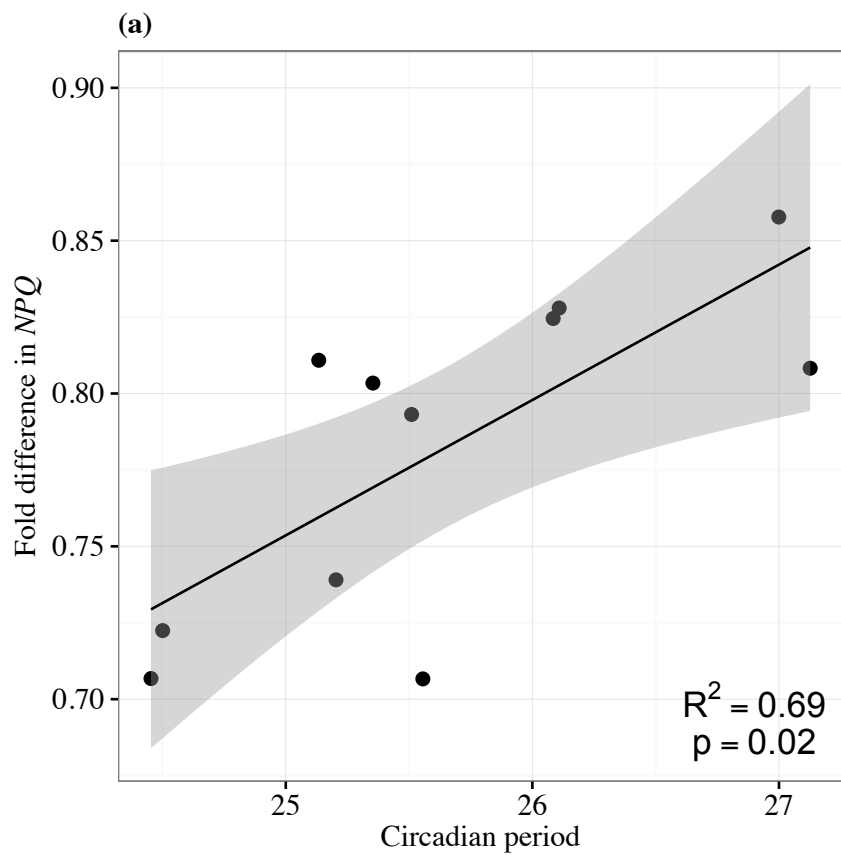
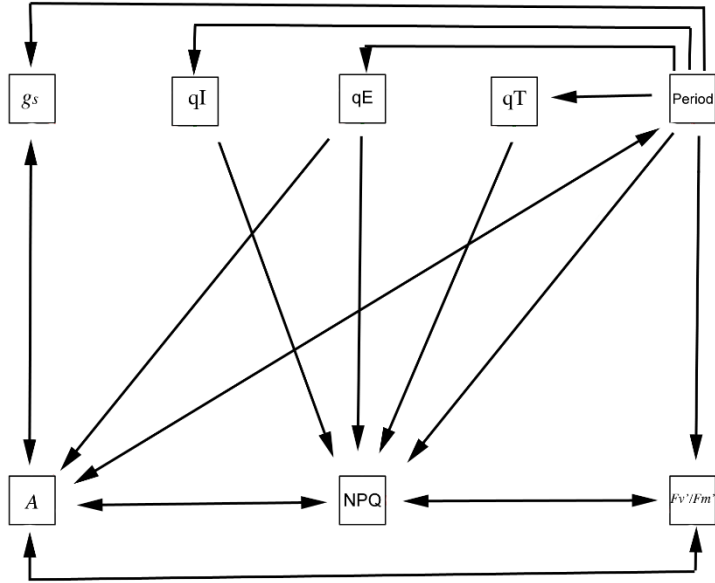


Figure 4. Association between circadian period and fold difference of *NPQ*.

(a) Association between circadian period and fold difference of total *NPQ* (values under LL / HL) for eleven *Arabidopsis thaliana* RIL genotypes. Each circle represents a genotype while *ws* and *ler* represents the parental genotypes. The line represents the following relationship: $R^2=0.44$, $p=0.02$

(b) Association between circadian period and transitional quenching (*qT*) for eleven *Arabidopsis thaliana* genotypes. Each triangle represents a genotype while *ws* and *ler* represents the parental genotypes. The line represents the following relationship: $R^2=0.38$, $p=0.04$

(a)



(b)

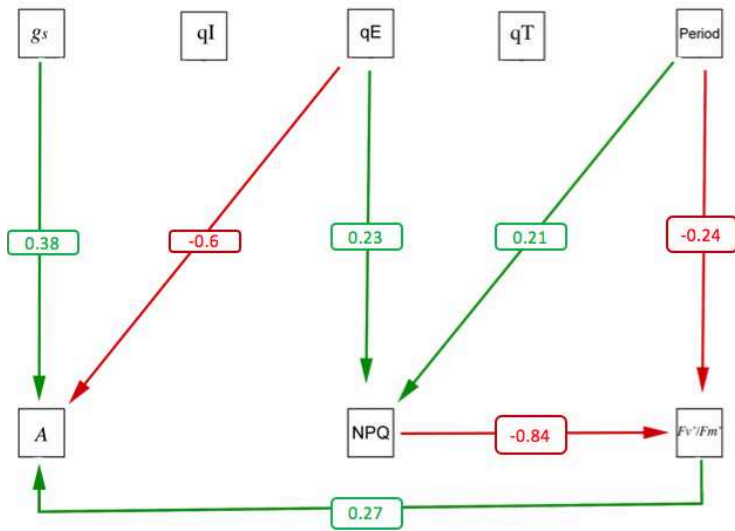


Figure 5. (a) Tested model **(b)** Path diagram of the relationships among physiological traits and circadian period of *A. thaliana* at 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (LL) light treatment. *Arrows* indicate significant relationships. Labels on arrows show standardized path coefficients. Paths are drawn with solid green lines if positive and red lines if negative, $n = 95$.

Table 1a. Analysis of variance for effects of circadian clock genotype on F_v'/F_m' and NPQ .

<i>F_v'/F_m'</i>					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genotype	2	0.04061530	0.02030765	3.32	0.0568

<i>NPQ</i>					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genotype	2	0.24957873	0.12478937	5.57	0.0125

Table 1b. Analysis of variance for effects of RIL genotype on circadian period.

<i>Circadian Period</i>					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genotype	31	215.0204579	6.9361438	11.58	<.0001

Table 1c. Analysis of variance for effects of genotype and treatment (LL and HL) on gas-exchange parameters and components of photochemical and non-photochemical quenching.

<i>Fm'</i>					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genotype	10	1790663.954	179066.395	9.15	<.0001
Treatment	1	2946925.899	2946925.899	150.6	<.0001
Genotype*Treatment	10	451899.95	45189.995	2.31	0.0148
A					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genotype	10	356.0066621	35.6006662	3.46	0.0004
Treatment	1	39.2847737	39.2847737	3.82	0.0522
Genotype*Treatment	10	68.6918485	6.8691849	0.67	0.7527
<i>g_s</i>					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genotype	10	0.20292674	0.02029267	5.78	<.0001
Treatment	1	0.00073165	0.00073165	0.21	0.6485
Genotype*Treatment	10	0.03881545	0.00388155	1.11	0.3603
<i>Fv'/Fm'</i>					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genotype	10	0.15260004	0.01526	18.3	<.0001
Treatment	1	0.38823759	0.38823759	465.49	<.0001
Genotype*Treatment	10	0.01960153	0.00196015	2.35	0.0132

NPQ

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genotype	10	8.27940223	0.82794022	26.43	<.0001
Treatment	1	7.27058433	7.27058433	232.06	<.0001
Genotype*Treatment	10	0.5065335	0.05065335	1.62	0.1068

qE

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genotype	10	0.05566935	0.00556694	4.86	<.0001
Treatment	1	0.02865255	0.02865255	25.01	<.0001
Genotype*Treatment	10	0.02556133	0.00255613	2.23	0.0189

qI

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genotype	10	0.0390058	0.00390058	5.48	<.0001
Treatment	1	0.04846508	0.04846508	68.1	<.0001
Genotype*Treatment	10	0.0091006	0.00091006	1.28	0.2475

qT

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genotype	10	0.02075992	0.00207599	6.43	<.0001
Treatment	1	0.0012175	0.0012175	3.77	0.054
Genotype*Treatment	10	0.00522974	0.00052297	1.62	0.106

Table 2. Phenotypic correlations between traits in *Arabidopsis* RIL population in LL light treatment. * P<0.05; ** P<0.01; *** P<0.001; ns not significant

	Period	A	G _s	F _v '/F _m '	F _o	F _v /F _m	NPQ	F _m	qE	qT	qI
Period	1	-0.18424 ^{ns}	-0.3255 ^{ns}	-0.73518 ^{**}	-0.34427 ^{ns}	-0.01184 ^{ns}	0.4895 ^{ns}	-0.34318 ^{ns}	0.18781 ^{ns}	-0.25292 ^{ns}	-0.03305 ^{ns}
A	-0.18424 ^{ns}	1	0.91449 ^{***}	0.66855 ^{**}	-0.05218 ^{ns}	0.78373 ^{**}	-0.77557 ^{**}	0.24886 ^{ns}	-0.42351 ^{ns}	-0.13583 ^{ns}	0.74798 ^{**}
G_s	-0.3255 ^{ns}	0.91449 ^{***}	1	0.65384 [*]	0.02668 ^{ns}	0.64053 [*]	-0.89794 ^{**}	0.2743 ^{ns}	-0.43889 ^{ns}	-0.1152 ^{ns}	0.75076 ^{**}
F_v'/F_m'	-0.73518 ^{**}	0.66855 [*]	0.65384 [*]	1	0.09073 ^{ns}	0.33412 ^{ns}	-0.71858 ^{**}	0.21581 ^{ns}	-0.39723 ^{ns}	0.04947 ^{ns}	0.53288 ^{ns}
F_o	-0.34427 ^{ns}	-0.05218 ^{ns}	0.02668 ^{ns}	0.09073 ^{ns}	1	-0.01753 ^{ns}	-0.19731 ^{ns}	0.92043 ^{***}	-0.32823 ^{ns}	0.37615 ^{ns}	0.12059 ^{ns}
F_v/F_m	-0.01184 ^{ns}	0.78373 ^{**}	0.64053 [*]	0.33412 ^{ns}	-0.01753 ^{ns}	1	-0.50506 ^{ns}	0.37281 ^{ns}	-0.45395 ^{ns}	0.20493 ^{ns}	0.46745 ^{ns}
NPQ	0.4895 ^{ns}	-0.77557 ^{**}	-0.89794 ^{**}	-0.71858 ^{**}	-0.19731 ^{ns}	-0.50506 ^{ns}	1	-0.38731 ^{ns}	0.6344 [*]	-0.13819 ^{ns}	-0.7946 ^{**}
F_m	-0.34318 ^{ns}	0.24886 ^{ns}	0.2743 ^{ns}	0.21581 ^{ns}	0.92043 ^{***}	0.37281 ^{ns}	-0.38731 ^{ns}	1	-0.48925 ^{ns}	0.44896 ^{ns}	0.28628 ^{ns}
qE	0.18781 ^{ns}	-0.42351 ^{ns}	-0.43889 ^{ns}	-0.39723 ^{ns}	-0.32823 ^{ns}	-0.45395 ^{ns}	0.6344 [*]	-0.48925 ^{ns}	1	-0.7331 ^{**}	-0.76113 ^{**}
qT	-0.25292 ^{ns}	-0.13583 ^{ns}	-0.1152 ^{ns}	0.04947 ^{ns}	0.37615 ^{ns}	0.20493 ^{ns}	-0.13819 ^{ns}	0.44896 ^{ns}	-0.7331 ^{**}	1	0.11686 ^{ns}
qI	-0.03305 ^{ns}	0.74798 ^{**}	0.75076 ^{**}	0.53288 ^{ns}	0.12059 ^{ns}	0.46745 ^{ns}	-0.7946 ^{**}	0.28628 ^{ns}	-0.76113 ^{**}	0.11686 ^{ns}	1