Photoperiodic Regulation of Sexual Reproduction in *Physcomitrella patens*

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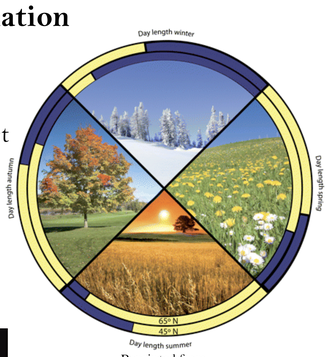
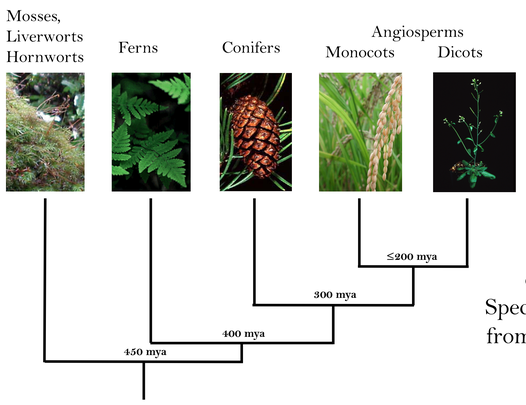
Abstract: Photoperiodism is an important regulatory mechanism of many living organisms in day-to-day functions as responses to environmental cues. In many land plants, the Circadian Clock interprets environmental cues regulating the necessary genes. The moss, *Physcomitrella patens*, controls reproduction through photoperiodic sensitivity, reproducing in short day conditions. Moss genes have been identified resembling those known in angiosperms to control photoperiod dependent reproduction. Whether the photoperiodic control in moss is due to a similar genetic pathway is unknown. If moss reproduction is tied to seasonal differences in daylight, reproduction tied to day length may have evolved with the first land plants. Two different approaches are being utilized to discover an evolutionary link. In one study, a moss plant with mutations in all three CONSTANS-like genes is being constructed. These genes are known in angiosperms to control the timing of reproduction. Exposing the mutant plants to inductive light conditions and examining resulting MFT expression may determine pathway conservation. Ongoing plant screens will locate a mutant plant. The second study aims to interrupt the timing of reproduction by interrupting the measurement of the received nighttime length. Disrupting the night of an angiosperm will stop accumulation of CONSTANS mRNA and resulting protein. Exposing moss to a night break may reveal a similar measurement control. Preliminary data suggests that moss exposed to a night break delays the development of reproductive structures.

Background Information

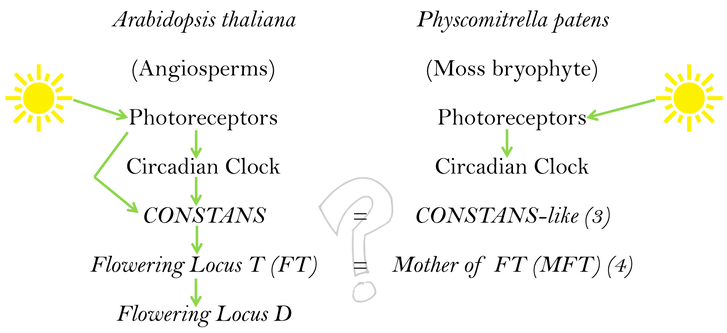
A main regulator of plant reproduction is the amount of daylight, or length of nighttime received, which varies with the passing of seasons. Moss reproduction occurs under similar control: short day conditions, less than 8 hours of light and greater than 16 hours of darkness.



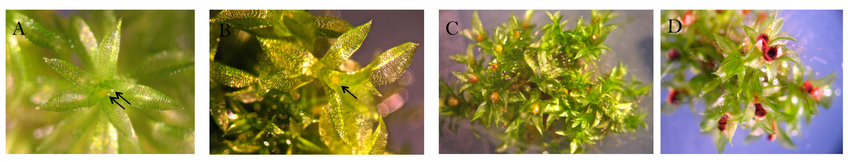
Reprinted from Lagercrantz, 2009[1]



Over the past 450 million years land plants have evolved from a common ancestor to the various Species we see today. Moss diverged from the common lineage about 450 million years ago.



Sequence similarity exists between both the CONSTANS of angiosperms and the CONSTANS-like genes of *Physcomitrella patens* as well as between FT genes and MFT. However, any connection to each other or to a reproductive pathway is unknown at this time.



P. patens reproductive structures. A. Archegonia, the female reproductive structures, are white stalks that hang over the antheridia, the male structures. B. Antheridia are yellow orange ball structures at the base of the steams. C. Spore capsules, the diploid offspring, emerge after fertilization of the archegonia by antheridia. Spores start out green, then turn brown, and eventually splitting open. D. Spore capsules which have opened to release spores.

Acknowledgements

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References

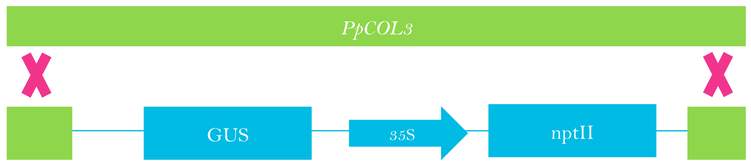
1-Lagercrantz, U. 2009.At the end of the day, a common molecular mechanism for photoperiod responses in plants? Journal of Experimental Biology 60: 2501-2515.
2- Hayama R., A Bhavna, E. Luley, and G Coupland. 2007. A Circaidan Rhythm Set by Dusk Determines the Expression of FT Homologs and the Short-Day Photoperiodic Flowering Response in Pharbitis. The Plant Cell 19: 2988-3000.
3-Ishikawa, R., S Tamaki, S Yokoi, T Shinomura, M Takano, K Shimamoto. 2005. Suppression of the Floral Activator of the Night Break Effect in Rice. The Plant Cell 17: 3326-3336.

CONSTANS-like Knockout Screens

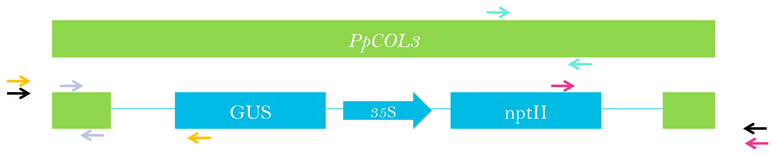
Determining evolutionary conservation between the genes controlling of reproduction in mosses and flowering plants may be found by knocking out the CONSTANS-like genes. The triple mutant plant will be exposed to inductive light conditions and MFT levels examined. Knockout plants exist for Δ1, Δ2, Δ3, Δ1Δ2, and Δ2Δ3, however, none show reproducibly different responses to inductive light conditions.

Methods

Linearized plasmid was transformed into PpCOLΔ1Δ2 protoplasts. The transformation construct includes PpCOL3 sequence at which homologous recombination can occur along with a reporter gene, promoter, and a gene for antibiotic resistance.

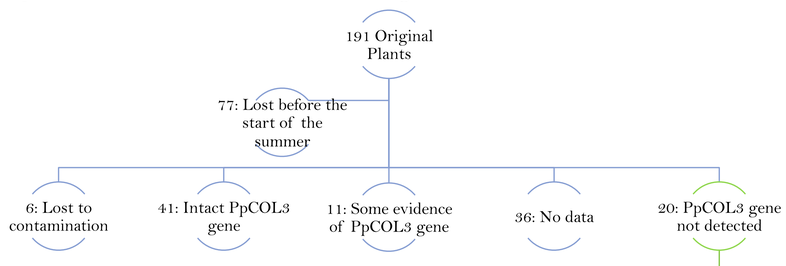


Transformed protoplasts were grown on selective media. Transformations were confirmed through three rounds of PCR analysis. The first round (teal arrows) confirmed interruption of the PpCOL3 gene. The second round (yellow and pink arrows) confirmed insertion at the correct genomic location. The third round (black arrows) confirmed insertion of the entire construct. DNA quality was confirmed through amplification within the 5' PpCOL3 genomic section of the construct (purple arrows).



Results

Top gel amplification is within the PpCOL3 gene, reactions were done in triplicate. Bottom gel is the positive DNA control, reactions were done in duplicate. Missing bands gene amplification has confirmed DNA in the bottom gel.



Of the 20 plants where *PpCOL3* was not detected, PCR confirmation of the correct insertion point is ongoing. Additionally, removal of antibiotic selection and homogenization of tissues to determine the nature of the insertion, if inserted at all is ongoing.

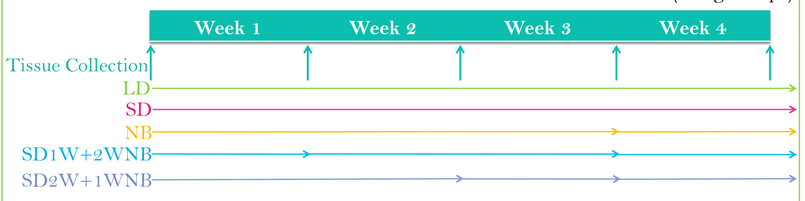
Concluding Statements and Future Work

Of the 191 total plants, 20 remain as putative triple mutants. The 36 plants with no data are undergoing screening and are an additional source of potential triple knockout plants. Future transformations are planned to increase the number of putative *PpCOL* triple knockout plants available. Additionally, transformations to create a Δ1Δ3 plant are also ongoing.

Night Break Study

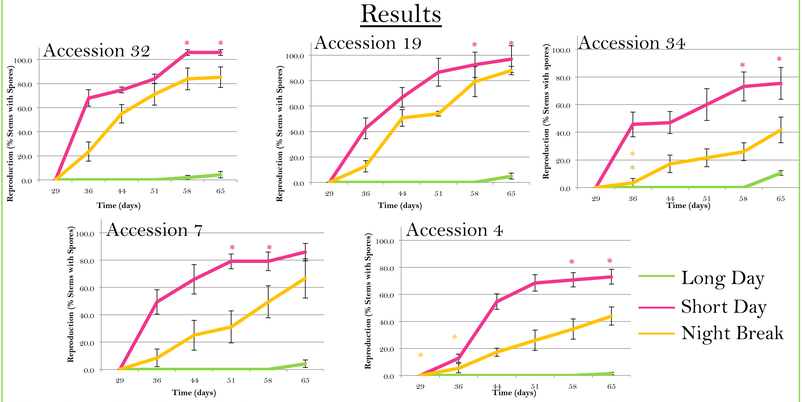
Many angiosperms will only flower under appropriate lighting conditions: short or long days. Studies have shown that interrupting a long night can halt reproduction[2,3]. To design a study in moss, night breaks of short day angiosperms were examined. Based upon past studies, plants were grown in short day conditions, and received one hour of light after the 8th hour of darkness. The moss plants used came from four different geographic locations. This study examines the physiological response to different lighting conditions, controlled potentially by the same genes.

(Google maps)

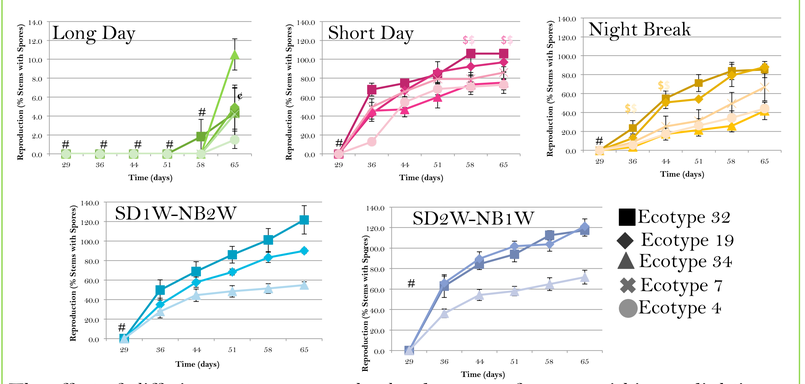


LD: long day, under conditions for the duration (16L:8D)
SD: short day, under conditions for the duration (8L:16D)
NB: Night Breaks for three weeks, into SD for the duration (8L:8D:1L:7D)
SD1W+2WNB: short day for one week, night break for 2, into SD for duration
SD2W+1WNB: short day for two weeks, night break for 2, into SD for duration

Results



The effect of differing lighting conditions upon the development of spores within one ecotype. Data was collected on a weekly basis. All data points significantly different from one another, unless noted otherwise. GLM $_{\text{date of collection, light condition, ecotype, interaction}}$ F=165.99, 741.39, 39.43,1.45; df=4,4,4,4; P= 0,0,0,0.042. Error bars=SEM. (* indicates means are the same, color indicates which means)



The effect of differing ecotypes upon the development of spores within one lighting conditions. All data points significantly different from one another unless noted. GLM $_{\text{date of collection, light condition, ecotype, interaction}}$ F=165.99, 741.39, 39.43,1.45; df=4,4,4,4; P= 0,0,0,0.042 Error bars=SEM. (# indicates all means are identical, $ indicates two means are the same, ¢ shows all means but 34 are the same)

Concluding Statements and Future Work

Differing both lighting conditions and geographic location had a significant effect upon the development of reproductive structures. Exposing moss to a night break had an universal delaying effect upon the appearance of reproductive structures. MFT RNA expression analysis from tissue collections is ongoing. Another study is underway, examining the effects of a night break upon the PpCOL knockout plants, tissue will also be collected for MFT expression analysis.