Uncovering Photoperiodic Regulation Mechanisms in *Neurospora crassa*

Center for Computational and Integrative Biology (CCIB), Rutgers University- Camden
Aleece Siner and Kwangwon Lee

RUTGERS CAMDEN

Abstract

Organisms adapt to seasonal changes driven by Earth's rotation around the sun, with photoperiod influencing their biological rhythms. While photoperiodism has been more extensively studied in plants, the mechanisms underlying seasonal responses in fungi remain largely unexplored. This study explores novel mechanisms and identifies genetic components involved in photoperiodic regulation in the model fungus *Neurospora crassa*, focusing on how it adapts reproductive cycles to changes in day length. We tested whether fungi share a general photoperiodic mechanism with flowering plants, which we termed the signal accumulation model. We also tested two alternative hypotheses: the balance model and the gating model. To examine these, we developed a systematic assay to monitor protoperithecia (female organ) formation under varying photoperiods, referred to as the Protoperithecia Assay (PPA), using protoperithecia development as a proxy for the organism's photoperiodic recognition. Interestingly, the data support a balance model, where day length is measured through a dynamic equilibrium between light and dark phases, rather than through signal accumulation. We also observed varying degrees of system sensitivity depending on the time of day, lending support to the gating model. To identify genetic components involved in this fungal day-length sensing, we screened approximately 200 *N. crassa* mutants targeting genes involved in signal transduction, under different photoperiodic conditions. Understanding this unique fungal mechanism may provide broader insights into how non-photosynthetic organisms use ambient light as a seasonal cue.

Background

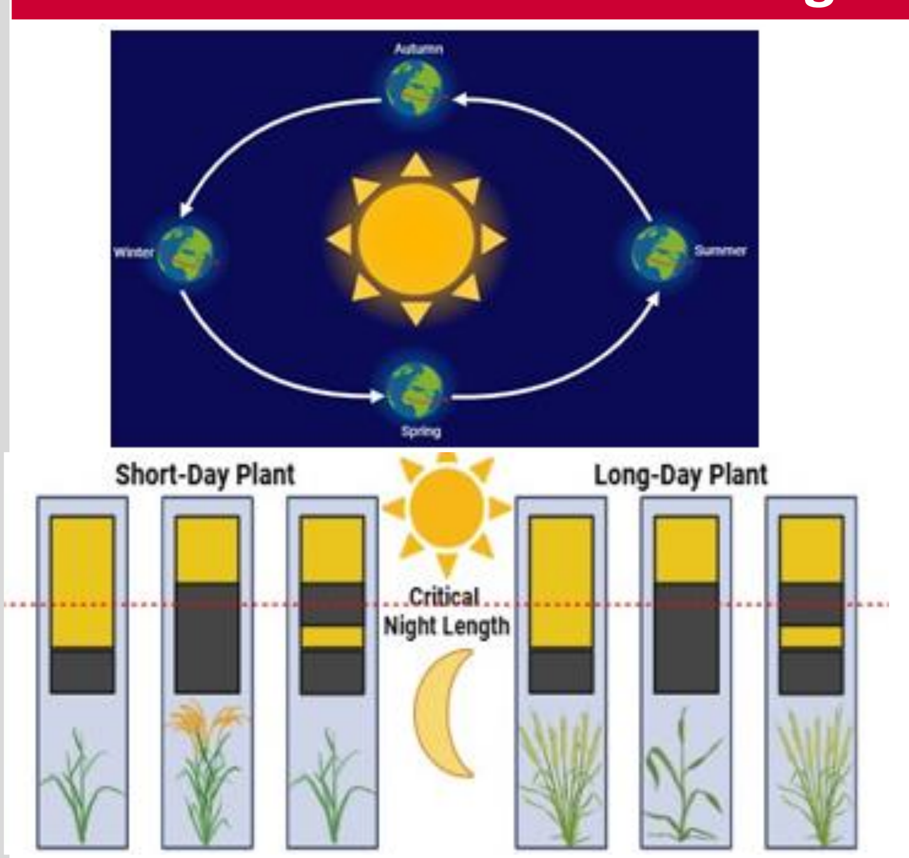


Figure 1. Photoperiodic Regulation. A. Earth's Rotation Around the Sun. B. The Photoperiodic Mechanism in Short-Day and Long-Day Plants

- Circannual rhythms help organisms adapt to seasonal changes by regulating many biological behaviors.
- Photoperiodism, the response to day length, is a key cue for synchronizing these rhythms with the changing seasons.
- Unlike plants, which rely on light as an energy source, fungi utilize unique mechanisms where a balance between light and dark phases, and the timing and duration of light and dark interruptions are crucial for photoperiodic recognition in fungi.

Methods and Materials

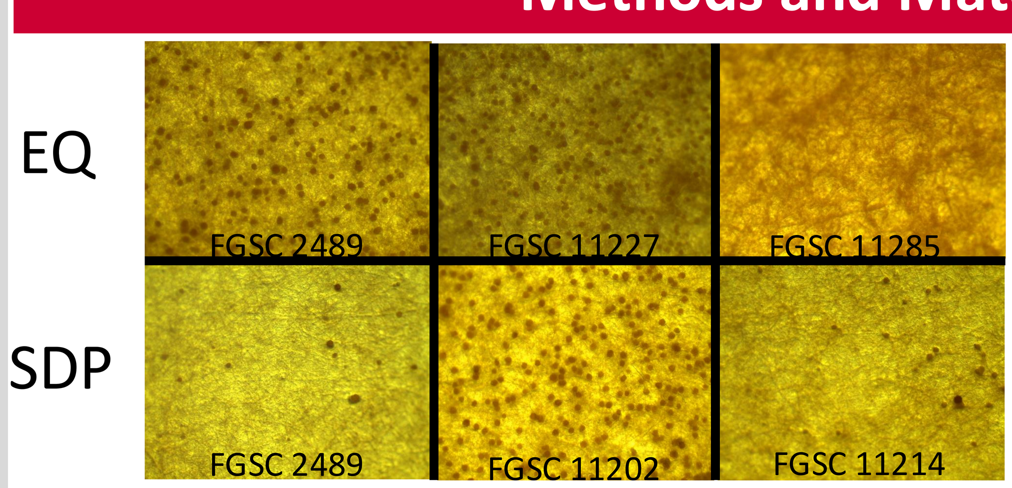


Figure 2. Protoperithecia Assay (PPA)
Wild-type FGSC 2489 is expected to produce higher protoperithecia (PP) concentrations in EQ and lower in SDP. Notable mutant strains showed significantly increased or decreased PP production compared to the wild type.

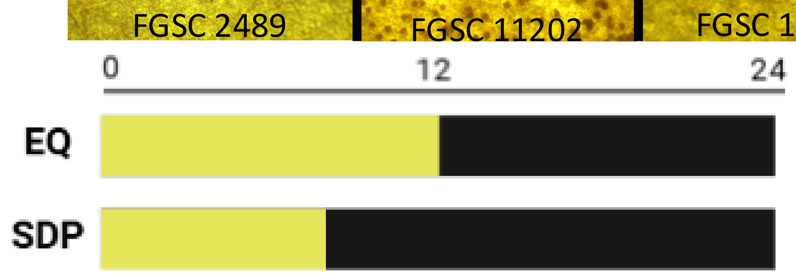


Figure 5. Light Conditions Model

Results

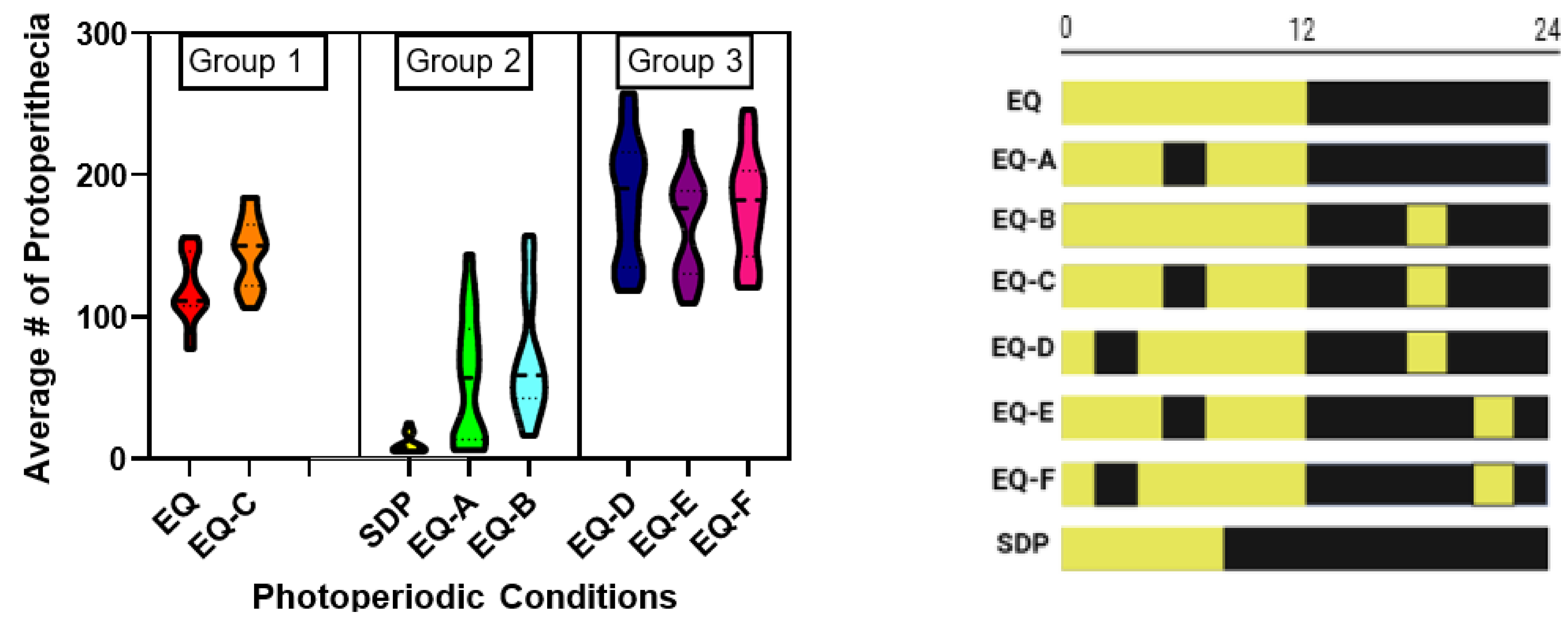
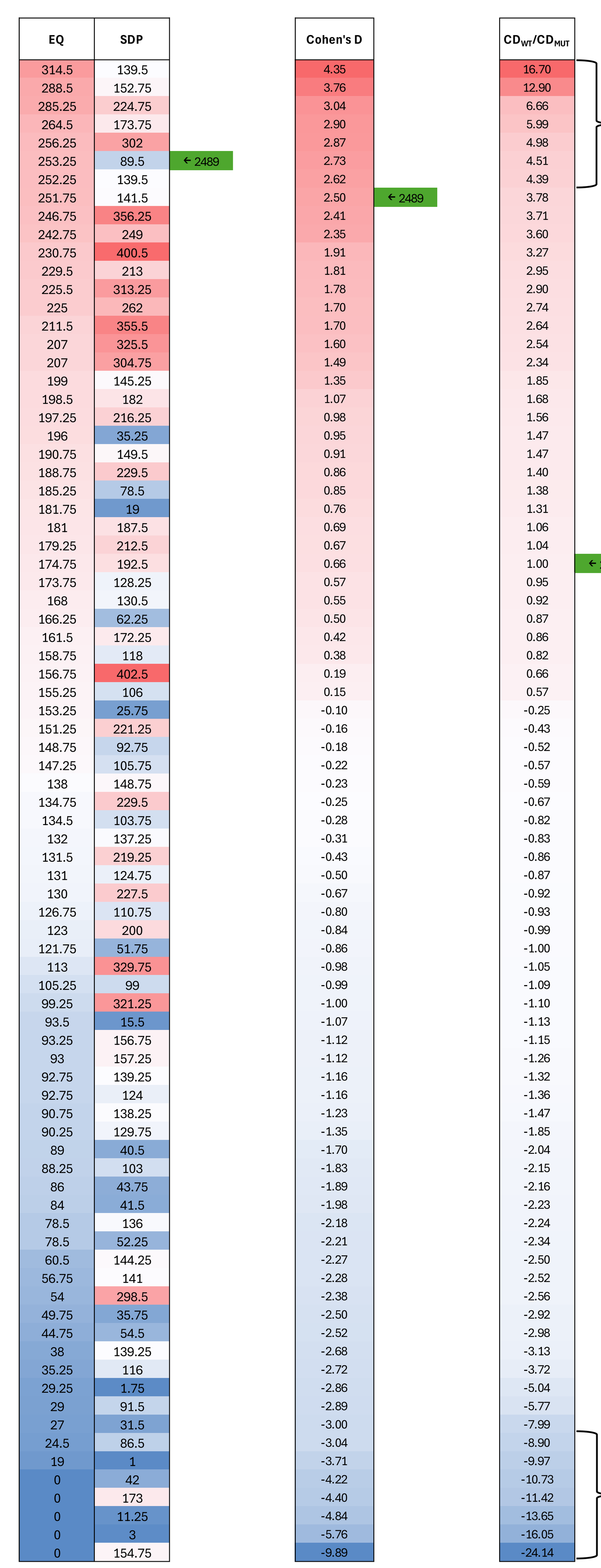


Figure 3. EQ Interruption Balance Model Experiment. Equinox (EQ), short- day (SDP), equinox-A (EQ-A), equinox-B (EQ-B), equinox-C (EQ-C), equinox- D (EQ-D), equinox-E (EQ-E), equinox- F (EQ-F). Group 1 exhibits an optimal production of protoperithecia, Group 2 exhibits a decrease in protoperithecia production, Group 3 exhibits an increase in protoperithecia production, nested one-way ANOVA test, p-value < 0.05, n=4.



Extreme difference (Candidate Genes)

Cohen's D = Group A Mean – Group B Mean / Pooled Standard Deviation

Extreme difference (Candidate Genes)

Figure 4. *N. crassa* mutants Effect Size Heat Map

Wild type FGSC 2489 and approximately 200 mutant strains were grown on Vogel's minimal media. These strains were inoculated onto the center of petri dishes with cross media (1 petri dish per strain). These petri dishes were kept in growth chambers at 25 degrees Celsius for seven days. The number of protoperithecia (female organ in *N.crassa*) produced was counted on Image J software.

Hypothesis

We hypothesize that candidate genes involved in light signal transduction mediate the balance model of photoperiodic regulation *in Neurospora crassa*, and their activity is selectively gated by circadian timing.

Conclusion

In conclusion, our study set out to uncover novel mechanisms by which fungi recognize and respond to photoperiods, with a particular emphasis on the molecular and genetic components underlying this regulation. Unlike plants, fungi appear to rely on distinct pathways for photoperiodic recognition, where the balance between light and dark phases—as well as the timing and duration of interruptions—plays a critical role. To explore these mechanisms, we screened approximately 200 *Neurospora crassa* mutants targeting genes involved in signal transduction and light perception. This approach led to the identification of several promising candidate genes that may contribute to photoperiodic regulation in fungi. These findings suggest that fungi possess unique signaling networks that interpret environmental light cues in ways not yet fully understood. As a future direction, we plan to genotype these candidate strains and perform functional analyses to validate their roles, ultimately deepening our understanding of the genetic architecture behind fungal photoperiodism and its broader implications for circadian biology.

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