Characterizing Genetic Mechanisms for Measuring Day-Length in *Neurospora crassa*

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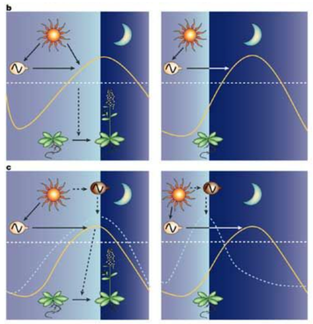
Abstract

Photoperiodism is a physiological response of an organism to changes of the ambient environment over a year. The day-length is one of the major environmental changes for most of the organisms on earth over a year. There is a lack of understanding on how an organism accurately measures the day-length. In the current study, we identified genes that are responsible for measuring the day-length in a model organism *Neurospora crassa*.

Photoperiodism and the Circadian Clock

Photoperiodism: the response of an organism to a change in day length

Circadian clock: regulates 24-hour cycles within an organism



External coincidence hypothesis: light is needed in order to regulate the circadian clock so it can respond to seasonal changes.

Internal coincidence hypothesis: internal oscillators regulate the circadian clock, while the affect of light on the circadian clock is very small.

Yanovsky, M.J., and Kay, S.A. (2003). Living by the calendar: how plants know when to flower. Nat. Rev. Mol. Cell Biol. *4*, 265–276.

$$Cohen's\ d = \frac{(M_2 - M_1)}{Pooled\ SD}$$

Cohen, 1962

We hypothesized that the Cohen's D value of the number of protoperithecia produced may reflect an organism's ability to measure day-length.

Hypotheses

The ability to measure day-length is a multi-gene trait, and thus Quantitative Trait Loci (QTL) analysis will lead us to identify genetic elements for photoperiodism. Also, genes that are known to be involved in the circadian rhythm, a biological process orchestrating the 24-hr period rhythm, play a role in photoperiodism.

Research Conducted

We have performed a protoperithecia assay (PPA) on 91 strains of an F1 population in *N. crassa* in order to determine how it measures day-length. In short, in the protoperithecia assay, the strains are exposed to different photoperiods which are long-day (16 hours of light:8 hours of dark), short-day (8 hours of light:16 hours of dark), and equinox (12 hours of light:12 hours of dark) for 12-14 days at a constant 25°C. They are then removed from exposure and the number of protoperithecia produced is counted for each strain in each photoperiod. The data from this was used in QTL analysis to find significant QTLs. A major QTL was found on chromosome 5. We also performed PPA on 21 knockout mutants in the target region on chromosome 5 to identify the possible causative gene for the photoperiod gene. We also performed PPA on 10 clock/photoreceptor mutants to test if the circadian clock was involved in measuring day-length. Both the target region knockout mutants and the clock/photoreceptor mutants used a wildtype strain with FGSC 2489 as the control.

Statistical Analyses

One-way and two-way ANOVA tests could not be done because some of the data failed the normality tests. Instead, paired t-tests were used in R. The Shapiro-Wilks test was used to check if the data was normally distributed, and only six out of thirty-three mutants failed the Shapiro-Wilks test. These six mutants were separated into a different analysis. Paired t-tests were conducted on all of the mutants that were normally distributed to determine which ones were statistically significant between photoperiods.

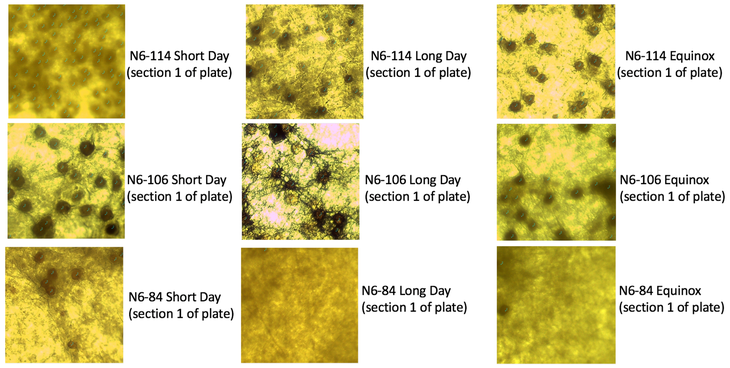


Figure 1: Images of the protoperithecia assay. Shown is protoperithecia grown on plates. Protoperithecia are female sexual reproductive structures in *N. crassa*.

Results

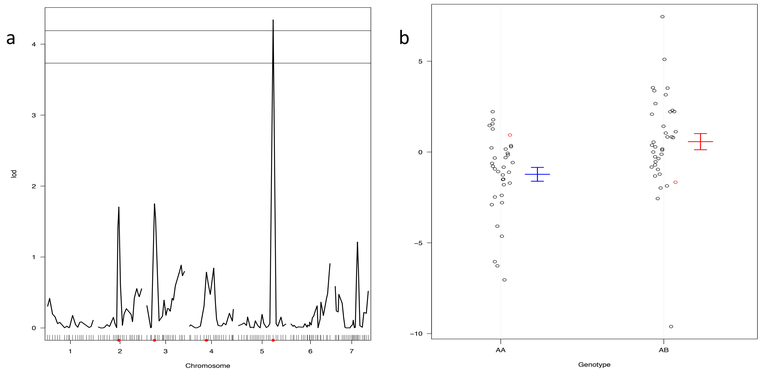


Figure 2: Results of QTL analysis. Figure 2a shows a diagram of the LOD scores for all chromosomes. The LOD score for chromosome 5 surpasses the thresholds, so chromosome 5 is a major QTL. Figure 2b shows that the genotypes can distinguish between different photoperiods.

Clock/Photoreceptor Mutant	Cohen's d LD	Cohen's d SD	Genotype Effect Size LD	Genotype Effect Size SD	PPA Image LD	PPA Image SD	PPA Image EQ
FGSC 2489	-1.28	-2.12	0	0			
Clock Mutant #1	1.35	0.84	2.63	2.96			
Clock Mutant #2	3.75	6.02	5.02	8.14			
Clock Mutant #3	2.21	0.59	3.48	2.71			
Clock Mutant #4	-5.66	N/A	-5.41	N/A		N/A	
Clock Mutant #5	-5.74	N/A	-5.49	N/A		N/A	
Clock Mutant #6	-3.43	N/A	-3.17	N/A		N/A	
Clock Mutant #7	0.41	N/A	0.66	N/A		N/A	
Clock Mutant #8	-2.06	N/A	-1.80	N/A		N/A	
Clock Mutant #9	2.18	N/A	2.43	N/A		N/A	
Clock Mutant #10	-2.64	N/A	-2.38	N/A		N/A	

Table 1 is a summary table of the effect sizes of the clock/photoreceptor mutants and the wildtype. Images taken of PPA are also shown to show how the amount of protoperithecia produced differs between photoperiods. (EQ means equinox, LD means long-day, SD means short-day). The images highlighted in orange were found to be statistically significant in the paired t-tests. N/A refers to the dataset that we do not have the SD data for.

Clock/Photoreceptor Mutant	EQ+LD p-value	EQ+SD p-value	LD+SD p-value
FGSC 2489	0.2417	0.08139	0.04708
Clock Mutant #1	Failed normality	Failed normality	0.3255
Clock Mutant #2	0.006631	0.004311	0.03342
Clock Mutant #3	0.02892	0.5929	0.2664
Clock Mutant #4	0.008659	N/A	N/A
Clock Mutant #5	0.000638	N/A	N/A
Clock Mutant #6	0.01897	N/A	N/A
Clock Mutant #7	0.6423	N/A	N/A
Clock Mutant #8	Failed normality	N/A	N/A
Clock Mutant #9	0.09279	N/A	N/A
Clock Mutant #10	0.01531	N/A	N/A

Table 2 is a summary table of the results of the paired t-tests for the clock/photoreceptor mutants and the wildtype. The table shows the mutants' p-values when their amounts of protoperithecia produced were compared between photoperiods. P-values that are highlighted in purple show a statistically significant difference between photoperiods.

Target Region Mutant	Cohen's d LD	Cohen's d SD	Genotype Effect Size LD	PPA Image LD	PPA Image EQ	PPA Image SD
Mutant#1	4.72	N/A	6.80			N/A
Mutant#2	-0.01	N/A	2.07			N/A
Mutant#3	0.93	0.17	3.01			
Mutant#4	6.47	1.46	8.54			
Mutant#5	3.64	0.58	5.72			
Mutant#6	1.92	N/A	4.00			N/A
Mutant#7	5.42	1.40	7.50			
Mutant#8	4.21	N/A	6.29			N/A
Mutant#9	4.52	N/A	6.59			N/A
Mutant#10	1.44	-2.16	3.52			
Mutant#11	3.05	-2.55	5.12			
Mutant#12	24.79	N/A	26.86			N/A
Mutant#13	8.07	2.77	10.15			
Mutant#14	12.61	N/A	14.69			N/A
Mutant#15	3.19	N/A	5.27			N/A
Mutant#16	0.04	-1.86	2.12			
Mutant#17	1.08	-2.17	3.16			
Mutant#18	1.45	N/A	3.53			N/A
Mutant#19	3.13	-1.13	5.21			
Mutant#20	-1.15	N/A	0.93			N/A
Mutant#21	1.10	-2.22	3.17			
FGSC 2489	-2.08	N/A	0.00			N/A

Table 3 shows a summary table of the effect sizes of the target region mutants and the wildtype. Images taken of PPA are also shown to show how the amount of protoperithecia produced differs between photoperiods. (EQ means equinox, LD means long-day, SD means short-day). Images highlighted in orange were found to be statistically significant in the paired t-tests. N/A refers to the dataset that we do not have the SD data for.

Target Region Mutant	EQ+LD p-value	EQ+SD p-value	LD+SD p-value
Mutant #1	0.007118	N/A	N/A
Mutant #2	0.9921	N/A	N/A
Mutant #3	0.2391	0.8711	0.1126
Mutant #4	0.005106	0.2092	0.05085
Mutant #5	Failed normality	Failed normality	0.01369
Mutant #6	0.1037	N/A	N/A
Mutant #7	0.01117	0.203	0.09966
Mutant #8	Failed normality	N/A	N/A
Mutant #9	0.01699	N/A	N/A
Mutant #10	0.08754	0.007906	0.009453
Mutant #11	0.01904	0.0297	0.0223
Mutant #12	7.66E-05	N/A	N/A
Mutant #13	0.002717	0.00714	0.0106
Mutant #14	0.0008832	N/A	N/A
Mutant #15	0.01009	N/A	N/A
Mutant #16	0.967	0.06063	0.07749
Mutant #17	0.3221	0.05983	0.01163
Mutant #18	0.08034	N/A	N/A
Mutant #19	0.03666	0.2469	0.02721
Mutant #20	0.2838	N/A	N/A
Mutant #21	0.1765	Failed normality	Failed normality
FGSC 2489	0.003614	N/A	N/A

Table 4 shows a summary table of the results of the paired t-tests for the target region mutants and the wildtype. The table shows the mutants and their p-values when their amounts of protoperithecia produced were compared between photoperiods. The p-values that are highlighted in purple show a statistically significant difference between photoperiods.

Conclusion and Future Study

In conclusion, in this study we identified a major QTL on chromosome 5 and performed PPA on 21 target region mutants on chromosome 5. We also performed PPA on 10 clock/photoreceptor mutants in order to determine if the circadian clock is involved in measuring photoperiod. We identified many candidate genes that may be involved in measuring day-length in *N. crassa* by performing paired t-tests on our data. We determined that 13 genes in the target region showed statistically significant photoperiodic responses. Also, 6 clock/photoreceptor genes showed statistically significant photoperiodic responses. It should be known that this data is preliminary, and we still need to repeat the protoperithecia assay to confirm our results. In the future, we plan on repeating all of the experiments to confirm our results. We also plan on continuing the analysis of our data using R.

Acknowledgements

This project was supported by the New Jersey Space Grant Consortium summer fellowship to SC. The authors appreciate support from Lee lab members, Ningyuan Zhu, Harjit Khaira, and William Myers.

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