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

3

4 Yulia Yarkhunova<sup>1</sup>, Carmela R. Guadagno<sup>1</sup>, Matthew J. Rubin<sup>2</sup>, Seth J. Davis<sup>4</sup>, Brent E.  
5 Ewers<sup>1</sup>, Cynthia Weinig<sup>1,3</sup>

6

7 <sup>1</sup>Department of Botany and Program in Ecology, University of Wyoming, Laramie, WY  
8 82071, USA

9 <sup>2</sup>Department of Biology, Syracuse University, Syracuse, NY 13244, USA

10 <sup>3</sup>Department of Molecular Biology, University of Wyoming, Laramie, WY 82071, USA

11 <sup>4</sup>Department of Biology, University of York, Heslington, York, YO10 5DD, UK

12

13

14 Author for correspondence:

15 *Cynthia Weinig*

16 *Tel: +1307 7666378*

17 *Email: [cweinig@uwyo.edu](mailto:cweinig@uwyo.edu)*

18

19 **Abstract.** The circadian clock regulates many aspects of leaf gas supply and biochemical  
20 demand for CO<sub>2</sub>, 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 ( $Fv'/Fm'$ ) 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  $Fv'/Fm'$ ,  
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  $Fv'/Fm'$  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  $Fv'/Fm'$  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 CO<sub>2</sub> 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<sup>-2</sup> s<sup>-1</sup>. 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<sup>-2</sup> s<sup>-1</sup> 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  $22 \pm 1$  °C and relative humidity of  
172  $50 \pm 1$  % for five days, a period of time sufficient for clock entrainment. After  
173 entrainment, 20 $\mu$ 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  $\text{VPD}_L$  (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 ( $\pm 4$  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<sub>m</sub>'*, *F<sub>v</sub>'/F<sub>m</sub>'*, *NPQ*, *qE*, *qT*, and *qI* ( $p < 0.001$ ;  
278 Table 1). As expected, *A* decreased in low light conditions, while *F<sub>v</sub>'/F<sub>m</sub>'* 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<sub>s</sub>*, *F<sub>m</sub>'*, *F<sub>v</sub>'/F<sub>m</sub>'*, *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  $F_v'/F_m'$ . Shorter circadian period (closer to 24 hrs) was associated with higher  
355  $F_v'/F_m'$  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  $\mu\text{mol}$   
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  $\text{vs. HL}$  (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

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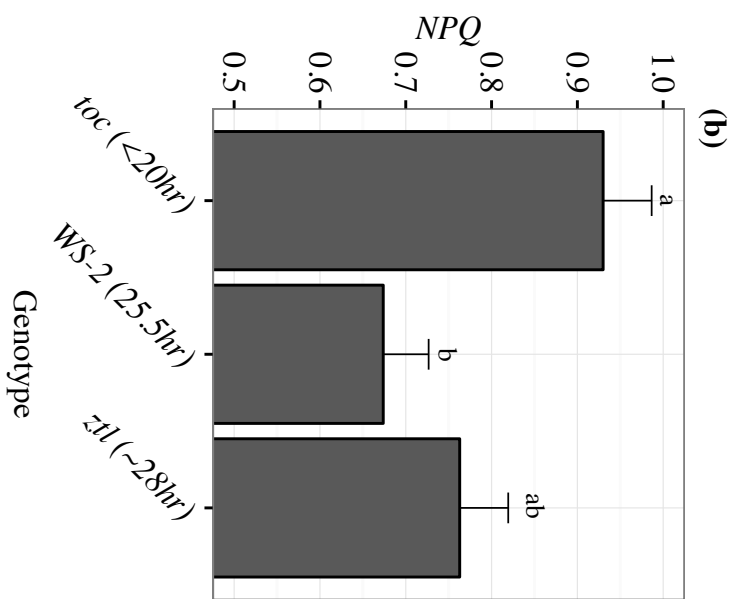
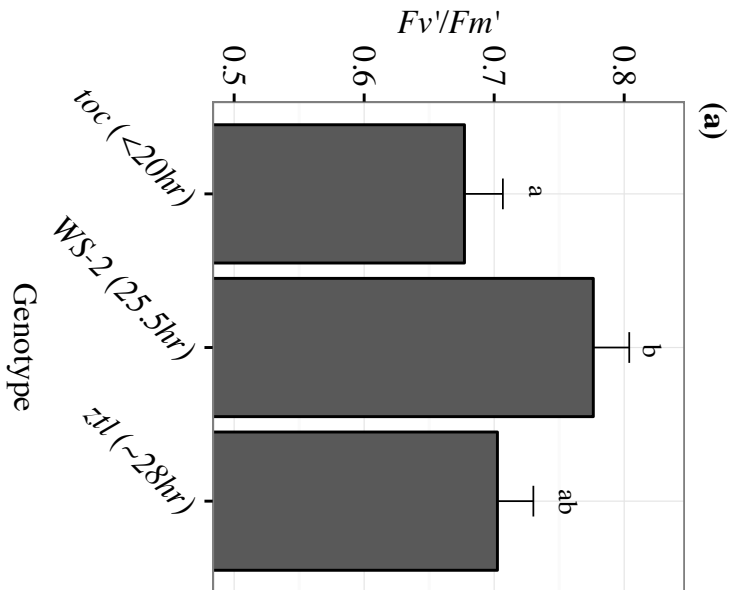
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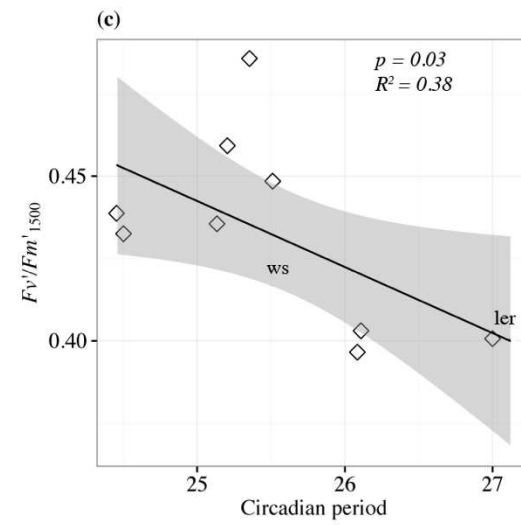
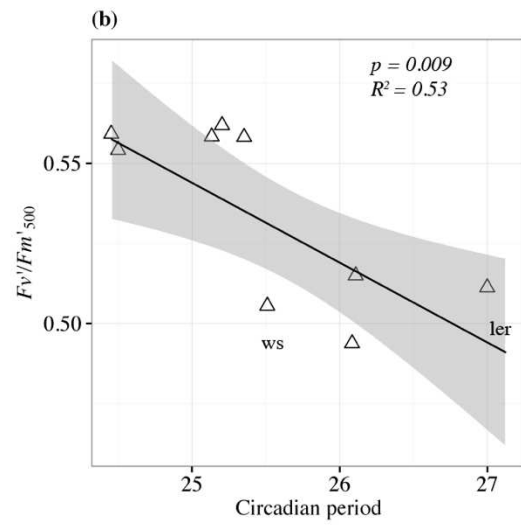
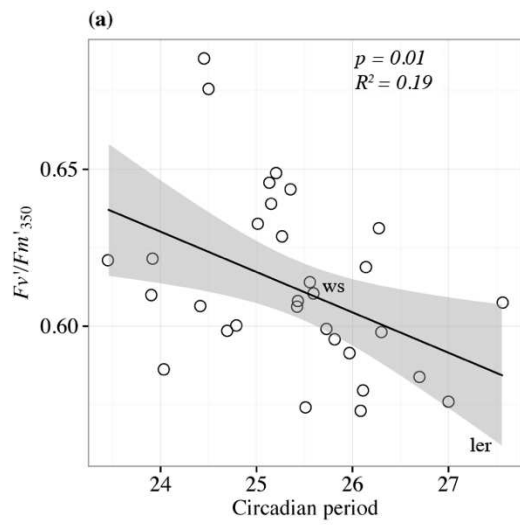
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**Figure 1.** Differences in quantum yield of PSII ( $Fv'/Fm'$ ) (**a**) and total non-photochemical quenching  $NPQ$  (**b**) among circadian clock mutant and wild type genotypes of *Arabidopsis thaliana* growing at 350  $\mu\text{mol photons m}^{-2}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



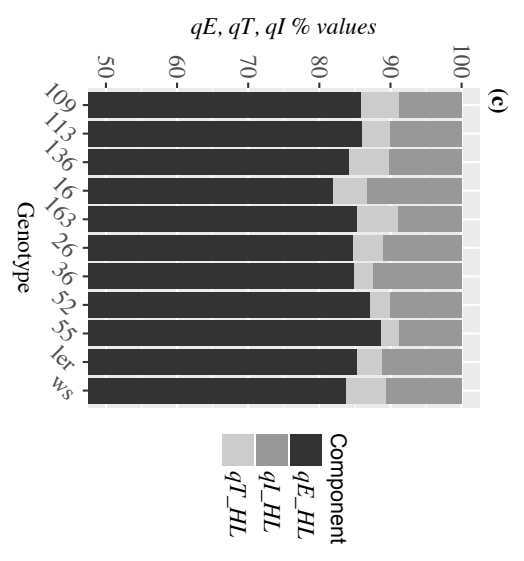
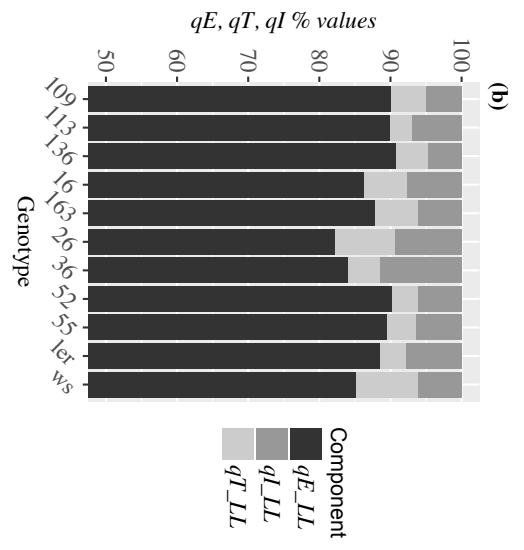
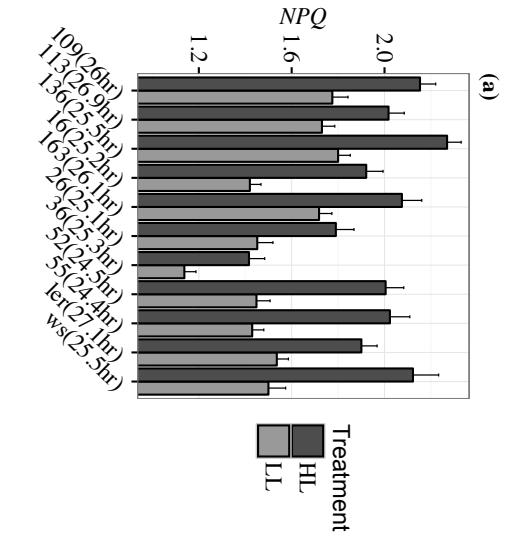
**Figure 2. Association between circadian period and quantum yield of photosystem II ( $F_v'/F_m'$ ) at different light levels.**

(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:  $R^2=0.53, p=0.0099$

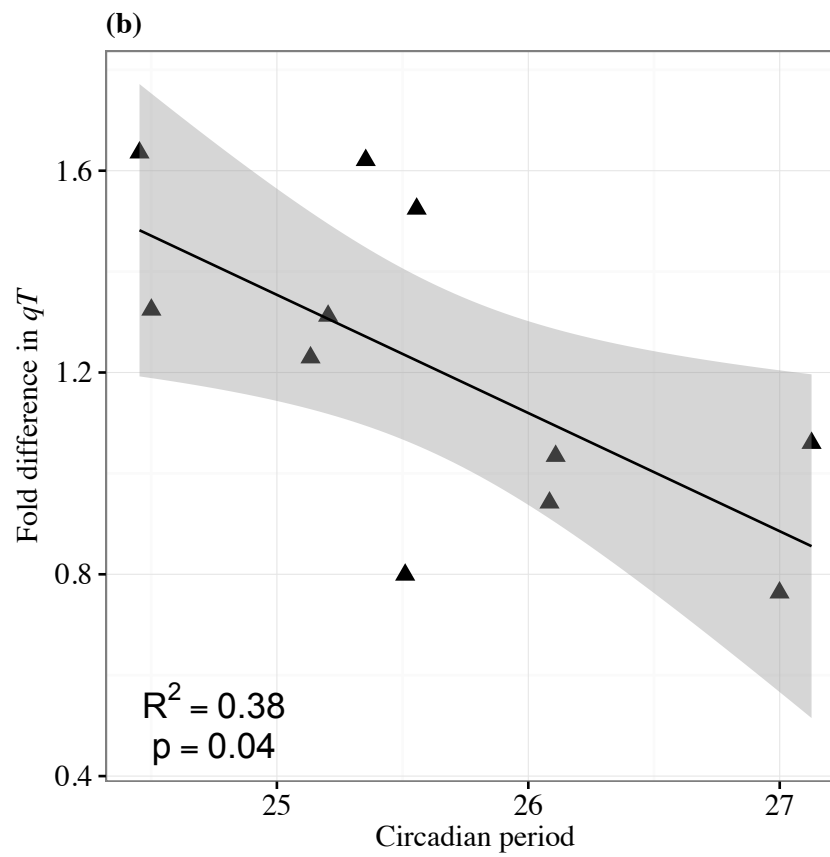
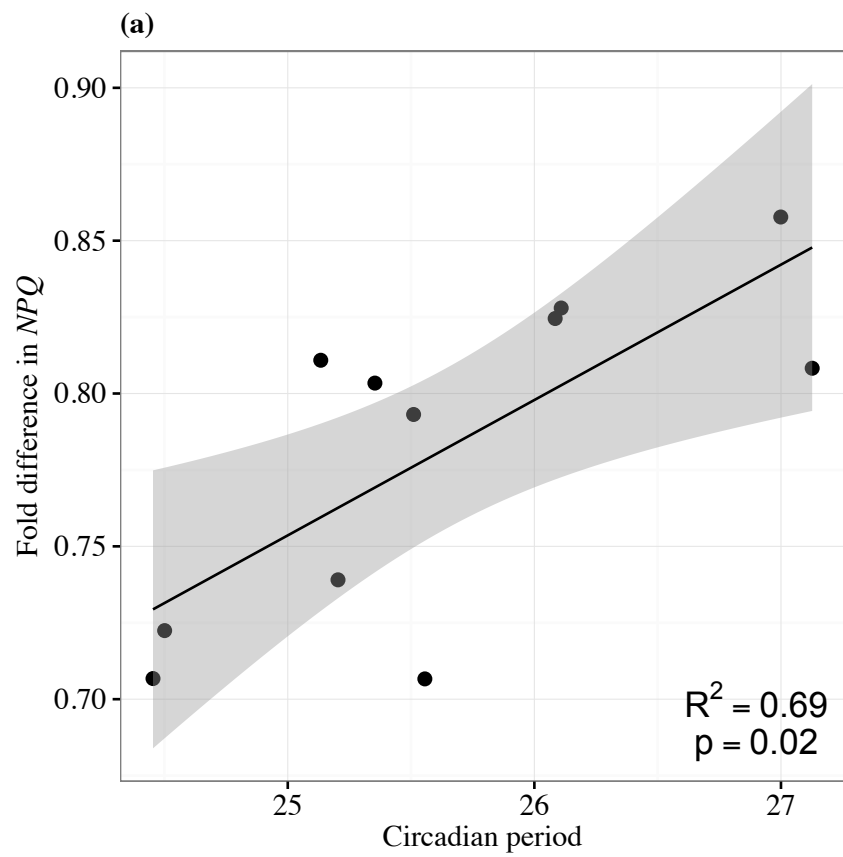
(c) Association between circadian period and  $F_v'/F_m'$  in high light conditions (1500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ; HL) for eleven *Arabidopsis thaliana* genotypes. Each diamond represents a genotype. The line represents the following relationship:  $R^2=0.38, p=0.03$





**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





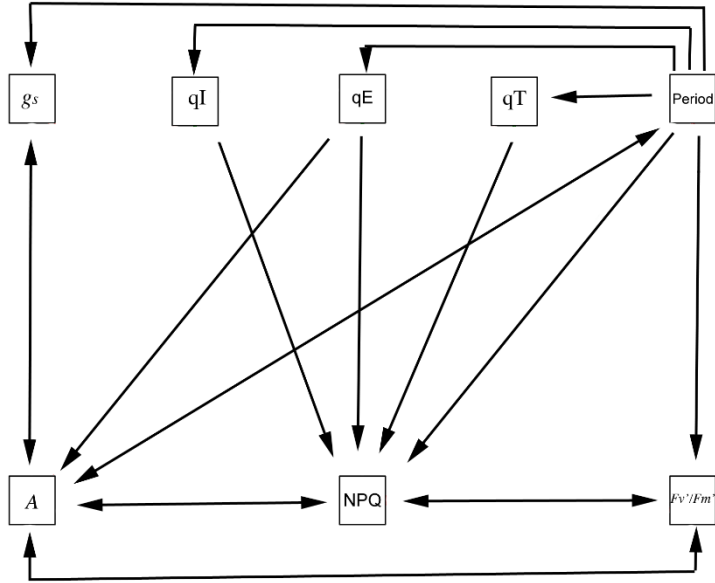


**Figure 4. Association between circadian period and fold difference of *NPQ*.**

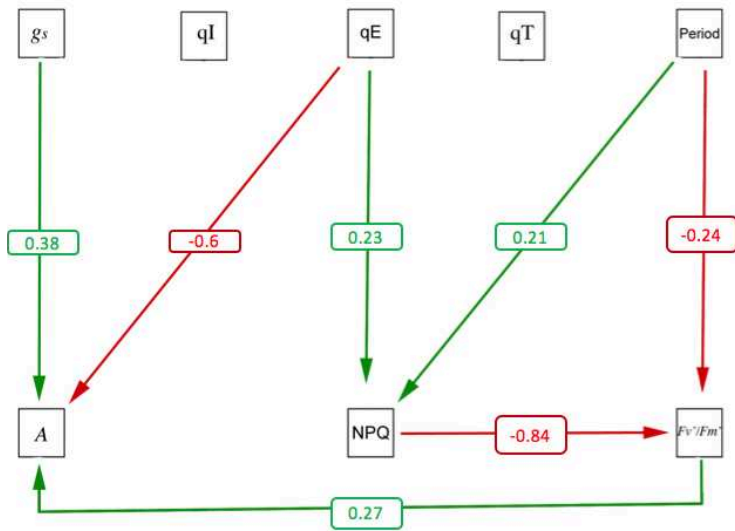
**(a)** Association between circadian period and fold difference of total *NPQ* (values under LL / HL) for eleven *Arabidopsis thaliana* RIL genotypes. Each circle represents a genotype while *ws* and *ler* represents the parental genotypes. The line represents the following relationship:  $R^2=0.44$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

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

*NPQ*

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

*qE*

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

*qI*

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

*qT*

Source	DF	Type III SS	Mean Square	F Value	Pr > F
<b>Genotype</b>	10	0.02075992	0.00207599	6.43	<.0001
<b>Treatment</b>	1	0.0012175	0.0012175	3.77	0.054
<b>Genotype*Treatment</b>	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 <sub>s</sub>	F <sub>v</sub> '/F <sub>m</sub> '	F <sub>o</sub>	F <sub>v</sub> /F <sub>m</sub>	NPQ	F <sub>m</sub>	qE	qT	qI
<b>Period</b>	1	-0.18424 <sup>ns</sup>	-0.3255 <sup>ns</sup>	-0.73518 <sup>**</sup>	-0.34427 <sup>ns</sup>	-0.01184 <sup>ns</sup>	0.4895 <sup>ns</sup>	-0.34318 <sup>ns</sup>	0.18781 <sup>ns</sup>	-0.25292 <sup>ns</sup>	-0.03305 <sup>ns</sup>
<b>A</b>	-0.18424 <sup>ns</sup>	1	0.91449 <sup>***</sup>	0.66855 <sup>**</sup>	-0.05218 <sup>ns</sup>	0.78373 <sup>**</sup>	-0.77557 <sup>**</sup>	0.24886 <sup>ns</sup>	-0.42351 <sup>ns</sup>	-0.13583 <sup>ns</sup>	0.74798 <sup>**</sup>
<b>G<sub>s</sub></b>	-0.3255 <sup>ns</sup>	0.91449 <sup>***</sup>	1	0.65384 <sup>*</sup>	0.02668 <sup>ns</sup>	0.64053 <sup>*</sup>	-0.89794 <sup>**</sup>	0.2743 <sup>ns</sup>	-0.43889 <sup>ns</sup>	-0.1152 <sup>ns</sup>	0.75076 <sup>**</sup>
<b>F<sub>v</sub>'/F<sub>m</sub>'</b>	-0.73518 <sup>**</sup>	0.66855 <sup>*</sup>	0.65384 <sup>*</sup>	1	0.09073 <sup>ns</sup>	0.33412 <sup>ns</sup>	-0.71858 <sup>**</sup>	0.21581 <sup>ns</sup>	-0.39723 <sup>ns</sup>	0.04947 <sup>ns</sup>	0.53288 <sup>ns</sup>
<b>F<sub>o</sub></b>	-0.34427 <sup>ns</sup>	-0.05218 <sup>ns</sup>	0.02668 <sup>ns</sup>	0.09073 <sup>ns</sup>	1	-0.01753 <sup>ns</sup>	-0.19731 <sup>ns</sup>	0.92043 <sup>***</sup>	-0.32823 <sup>ns</sup>	0.37615 <sup>ns</sup>	0.12059 <sup>ns</sup>
<b>F<sub>v</sub>/F<sub>m</sub></b>	-0.01184 <sup>ns</sup>	0.78373 <sup>**</sup>	0.64053 <sup>*</sup>	0.33412 <sup>ns</sup>	-0.01753 <sup>ns</sup>	1	-0.50506 <sup>ns</sup>	0.37281 <sup>ns</sup>	-0.45395 <sup>ns</sup>	0.20493 <sup>ns</sup>	0.46745 <sup>ns</sup>
<b>NPQ</b>	0.4895 <sup>ns</sup>	-0.77557 <sup>**</sup>	-0.89794 <sup>**</sup>	-0.71858 <sup>**</sup>	-0.19731 <sup>ns</sup>	-0.50506 <sup>ns</sup>	1	-0.38731 <sup>ns</sup>	0.6344 <sup>*</sup>	-0.13819 <sup>ns</sup>	-0.7946 <sup>**</sup>
<b>F<sub>m</sub></b>	-0.34318 <sup>ns</sup>	0.24886 <sup>ns</sup>	0.2743 <sup>ns</sup>	0.21581 <sup>ns</sup>	0.92043 <sup>***</sup>	0.37281 <sup>ns</sup>	-0.38731 <sup>ns</sup>	1	-0.48925 <sup>ns</sup>	0.44896 <sup>ns</sup>	0.28628 <sup>ns</sup>
<b>qE</b>	0.18781 <sup>ns</sup>	-0.42351 <sup>ns</sup>	-0.43889 <sup>ns</sup>	-0.39723 <sup>ns</sup>	-0.32823 <sup>ns</sup>	-0.45395 <sup>ns</sup>	0.6344 <sup>*</sup>	-0.48925 <sup>ns</sup>	1	-0.7331 <sup>**</sup>	-0.76113 <sup>**</sup>
<b>qT</b>	-0.25292 <sup>ns</sup>	-0.13583 <sup>ns</sup>	-0.1152 <sup>ns</sup>	0.04947 <sup>ns</sup>	0.37615 <sup>ns</sup>	0.20493 <sup>ns</sup>	-0.13819 <sup>ns</sup>	0.44896 <sup>ns</sup>	-0.7331 <sup>**</sup>	1	0.11686 <sup>ns</sup>
<b>qI</b>	-0.03305 <sup>ns</sup>	0.74798 <sup>**</sup>	0.75076 <sup>**</sup>	0.53288 <sup>ns</sup>	0.12059 <sup>ns</sup>	0.46745 <sup>ns</sup>	-0.7946 <sup>**</sup>	0.28628 <sup>ns</sup>	-0.76113 <sup>**</sup>	0.11686 <sup>ns</sup>	1