elements or by nucleosomal eviction by recruited proteins, and nucleosomes are subsequently well-positioned between nearby NFRs because of structural constraints imposed by packaging short stretches of sequence with nucleosomes.

It will be interesting to determine whether the accessible transcription factor binding sites, highly positioned nucleosomes, and stereotyped promoter architecture found in yeast chromatin will be conserved features of metazoan chromatin.

References and Notes

1. J. D. Anderson, J. Widom, *J. Mol. Biol.* **296**, 979 (2000).
2. L. L. Wallrath, Q. Lu, H. Granok, S. C. Elgin, *Bioessays* **16**, 165 (1994).
3. U. Venter, J. Svaren, J. Schmitz, A. Schmid, W. Horz, *EMBO J.* **13**, 4848 (1994).
4. W. Stunkel, I. Kober, K. H. Seifart, *Mol. Cell. Biol.* **17**, 4397 (1997).
5. J. L. DeRisi, V. R. Iyer, P. O. Brown, *Science* **278**, 680 (1997).
6. K. E. Van Holde, *Chromatin*, Springer series in molecular biology (Springer-Verlag, New York, 1989).
7. R. Durbin, *Biological Sequence Analysis: Probabalistic Models of Proteins and Nucleic Scids* (Cambridge Univ. Press, Cambridge, 1998).
8. Y. Teng, S. Yu, R. Waters, *Nucleic Acids Res.* **29**, E64 (2001).
9. M. J. Saunders, E. Yeh, M. Grunstein, K. Bloom, *Mol. Cell. Biol.* **10**, 5721 (1990).
10. J. M. Moreira, S. Holmberg, *EMBO J.* **17**, 6028 (1998).
11. S. Ercan, R. T. Simpson, *Mol. Cell. Biol.* **24**, 10026 (2004).
12. C. K. Lee, Y. Shibata, B. Rao, B. D. Strahl, J. D. Lieb, *Nat. Genet.* **36**, 900 (2004).
13. B. E. Bernstein, C. L. Liu, E. L. Humphrey, E. O. Perlstein, S. L. Schreiber, *Genome Biol.* **5**, R62 (2004).
14. P. T. Lowary, J. Widom, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1183 (1997).
15. D. E. Gottschling, O. M. Aparicio, B. L. Billington, V. A. Zakian, *Cell* **63**, 751 (1990).
16. L. Z. Osherovich, J. S. Weissman, *Dev. Cell* **2**, 143 (2002).
17. M. A. Schwabish, K. Struhl, *Mol. Cell. Biol.* **24**, 10111 (2004).
18. A. Kristjuhan, J. Q. Svejstrup, *EMBO J.* **23**, 4243 (2004).
19. R. Belotserkovskaya *et al.*, *Science* **301**, 1090 (2003).
20. J. D. Lieb, X. Liu, D. Botstein, P. O. Brown, *Nat. Genet.* **28**, 327 (2001).
21. C. T. Harbison *et al.*, *Nature* **431**, 99 (2004).
22. M. Kellis, N. Patterson, M. Endrizzi, B. Birren, E. S. Lander, *Nature* **423**, 241 (2003).
23. K. Struhl, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 8419 (1985).
24. J. D. Anderson, J. Widom, *Mol. Cell. Biol.* **21**, 3830 (2001).
25. H. C. Nelson, J. T. Finch, B. F. Luisi, A. Klug, *Nature* **330**, 221 (1987).
26. S. Karlin, B. E. Blaisdell, R. J. Sapolsky, L. Cardon, C. Burge, *Nucleic Acids Res.* **21**, 703 (1993).
27. W. Horz, W. Altenburger, *Nucleic Acids Res.* **9**, 2643 (1981).
28. R. D. Kornberg, L. Stryer, *Nucleic Acids Res.* **16**, 6677 (1988).
29. Alignment scores were downloaded from http://genome.ucsc.edu.

30. We thank N. Francis, T. Maniatis, K. Thorn, N. Friedman, J. Oshiro, T. Kaplan, J. Rinn, H. Chang, J. Lieb, and A. Gasch for critical reading of the manuscript; T. Aldredge, C. Reardon, and C. Daly for technical help; and A. Murray and the Bauer Center community for advice and support. This work was supported by the Bauer Center and by National Institute of General Medical Sciences.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1112178/DC1
Materials and Methods
SOM Text
Figs. S1 to S10
Table S1
References and Notes

14 March 2005; accepted 2 June 2005
Published online 16 June 2005;
10.1126/science.1112178
Include this information when citing this paper.

Plant Circadian Clocks Increase Photosynthesis, Growth, Survival, and Competitive Advantage

Antony N. Dodd,[1] Neeraj Salathia,[2]* Anthony Hall,[2]† Eva Kévei,[3] Réka Tóth,[3] Ferenc Nagy,[3] Julian M. Hibberd,[1] Andrew J. Millar,[2]‡ Alex A. R. Webb[1]§

Circadian clocks are believed to confer an advantage to plants, but the nature of that advantage has been unknown. We show that a substantial photosynthetic advantage is conferred by correct matching of the circadian clock period with that of the external light-dark cycle. In wild type and in long– and short–circadian period mutants of *Arabidopsis thaliana*, plants with a clock period matched to the environment contain more chlorophyll, fix more carbon, grow faster, and survive better than plants with circadian periods differing from their environment. This explains why plants gain advantage from circadian control.

Circadian clocks produce an internal estimate of time that synchronizes biological events with external day-night cycles (*1*). Clocks with similar properties and regulatory architecture have evolved at least four times, indicating that circadian rhythms confer a selective advantage (*2*). In plants, circadian rhythms control gene expression, stomatal opening, and the timing component of photoperiodism, which regulates seasonal reproduction, but the basis for their contribution to fitness during vegetative growth remains undetermined (*3*, *4*). Indirect evidence suggests a physiological benefit from circadian rhythms during growth under unnaturally short photoperiods (*5*). Cyanobacteria and higher plants gain an advantage when the endogenous period is matched to the light-dark cycle (*6*–*8*). Rhythmic growth inhibitor secretion might cause the growth advantage in cyanobacteria (*7*, *8*), but this hypothesis may not apply to multicellular eukaryotes. We demonstrate that when correctly tuned, the *Arabidopsis* circadian system enhances chlorophyll content, photosynthetic carbon fixation, and growth. We also show that circadian enhancement of photosynthesis leads to improved survival and competitive advantage.

Biological clocks have evolved so that clock outputs are in phase with the Earth's rotation. We wished to identify and quantify mechanisms by which the clock confers advantage in light-dark cycles. We hypothesized that matching the endogenous clock period (τ) with the period of exogenous light-dark cycles (T) [so called "circadian resonance" (*7*)] provides an advantage by optimizing the phase relation between clock-controlled biology and exogenous day-night cycles. Plants having clocks that are dissonant from the environment, therefore, may be disadvantaged. To test this hypothesis, we compared the performance of wild-type plants with lines having mutations that alter clock period length, in a range of environmental period lengths ("T cycles") that were either matched or unmatched to the endogenous clock period.

We used three experimental approaches to test this hypothesis (*9*). First, wild-type plants with a circadian period of about 24 hours were grown in 10 hours light–10 hours dark (T20), 12 hours light–12 hours dark (T24) and 14 hours light–14 hours dark (T28) cycles. Second, we grew the long- and short-period mutants *ztl-1* [τ = 27.1 hours–32.5 hours; (*10*)] and *toc1-1* [τ = 20.7 hours; (*11*)] in T cycles that were similar to, or dissimilar from, their endogenous clock periods (T20 and T28). In these T-cycle experiments, relative performance was measured within, not between, genotypes, which specifically quantified the benefit

[1]Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge, CB2 3EA, UK. [2]Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, UK. [3]Plant Biology Institute, Biological Research Centre of the Hungarian Academy of Sciences, Post Office Box 521, H-6701 Szeged, Hungary.

*Present address: Bauer Center for Genomics Research, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138, USA.
†Present address: School of Biological Sciences, University of Liverpool, Crown Street, Liverpool L69 7ZB, UK.
‡Present address: Institute of Molecular Plant Sciences, Mayfield Road, University of Edinburgh, Edinburgh EH9 3JH, UK.
§To whom correspondence should be addressed. E-mail: alex.webb@plantsci.cam.ac.uk

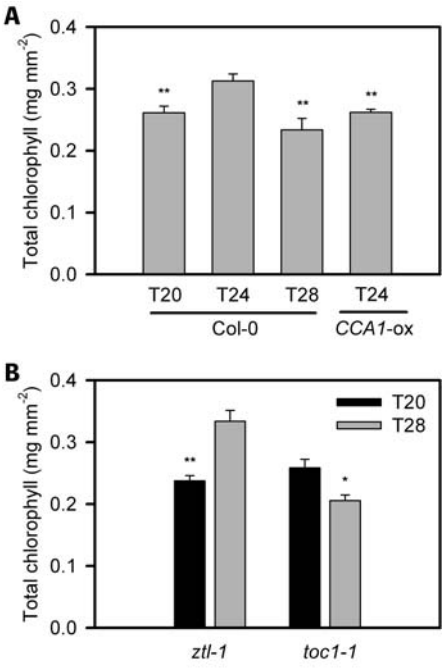

Fig. 1. Leaves contain more chlorophyll when their clock period matches the environmental period. (**A**) Total chlorophyll in Col-0 wild type grown under T20, T24, and T28, and in arrhythmic line *CCA1*-ox under T24. (**B**) Total chlorophyll in *ztl-1* (long-period mutant) and *toc1-1* (short-period mutant) grown in T20 and T28. For all groups, $n = 5$; data are means + SEM. In two-sample *t* tests comparing chlorophyll concentrations to the line having the period matched to the environment, significance of results: $*P < 0.05$, $**P < 0.01$.

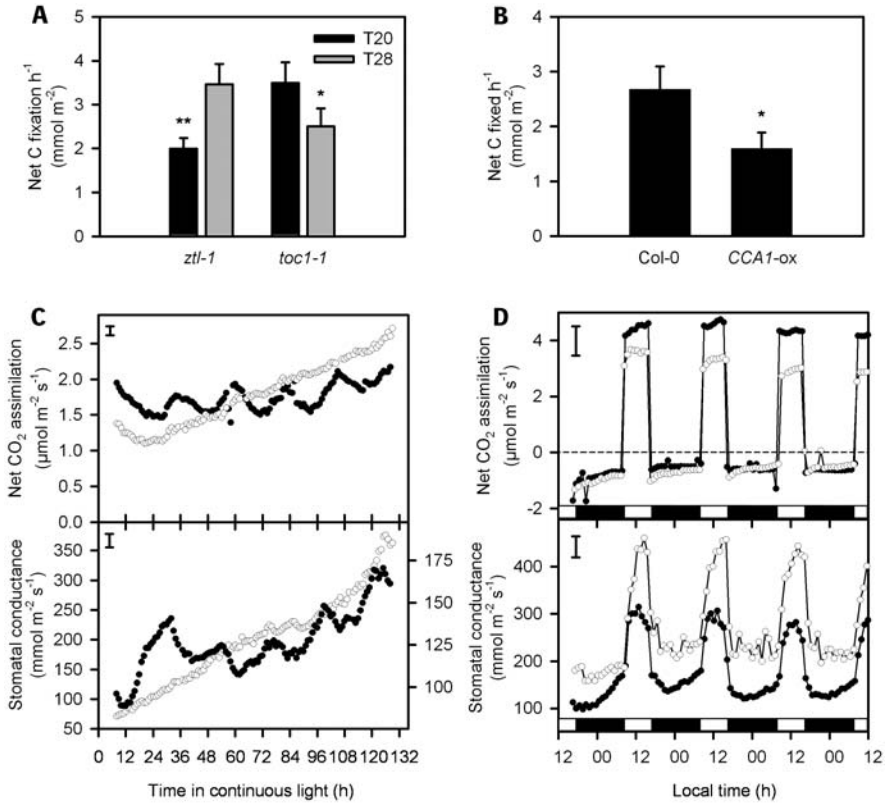

Fig. 2. The circadian clock enhances photosynthetic carbon fixation. (**A**) Mean C fixation per hour in *ztl-1* and *toc1-1* grown in T20 and T28. (**B**) Mean C fixation per hour in Col-0 wild type and arrhythmic *CCA1*-ox, in T24. (**C**) *CCA1* overexpression (open circles) abolishes the circadian rhythms of CO_2 fixation and stomatal opening that occur in Col-0 wild type (filled circles). (**D**) CO_2 assimilation and stomatal conductance in *CCA1*-ox (open circles) and Col-0 wild type (filled circles) under light-dark cycles (indicated by bars on the *x* axis). For these experiments, $n = 6$; data are means ± SEM (A and B) or largest standard error (C and D). In two-sample *t* tests comparing net C fixation per hour to the line with the clock period matched to the environment, significance of results: $*P < 0.05$, $**P < 0.01$.

of circadian resonance and excluded effects not associated with the clock. Last, the effect of circadian arrhythmia on growth and physiology was investigated in well-characterized arrhythmic plants overexpressing the molecular oscillator component *CCA1* (*CCA1*-ox), and compared with rhythmic wild types (*8*, *12*). Experiments were conducted during vegetative growth, to assess the contribution of circadian resonance to growth and fitness, and to eliminate pleiotropic effects on life history due to the flowering time alterations that arise in circadian period mutants (*13*). We assessed the contribution of circadian resonance to carbon fixation, biomass, and leaf chlorophyll. Carbon fixation rates (*14*–*16*) and biomass (*17*) are traits associated with plant fitness; therefore, our study provides information concerning specific mechanisms by which the clock contributes to fitness.

In wild type and in short- and long-period mutants, leaves contained more chlorophyll when the oscillator period matched that of the environment. Leaves of Columbia-0 (Col-0) wild-type plants grown for 30 days in T24 contained more chlorophyll than Col-0 grown in T20 or T28 (Fig. 1A). When *ztl-1* and *toc1-1* were grown under T20 and T28, the long-period mutant *ztl-1* contained more chlorophyll after growth in T28 than T20, whereas the short-period mutant *toc1-1* contained more chlorophyll after growth in T20 than T28 (Fig. 1B). Therefore, correct matching of the circadian period with the external period increases chlorophyll accumulation. When the Col-0 clock was stopped by *CCA1* overexpression, less chlorophyll was present compared with wild-type Col-0 under T24, which confirmed the dependence of chlorophyll accumulation on clock function (Fig. 1A).

There are circadian rhythms in transcript abundance of genes associated with chlorophyll synthesis, heme production, chlorophyll accumulation, and synthesis of chlorophyll-binding proteins (*11*, *18*–*20*). Virtually all clock-controlled genes associated with chlorophyll synthesis and the light-harvesting apparatus exhibit peak circadian transcript abundance 4 hours after subjective dawn (*18*), which suggests that circadian expression of these genes could be important for enhancing light-harvesting capacity. Although the amount of light-harvesting complex pigments and proteins remains uniform over the diel cycle (*19*), the clock might sustain steady-state levels of proteins that exhibit light-induced degradation, by way of circadian changes in turnover. This could explain why chlorosis can occur under very long photoperiods (*21*), because the duration of light-induced degradation of light-harvesting complex proteins extends beyond their period of clock-enhanced transcription.

Because chlorophyll content was greatest under circumstances of matched endogenous and environmental periods, we examined whether circadian resonance improves photosynthesis. We compared net carbon fixation of long- and short-period mutants and *CCA1*-ox, under T20, T24, and T28. The long-period mutant *ztl1* fixed 42% more carbon under T28 than T20, whereas the short-period mutant *toc1-1* fixed 40% more carbon under T20 than T28 (Fig. 2A). Circadian resonance, therefore, increases CO_2 fixation. Col-0 wild type fixed 67% more carbon than arrhythmic *CCA1*-ox (Fig. 2B). The reduction in carbon fixation associated with circadian arrhythmia was, therefore, greater than the disadvantage caused by the ~8-hour period mismatch with the environment that occurred in *ztl1* and *toc1-1*. Under continuous light, the rate of CO_2 fixation was lower in *CCA1*-ox than wild type for the first 48 hours

of continuous light, but during prolonged constant light, assimilation was higher in *CCA1*-ox than wild type (Fig. 2C). This was reminiscent of the outcompetition of rhythmic cyanobacterial lines by the arrhythmic line CLAb under continuous light (*8*). *CCA1* overexpression abolished circadian rhythms of CO_2 fixation and stomatal opening in constant conditions. Fourier analysis (*22*) estimated period lengths of 24.1 ± 0.6 hours and 23.5 ± 0.3 hours for stomatal conductance and carbon assimilation, respectively, in Col-0 wild type, but failed to detect circadian regulation for *CCA1*-ox. Similarly, the *toc1-1* and *ztl1* mutations cause respective shortening and extension of the circadian period of CO_2 fixation and stomatal opening rhythms that occur in continuous light (*23*, *24*).

Under light-dark cycles, rhythmic stomatal opening and closure was restored in *CCA1*-ox, but anticipation of dawn and dusk was absent, which demonstrated that the clock remained stopped in *CCA1*-ox, even in light-dark cycles (Fig. 2D). The stomata continued to open for the entire photoperiod in *CCA1*-ox, whereas in Col-0, stomatal opening ceased around midday. *CCA1*-ox, therefore, had higher total transpiration than Col-0 during the light period (Fig. 2D). Thus, the clock allows stomata to anticipate dusk and might participate in enhancement of water-use efficiency.

Because photosynthesis increased when exogenous and endogenous periods were similar (Fig. 2) and because long-term carbon fixation is correlated to leaf chlorophyll content (*25*), we reasoned that circadian resonance might increase vegetative growth. Col-0 wild type grown under T20, T24, and T28 had greatest vegetative biomass in T24 (Fig. 3, A and B). Under T20, aerial biomass was reduced by 47% relative to T24, and growth under T28 resulted in 42% less biomass relative to T24. When the long- and short-period mutants *ztl1* and *toc1-1* were grown under T20 and T28, *ztl1* had maximum aerial biomass and leaf area under T28. In *toc1-1*, aerial biomass and leaf area were maximal under T20 (Fig. 3, C and D). These measures of growth were lower in *toc1-1* than *ztl1-1* under all conditions. Separately, we compared growth of Col-0 and CCA1-ox under T24. After 32 days, the aerial biomass of *CCA1*-ox was 53% lower than that of Col-0 (Fig. 3, E and F). We did not compare growth of *CCA1*-ox and Col-0 in continuous light, because wild-type plants become arrhythmic under extended continuous light (*26*). Therefore, circadian resonance enhances growth in wild-type plants and mutants with altered circadian period, and stopping the clock further reduces growth. Enhancement of biomass and photosynthesis by the circadian clock consequently indicates processes by which the clock increases fitness (*14*–*17*).

Fitness arising from circadian resonance might be reported by seedset (*5*). We compared seedset from short- and long-period mutants and wild type, under T24. In a single experiment, there were no large or consistent differences in mean seed production (*toc1-1*, 16200 seeds per plant; wild type, 15757 seeds per plant; *ztl-1*, 15005 seeds per plant; $n = 15$). Because the clock determines flowering time, which becomes altered in period mutants (*13*), seedset is likely to be an ambiguous marker for the fitness implications of circadian resonance. However, we have demonstrated that circadian resonance increases the established fitness traits of photosynthesis and biomass (Figs. 1 to 3) (*14*–*17*).

We performed reciprocal competitions between long- and short-period plants (*7*), using two short-period mutants of *TOC1* [*toc1-1* and *toc1-2*, $\tau \approx 20$ hours (*27*)] and two long-period mutants of *ZTL* [*ztl-1* and *ztl-27*, $\tau \approx 28$ hours (*9*, *10*)]. Mixed populations of *toc1* and *ztl* were grown under T20 and T28, which generated a crowded lawn of plants. In these conditions, interactions among neighboring plants of differing genotypes affect physiological outcomes, in addition to the interaction between each plant and the light-dark cycle that we tested previously. We grew monocultures of each line, to assess the importance of differing growth rates among neighbors compared with growth rates among lines. Testing for competitive advantage derived from circadian resonance, using reciprocal competition, requires a mixed population of two period-length genotypes; therefore, it cannot be assessed in just wild type. The period-length mutants were appropriate for this experiment because the growth disadvantage that occurred when $T \neq \tau$ in wild type also occurred with the period mutants (Figs. 1 to 3).

In two separate competition experiments, using different *ZTL* and *TOC1* mutants, under T20, *TOC1* mutants grew more successfully than *ZTL* mutants, as indicated by multiple parameters including chlorophyll content, leaf number, rosette diameter, and aerial biomass (Fig. 4). Conversely, under T28, growth of *ZTL* mutants was enhanced compared with *TOC1*. This was similar to results obtained when plants were grown without competition (Figs. 1 to 3). However, competition caused mortality of some plants, which did not occur in the absence of competition. Mortality was greater in *ztl-27* under T20, and greater in *toc1-2* under T28 (Fig. 4B). Circadian resonance, therefore, en-

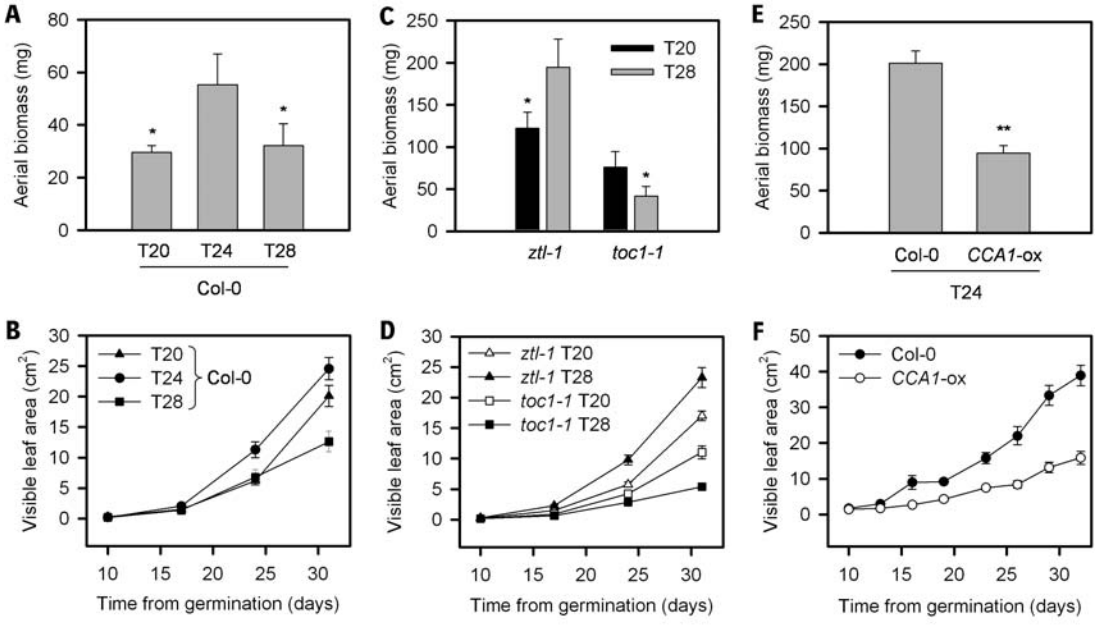

Fig. 3. Environmentally matched clock period enhances vegetative growth. (**A** and **B**) Dry aerial biomass (A) and visible leaf area (B) in Col-0 wild type after growth under T20, T24, and T28. (**C** and **D**) Dry aerial biomass (C) and visible leaf area (D) after growth of the long-period mutant *ztl-1* and short-period mutant *toc1-1* under T20 and T28. (**E** and **F**) Dry aerial biomass (E) and visible leaf area (F) after 35 days' growth of Col-0 wild type and arrhythmic *CCA1*-ox line in T24. Biomass was measured after 32 days (A and C) or 35 days (E). For all groups, $n = 5$; data are means + or ± SEM. (A, C, and E) In two-sample *t* tests comparing aerial biomass to the line with the clock period matched to the environment, significance of results: $^*P < 0.05$, $^{**}P < 0.01$.

hanced growth and survival, and this was more pronounced under competition than during monoculture (Fig. 4A). In two separate monoculture experiments, there was no consistent pattern of T cycle–dependent mortality (Fig. 4C). Therefore, both poor individual growth and outcompetition confer a disadvantage under dissonant T cycles. Our data underestimate the true growth advantage that occurs under competition, because physiological parameters were not measured in dead plants. Neither genotype had an advantage in all conditions, which implicated circadian effects of the mutations rather than secondary phenotypes. Competition between *toc1-1* and *ztl-1*, and between *toc1-1* or *toc1-2* and *ztl-27*, gave the same result, which discounts the likelihood of background mutations or allele-specific effects (*28*). *Arabidopsis* entrains stably to T cycles far from τ (*24*, *29*, *30*), so the long-term growth advantage was likely due to correct phasing of rhythmic processes relative to the environment in one genotype, and an incorrect phase in its competitor (*7*). This suggests that a correctly matched circadian clock confers a competitive advantage, whereas the enhancement of two key fitness traits [biomass and photosynthesis; (*14*–*17*)] by circadian resonance indicates that enhanced photosynthesis is one mechanism by which the clock increases fitness.

Our experiments demonstrate that the circadian clock allows plants to increase photosynthesis and that the clock underlies a doubling of *Arabidopsis* productivity. This may derive from correct anticipation of dawn and dusk, and synchronization of the synthesis of light-harvesting complex proteins and chlorophyll, both of which are unstable in their unbound state (*18*). Incorrect matching of endogenous rhythms to environmental rhythms reduced leaf chlorophyll content, reduced assimilation, reduced growth, and increased mortality. Optimization of these parameters by circadian resonance could represent one of the mechanisms that has selected for circadian clock function during plant evolution. We suggest that selective plant breeding for enhanced crop performance must be performed carefully, because phase and period changes could arise from the close genetic linkage of phase and period loci (*31*) to the trait under selection, and cause alterations to clock function that might reduce vegetative yield. Clock manipulation could enhance food production during exploration of space and other planets, where the light-dark cycle may differ from the terrestrial 24-hour period. Circadian resonance is likely to provide an advantage in all kingdoms, because resonance of the internal clock with the external light-dark cycle ensures an optimal phase relation between physiology and the day-night cycle and provides the basis for anticipation of changes in environmental conditions.

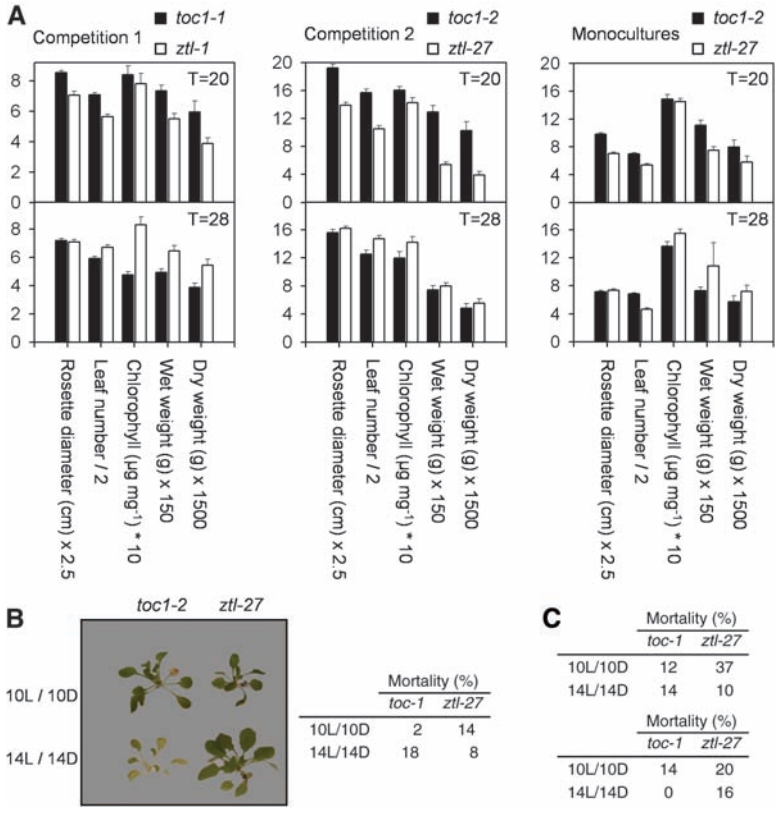

Fig. 4. Correct circadian period enhances growth and survival. (**A**) In two separate competition experiments using different mutant lines and one monoculture experiment, comparative growth and survival of *toc1* and *ztl* under T20 and T28; $n = 49$, except $n = 17$ to 20 for dry biomass values; data are means ± SEM. (**B**) Representative individuals from a competition experiment. Mortality in short- and long-period lines after competition (B) or monoculture (**C**) in T20 and T28.

References and Notes

1. C. H. Johnson, M. Knight, A. Trewavas, T. Kondo, in *Biological Rhythms and Photoperiodism in Plants*, P. L. Lumsden, A. J. Millar, Eds. (Bios Scientific, Oxford, 1998), pp. 1–34.
2. M. W. Young, S. A. Kay, *Nat. Rev. Genet.* **2**, 702 (2001).
3. M. J. Yanovsky, S. A. Kay, *Curr. Opin. Plant Biol.* **4**, 429 (2001).
4. T. P. Michael *et al.*, *Science* **302**, 1049 (2003).
5. R. M. Green, S. Tingay, Z. Y. Wang, E. M. Tobin, *Plant Physiol.* **129**, 576 (2002).
6. H. R. Highkin, J. B. Hanson, *Plant Physiol.* **29**, 301 (1954).
7. Y. Ouyang, C. R. Andersson, T. Kondo, S. S. Golden, C. H. Johnson, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 8660 (1998).
8. M. A. Woelfle, Y. Ouyang, K. Phanvijhitsiri, C. H. Johnson, *Curr. Biol.* **14**, 1481 (2004).
9. Materials and methods are available on *Science* Online.
10. D. E. Somers, T. F. Schultz, M. Milnamow, S. A. Kay, *Cell* **101**, 319 (2000).
11. A. J. Millar, I. A. Carré, C. A. Strayer, N.-H. Chua, S. A. Kay, *Science* **267**, 1161 (1995).
12. Z. Y. Wang *et al.*, *Plant Cell* **9**, 491 (1997).
13. R. Hayama, G. Coupland, *Curr. Opin. Plant Biol.* **6**, 13 (2003).
14. A. M. Arntz, E. H. DeLucia, N. Jordan, *Ecology* **81**, 2567 (2000).
15. J. K. Ward, J. K. Kelly, *Ecol. Lett.* **7**, 427 (2004).
16. P. S. Curtis, X. Wang, *Oecologia* **113**, 299 (1998).
17. T. Mitchell-Olds, *Evol. Int. J. Org. Evol.* **50**, 140 (1996).
18. S. L. Harmer *et al.*, *Science* **290**, 2110 (suppl. data) (2000).
19. A. Prombona, J. Argyroudi-Akoyunoglou, *Plant Sci.* **167**, 117 (2004).
20. K. Kaasik, C.-C. Lee, *Nature* **430**, 467 (2004).
21. A. P. Withrow, R. B. Withrow, *Plant Physiol.* **24**, 657 (1949).
22. J. D. Plautz *et al.*, *J. Biol. Rhythms* **12**, 204 (1997).
23. A. N. Dodd, K. Parkinson, A. A. R. Webb, *New Phytol.* **162**, 63 (2004).
24. D. E. Somers, A. A. R. Webb, M. Pearson, S. A. Kay, *Development* **125**, 485 (1998).
25. H. Griffiths *et al.*, in *Physiological Plant Ecology*, M. C. Press, J. D. Scholes, M. G. Barker, Eds. (Blackwell, Oxford, 1998), pp. 415–441.
26. T. L. Hennessey, C. B. Field, *Plant Physiol.* **96**, 831 (1991).
27. D. Alabadí *et al.*, *Science* **293**, 880 (2001).
28. N. Salathia *et al.*, unpublished observations.
29. M. J. Yanovsky, S. A. Kay, *Nature* **419**, 308 (2002).
30. L. C. Roden, H. R. Song, S. Jackson, K. Morris, I. A. Carré, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 13313 (2002).
31. D. E. Somers, W.-Y. Kim, R. Geng, *Plant Cell* **16**, 769 (2004).

32. Authors E.K., R.T., A.H., and A.J.M. identified *ztl-27*; N.S. and A.J.M. conceived and performed competition and seedset experiments with A.H.; A.N.D., J.M.H., and A.A.R.W. performed all other experiments. We thank the U.K. Biotechnology and Biological Sciences Research Council (A.A.R.W., J.M.H., and A.J.M.); the Royal Society of London and the Isaac Newton Trust (A.A.R.W.); and the Howard Hughes Medical Institute (F.N.). We are grateful to E. Tobin (UCLA) and S. Kay (Scripps) for collaboration and donation of *CCA1*-ox and *toc1-2*, respectively.

Supporting Online Material

www.sciencemag.org/cgi/content/full/309/5734/630/DC1
Materials and Methods
References and Notes

1 April 2005; accepted 7 June 2005
10.1126/science.1115581

***Science* Supporting Online Material**

Plant Circadian Clocks Increase Photosynthesis, Growth, Survival and Competitive Advantage

Antony N. Dodd, Neeraj Salathia, Anthony Hall, Eva Kévei, Réka Tóth, Ferenc Nagy, Julian M. Hibberd, Andrew J. Millar, Alex A. R. Webb

Materials and Methods

Plant growth for period length manipulations and gas exchange. Surface sterilized *Arabidopsis thaliana* seeds were germinated on 0.5× Murashige and Skoog (MS) nutrient mix dissolved in 0.8 % (w/v) agar. Seedlings (10 days old) were transferred to a 1:1 mix of potting compost and vermiculite, and grown under a photon flux density of 160 mmol/m^2 per s, 65% relative humidity, and 20°C by day and night. Period lengths were manipulated by repeated advancement or reversal of the growth chamber timer clocks. During the growth period leaf area was measured. Leaf rosettes were imaged at regular intervals using a digital camera, and visible leaf area calculated from images using the ImageJ software package (rsb.info.nih.gov/ij/). Plants were selected for gas exchange measurements after 3 weeks of growth, and the growth experiment was terminated upon appearance of the first nascent inflorescence. At this point, plant material was harvested and dried for 24 hours for biomass measurement.

Total chlorophyll measurement. Chlorophyll was extracted from mature leaves in 96% ethanol, and measured (*S1*), using a minimum of five replicates for each measurement.

Photosynthetic gas exchange. Leaf gas exchange was measured using a six-cuvette custom-built infra-red gas analyzer [*Arabidopsis* Special System, PP Systems, Hitchin, UK; (*S2*)]. Cuvettes enclosed the entire leaf rosette, and conditions within the cuvette were automatically controlled so that relative humidity remained within 1% of 65% and [CO_2] was 365 ± 5 μl/liter. Each cuvette was measured for 10 min, at hourly intervals. Measurements represent the mean from 3 min of continuous sampling. When required, Fast Fourier Transform (nonlinear least squares method) was performed (*S3*). Results from each experiment, which were side-by-side comparisons of two lines, were independently verified by conducting two identical repeats of each experiment.

Reciprocal competition experiment. A. thaliana was germinated on MS agar in constant dim light (17–20 mmol/m^2 per s). Seedlings were transferred after 10 days to a 1:1 mix of vermiculite and compost, and grown in an array of alternating *toc1-2* and *ztl-27* genotypes (or single genotype for monocultures) at 1–cm spacing (9 plants × 11 plants array), with a row of guard plants. Plants were grown under cool white fluorescent lights (40 μmol/m^2 per s) at 19°–21°C, under 10 hours light/10 hours dark or 14 hours light/14 hours dark cycles and harvested for morphological analysis after 3 weeks. Chlorophyll concentrations were quantified, and plants scored as "dead" when they had no visible chlorophyll.

ztl-27 was identified as a late-phase EMS mutant of the *CAB:LUC* transgenic line in the C24 ecotype, essentially as described previously for *ztl-1* (*S4*, *S5*), and back-crossed

twice to the *CAB:LUC* parent. The mutation is predicted to change Gly^{452} to Asp in the fourth kelch repeat of ZTL. *ztl-27* has a long-period phenotype very similar to *ztl-1*, which will be described in detail elsewhere.

References and Notes

S1. H. K. Lichtenthaler, A. R. Wellburn, *Biochem. Soc. Trans.* **11**, 591 (1983).
S2. A. N. Dodd, K. Parkinson, A. A. R. Webb, *New Phytol.* **162**, 63 (2004).
S3. J. D. Plautz *et al*., *J. Biol. Rhythm* **12**, 204 (1997).
S4. A. J. Millar, I. A. Carré, C. A. Strayer, N.-H. Chua, S. A. Kay, *Science* **267**, 1161 (1995).
S5. D. E. Somers, T. F. Schultz, M. Milnamow, S. A. Kay, *Cell* **101**, 319 (2000).