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

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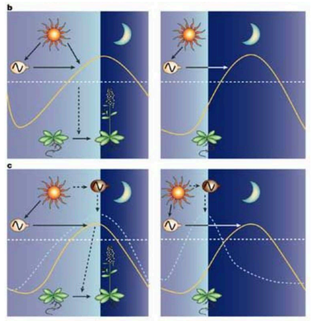
Abstract

Photoperiodism is a physiological response of an organism to changes of the ambient environment over a year and plays a major role in fitness of an organism in nature. There are two theories that explain how photoperiodism and a related process, the circadian rhythm, may be related. The first theory is called the external coincidence hypothesis, and the second theory is called the internal coincidence hypothesis. Unlike the mechanism of photoperiodism, that of the circadian rhythm (24-hour cycles within an organism) is well characterized. We hypothesized that there are multiple genes that are involved in photoperiodism, and that the genes involved in the circadian clock might also be involved in photoperiodism. We also hypothesized that there may be two different mechanisms that *N.crassa* uses to measure the day-length. To test our hypotheses, we developed the protoperithecia assay (PPA). Protoperithecia is a female sexual reproductive structure in *N. crassa and* is known to be responsive to different photoperiods. This data was used as the readout for an organism's ability to determine the day-length. We performed Quantitative Trait Loci (QTL) analysis on the number of protoperithecia at different photoperiods using 91 F1 progeny of *N. crassa*. We found a major QTL on chromosome (Chr) 5, and multiple minor QTLs on other Chr. We characterized 21 knockout mutants in the target region of Chr 5 and 17 knockout mutants on one minor QTL in Chr 1. We found that 12 genes on Chr 5 were statistically significant between at least two photoperiods, and 5 genes on Chr 1 were statistically significant between at least two photoperiods. We also performed PPA on 10 known clock/photoreceptor mutants to test our hypothesis that the circadian clock is involved in photoperiodism. We found that 5 genes were statistically significant between at least two photoperiods. Our data support the view that the circadian clock is a part of the day-length measuring mechanism. Our data also supported the hypothesis that there are multiple genes involved in photoperiodism because we identified 22 genes in total that showed statistically significant photoperiodic responses in our study. The current study will provide a comprehensive view on the possible genetic mechanisms of photoperiodism.

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.

Marcelo J. Yanovsky, Nature Reviews Molecular Cell Biology volume 4 pages 265-276 (2003).

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

https://toptipbio.com/cohens-d/

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

Methods

A protoperithecia assay was performed 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 (PPA), 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 25ºC. 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. One of the minor QTLs found was on chromosome 1. PPA was performed on 21 knockout mutants in the target region on chromosome 5 to identify the possible causative gene for the photoperiod gene. PPA was also performed on 17 knockout mutants located on minor QTL chromosome 1, in order to determine if there was a possible causative gene for the photoperiod gene on this chromosome as well. We also performed PPA on 10 clock/photoreceptor mutants to test if the circadian clock was involved in measuring day-length. The target region mutants on chromosome 5, the knockout mutants on chromosome 1, and the clock/photoreceptor mutants used a wildtype strain with FGSC 2489 as the control.

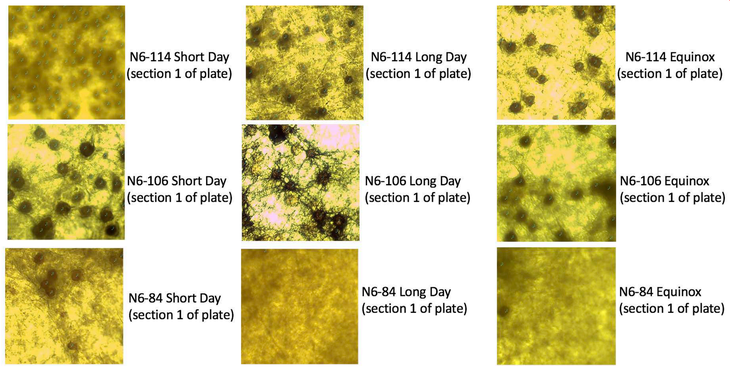


Figure 1: Images of the protoperithecia assay. Shown is protoperithecia grown on plates.

Results

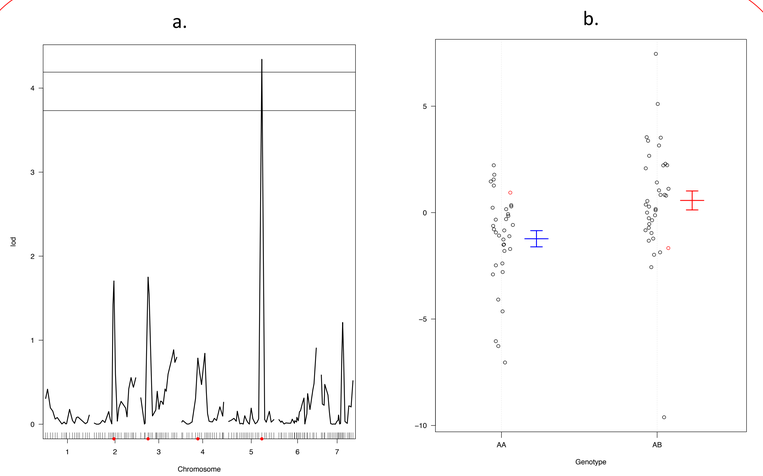


Figure 2: Results of QTL analysis. Figure 2a shows a diagram of the LOD scores for all chromosomes. The LOD score for chromosome 5 surpasses the thresholds, so chromosome 5 is a major QTL. Figure 2b shows that the genotypes can distinguish between different photoperiods.

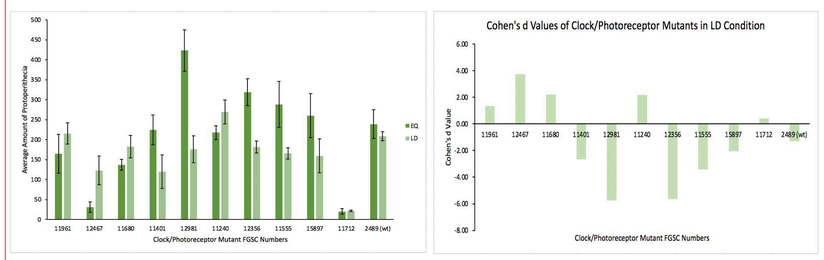


Figure 3: Results of the protoperithecia assay for clock/photoreceptor mutants. Figure 3a shows a graph of the raw data. Figure 3b shows a graph of the Cohen's d effect sizes for each mutant. FGSC 12467 is a candidate gene for the clock/photoreceptor mutants because it has the highest effect size going in the opposite direction from the wild type effect size.

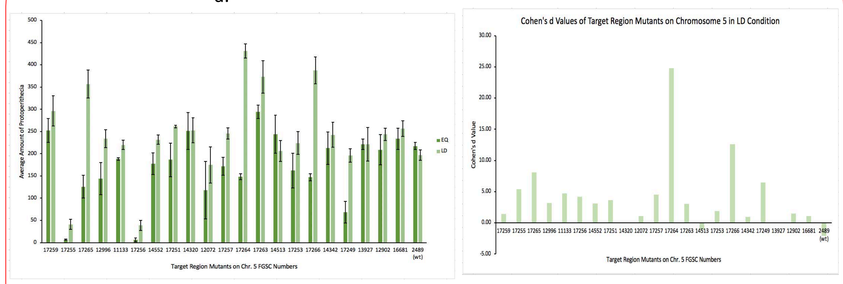


Figure 4: Results of the protoperithecia assay for the target region mutants on chromosome 5. Figure 4a shows a graph of the raw data. Figure 4b shows a graph of the Cohen's d effect sizes for each mutant. FGSC 17264 is a candidate gene on chromosome 5 because it has the highest Cohen's d effect size compared to the wild type.

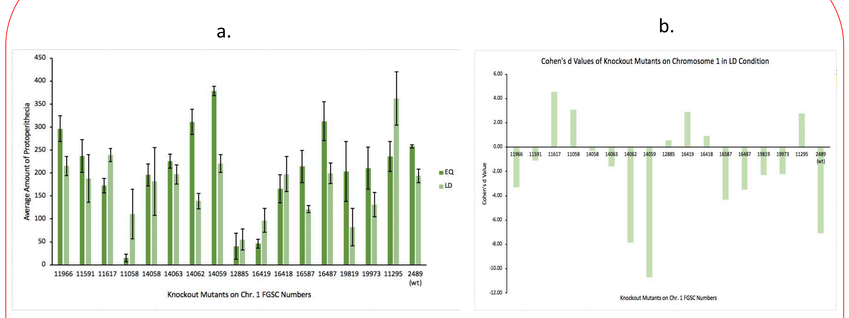


Figure 5: Results of the protoperithecia assay for the knockout mutants on chromosome 1. Figure 5a shows a graph of the raw data. Figure 5b shows a graph of the Cohen's d effect sizes for each mutant. FGSC 11617 is a candidate gene on chromosome 1 because it has the highest effect size and is going in the opposite direction than the wild type effect size.

Conclusion and Future Study

In conclusion, in this study we identified a major QTL on chromosome 5 and several minor QTLs, one of them being on chromosome 1. We also identified candidate genes in these QTL whose knockout mutants show different photoperiodic responses from that of the wild type. Also, our data supports a view that some but not all circadian clock genes are involved in photoperiodic responses. In the future, we will complete the data for the short-day photoperiod, repeat our PPA experiments to verify our data, and conduct experiments on all the candidate genes. It should be known that this data is preliminary, and we still need to repeat the protoperithecia assay in the future to confirm our results.

Acknowledgements

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