

Supplemental Figure 1. Mean traces of mutants tested for temperature compensation defects.

The circadian behavior of all mutants (toc1-101 (**A**), ztl-4 (**B**), lhy-20 (**C**), prr5 prr7 (**D**), arr3 arr4 (**E**), elf3-1 (**F**) and flc-3 (**G**)) was tested at 16°C, 22°C and 30°C. All traces were normalized to the mean value for each genotype, and shown as the average of 12-24 seedlings ± s.e.m..



Supplemental Figure 2. Mean traces of *gigantea* mutants tested for temperature compensation defects.

The circadian behavior of all mutants (gi-1 (**A**), gi-2 (**B**) and gi-201 (**C**)) was tested at 16°C, 22°C and 30°C. All traces were normalized to the mean value for each genotype, and shown as the average of 12-24 seedlings ± s.e.m..



Supplemental Figure 3. Normalized changes in free-running period in mutants.

Periods in all mutant backgrounds were normalized to wild-type period for each temperature (by subtracting Col-2 free-running period at each temperature from all mutant periods) and plotted as h difference relative to wild type. Negative values indicate short periods in the mutants, while positive values represent long periods.

Supplemental Data. Salomé et al. (2010). Plant Cell 10.1105/tpc.110.079087



Supplemental Figure 4. Mean traces of Col-0, *prr7 prr9* and *prr7 prr9 amiR* lines tested for temperature compensation defects.

The circadian behavior of all lines was tested at 12°C, 22°C and 30°C. All traces were normalized to the mean value for each genotype, and shown as the average of 12-24 seedlings ± s.e.m.



Supplemental Figure 5. Mean circadian periods in Col-0 at 22°C and *prr7 prr9* at 30°C for all lines tested.

Seedlings were grown under light-dark cycles at 22°C before being released in constant light at 22°C (for Col-0 lines) or at 30°C (for *prr7 prr9* lines). All T_2 lines tested are shown here. Selected T_2 lines in the Col-0 and *prr7 prr9* backgrounds included in all analyses are shaded in grey.

All period values are given as means \pm s.e.m. (n=12).



Supplemental Figure 6. Expression of clock genes in Col-0 and prr7 prr9.

(A) Expression of *CCA1* in Col-0 and *prr7 prr9* at 12°C, 22°C and 30°C. Mean \pm s.d. (n=2). (B) Expression of *LHY* in Col-0 and *prr7 prr9* at 12°C, 22°C and 30°C.Mean \pm s.d. (n=2). (C) Expression of *TOC1* in Col-0 and *prr7 prr9* at 12°C, 22°C and 30°C.Mean \pm s.d. (n=2). (D) Expression of *GI* in Col-0 and *prr7 prr9* at 12°C, 22°C and 30°C.Mean \pm s.d. (n=2). (E) Expression of the clock genes does not significantly respond to changes in ambient temperature. Expression levels were taken from (Gould et al., 2006), and are shown as means \pm s.d. (n=3).



Supplemental Figure 7. Daily temperature variation across 9 locations for the year 2005.

The daily maximum (red), mean (black) and minimum (blue) temperatures for quality controlled data were downloaded from the National Climatic Data Center (http://www7.ncdc.noaa.gov/CDO/cdoselect.cmd?datasetabbv=GSOD&resolution=40) for the year 2005, or for 2006 and 2007 in the case of Norwich. When possible, the weather station closest to an accession collection site was chosen. Flowering time data for Ler, Col-0, *gi-2* and *FRI*-Sf2 lines are replotted from (Wilczek et al., 2009). Each horizontal bar indicates the number of days until bolting from the time of sowing for the Summer 2006, Fall 2006, Spring 2007 and Summer 2007 cohorts. From top to bottom, the order of genotypes is Ler, Col-0, *gi-2, FRI*-Sf2.

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genotype	temperature	period (h)	RAE	RAE range	n
Col-2	16°C	25.9 ± 0.1	0.20	0.15 – 0.28	12
	22°C	24.3 ± 0.2	0.23	0.13 – 0.49	12
	30°C	23.5 ± 0.3	0.24	0.12 – 0.38	12
prr7 prr9	16°C	28.1 ± 0.3	0.24	0.15 – 0.32	12
	22°C	32.1 ± 0.5	0.23	0.10 - 0.42	12
	30°C	36.4 ± 0.5	0.21	0.12 – 0.27	12
lhy-20	16°C	24.5 ± 0.3	0.18	0.11 – 0.40	12
	22°C	22.2 ± 0.1	0.16	0.09 - 0.24	12
	30°C	20.7 ± 0.1	0.25	0.13 – 0.45	12
toc1-101	16°C	22.4 ± 0.4	0.37	0.26 – 0.65	11
	22°C	19.5 ± 0.2	0.23	0.14 – 0.33	10
	30°C	18.8 ± 0.1	0.42	0.30 – 0.61	12
ztl-4	16°C	28.6 ± 0.3	0.28	0.13 – 0.57	12
	22°C	26.9 ± 0.15	0.15	0.07 – 0.31	11
	30°C	26.9 ± 0.2	0.15	0.09 - 0.23	7
prr5 prr7	16°C	22.1 ± 0.2	0.41	0.25 – 0.62	12
	22°C	22.8 ± 0.9	0.56	0.50 – 0.62	11
	30°C	20.3 ± 1.3	0.62	0.45 – 0.71	12
arr3 arr4	16°C	26.9 ± 0.15	0.20	0.14 – 0.40	12
	22°C	27.1 ± 0.25	0.20	0.12 – 0.29	12
	30°C	27.2 ± 0.2	0.20	0.10 – 0.30	12
flc-3	16°C	25.6 ± 0.1	0.25	0.12 – 0.39	12
	22°C	24.3 ± 0.2	0.19	0.11 – 0.27	12
	30°C	23.4 ± 0.2	0.26	0.19 – 0.37	12
gi-1	16ºC	23.9 ± 0.3	0.27	0.19 – 0.35	12
	22°C	23.1 ± 1.0	0.38	0.23 – 0.51	12
	30°C	20.1 ± 0.8	0.43	0.22 – 0.78	12
gi-2	16°C	24.9 ± 0.3	0.43	0.24 – 0.66	12
	22°C	25.3 ± 0.1	0.52	0.35 – 0.76	12
	30°C	24.1 ± 0.7	0.57	0.45 – 0.69	12
gi-201	16°C	23.7 ± 0.2	0.40	0.25 – 0.58	12
	22°C	24.8 ± 0.3	0.30	0.22 – 0.36	12
	30°C	24.1 ± 0.5	0.58	0.44 – 0.69	12

Supplemental Table 1. Circadian periods (mean \pm s.e.m.) and Relative Amplitude Errors (RAE) of all genotypes in this study.

Supplemental Table 2. List of constructs.

Clone name	Target gene	Type of clone, selection*	
pINT098	CCA1	Entry clone, kanamycin	
pINT294	LHY	Entry clone, kanamycin	
pINT307	CCA1-LHY	Entry clone, kanamycin	
pINT511	TOC1	Entry clone, kanamycin	
pINT314	ZTL	Entry clone, kanamycin	
pINT315	PRR7-PRR9	Entry clone, kanamycin	
pINT275	NA (destination vector)	Binary, kanamycin	
pINT276	NA (destination vector)	Binary, BASTA	
pINT425	CCA1	Binary, Basta	
pINT406	LHY	Binary, Basta	
pINT346	CCA1-LHY	Binary, kanamycin	
pINT480	CCA1-LHY	Binary, Basta	
pINT514	TOC1	Binary, Basta	
pINT408	ZTL	Binary, Basta	
pINT444	PRR7-PRR9	Binary, Basta	

* Listed selection for Entry clones refer to antibiotic selection in *E. coli*, and selection for Binary clones to the selection for transgenic plants.

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gene	amiRNA sequence (5' to 3')
CCA1	UGAUCGGUUAUAUUGGAGCUC
LHY	UAGUUGAGAAUGGAACGCACG
TOC1	UAGUCGCCUAAUUUAUCCCGU
ZTL	UGUCAACUAAUGGAUGCGCUC
PRR7	UAACGAUAUAACGAGUGCGGU
PRR9	UUAAAUACUAUCAGGACGCCU

Supplemental Table 3. amiRNA sequences.

Supplemental Table 4. qPCR primers.

ID	Gene	Sequence (5' to 3')	Direction
G-27440	CCA1 ¹	CCT CAA ACT TCA GAG TCC AAT GC	sense
G-27441	CCA1 ¹	GAC CCT CGT CAG ACA CAG ACT TC	antisense
G-27442	LHY ¹	GAA GTC TCC GAA GAG GGT CG	sense
G-27443	LHY ¹	TAT TCA CAT TCT CTG CCA CTT GAG	antisense
G-26630	TOC1	GCT ATG AAC AGA AGT AAA GAT TCG	sense
G-16718	TOC1	GGA TAT CCC GTC ATT CCA TTC GGA	antisense
G-19623	GI ²	ACC AAC GGA TAG TCA AAG TGT TGG	sense
G-19624	GI ²	AAG CTA GCA CCG GCT GTA TTG CTC	antisense
G-9789	HSP70 ³	AGG AGC TCG AGT CTC TTT GC	sense
G-9790	HSP70 ³	AGG TGT GTC GTC ATC CAT TC	antisense
G-26631	ElF4a	GCG CAT CCT CCA AGC TGG TGT CC	sense
G-26632	ElF4a	GGT GGA AGA AGC TGG AAT ATG TCA TAG ATC	antisense

¹ from (Kanno et al., 2010).

² correspond to *gi-1* genotyping primers; *gi-1* carries a small 5-bp deletion in the 3' end of the GI gene (Park et al., 1999).

³ from (Balasubramanian et al., 2006).

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