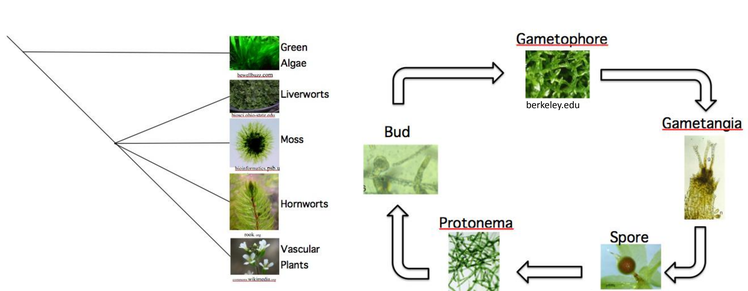
A functional comparison of photoperiodically-regulated genes in *Physcomitrella patens* and flowering plants

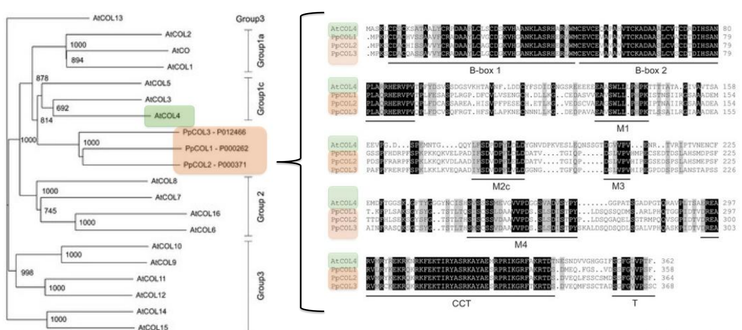
Sarah B. Cook, Karen A. Hicks, Biology Department, Kenyon College, Gambier, OH

Abstract

The timing of sexual reproduction in plants is highly dependent on various environmental conditions, such as light, temperature, and day length. In flowering plants, sexual reproduction is regulated by day length via a pathway involving many genes, including *CONSTANS.* Interestingly, in the moss *Physcomitrella patens*, which diverged from flowering plants over 450 million years ago, reproduction is also regulated by day length, and similar genes to the those in flowering plants have been recently determined. The question that we pose is whether the genes involved in this pathway in *Physcomitrella* have the same function as those in flowering plants. In this study, we focused on three photoperiodically-regulated genes in *Physcomitrella* called the *CONSTANS-like* genes (*PpCOL1, PpCOL2,* and *PpCOL3*) related to *CONSTANS* in flowering plants. Single and double *PpCOL* mutants were grown under SD and LD conditions to determine their requirement in reproductive induction, but data has not yet been collected. These mutants were also used to assess tissue-specific expression of the *PpCOL* genes in GUS assay experiments, but no discernable difference based on day length condition has yet been recognized. Lastly, crosses of different mutant strains were used to create a putative *PpCOL* triple mutant, but the mutations have yet to be confirmed by PCR. Further analysis of these genes is ongoing to determine their involvement in sexual reproduction in *Physcomitrella.*



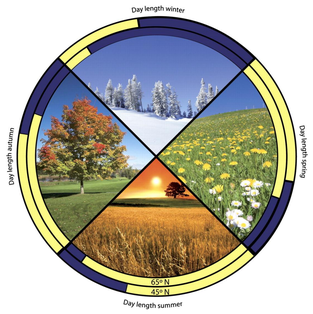
Mosses and flowering plants diverged phylogenetically over 450 million years ago. The moss life cycle consists of a haploid gametophyte which produces gametes and a diploid sporophyte that produces diploid sprores.



Zobell *et al.* 2005

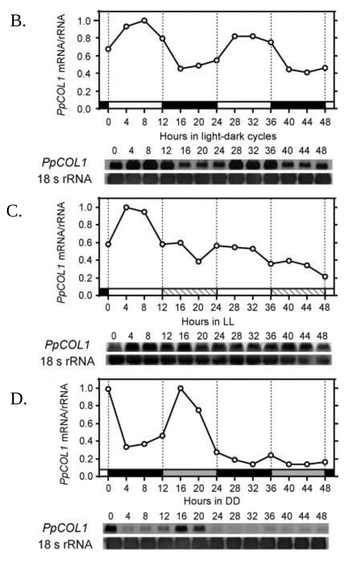
Three genes (*PpCOL1, PpCOL2, PpCOL3*) closely related to the *CO-like* genes in the angiosperm *Arabidopsis thaliana* were found in the moss *Physcomitrella* (Zobell *et al*. 2005). A comparison of protein sequence shows many conserved domains (underlined), conserved amino acids (black), and similar amino acids (grey) between *AtCOL4, PpCOL1, PpCOL2,* and *PpCOL3.*

A.



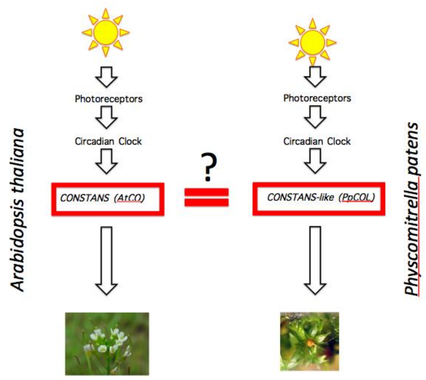
Lagercrantz 2009

Sexual reproduction can be timed to correlate with changing seasons based on day length (A). Sexual reproduction in *Physcomitrella* is strictly regulated by photoperiod, where reproduction occurs preferentially under short-day conditions (Hohe et al. 2002). mRNA levels of *PpCOL1* were found to coincide with light-dark cycles (B), while this pattern was disrupted in constant light (C) or constant dark (D) conditions (Shimizu *et. al.* 2004).

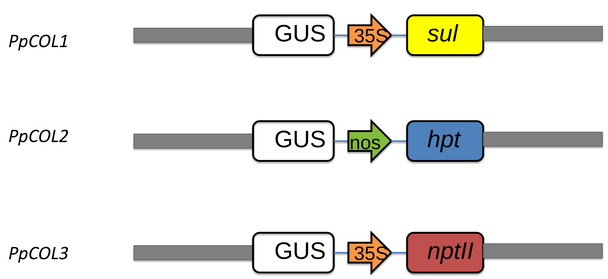


Shimizu *et. al.* 2004

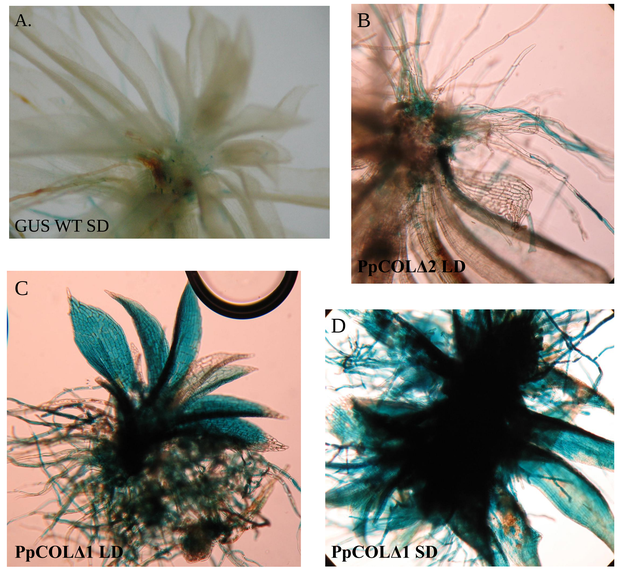
Are these photoperiodically-regulated genes evolutionarily conserved?



Sexual reproduction is regulated by similar pathways in *Physcomitrella* and flowering plants like *Arabidopsis*. The pathway consists of photoreceptors that receive light and activate both the circadian clock and *CONSTANS* (*CO*) or *CONSTANS-like* (*COL)* genes in *Arabidopsis and Physcomitrella*, respectively. *CO* and *COL* genes trigger the expression of other genes downstream in the pathway which induce reproduction.



Three transformants were prepared by inserting the above replacement vectors in place of the three genes *PpCOL1, PpCOL2,* and *PpCOL3*. Thus, activation of the *PpCOL1, PpCOL2,* or *PpCOL3* promoter turns on the GUS reporter gene and selectable marker. These transformants were prepared by Oliver Zobell and were used to assess gene function.



GUS staining was used to investigate *PpCOL* transcription levels in long-day versus short-day conditions in many mutant strains. Staining indicates *PpCOL* expression. Staining was typically strongest in axils (A) and filaments (B). In moss grown under long day (LD) (C) or short day (SD) (D) conditions, differences in *PpCOL* expression could not be discerned.

Concluding Statements

Experiments to investigate *PpCOL1, PpCOL2,* and *PpCOL3* expression are ongoing. Results from a current experiment will assess sporophyte induction in long-day versus short-day conditions to confirm that reproduction is photoperiodically-regulated. Lastly, a putative triple mutant via a cross of a double and single mutant will further help to discern gene function as compared to that in flowering plants.

Acknowledgements

I would like to thank Karen Hicks for her support and ongoing assistance throughout this research project. I also thank Kenyon College for giving me the opportunity to spend the summer pursuing this research.

References

Lagercrantz, U. 2009. At the end of the day: a common molecular mechanism for photoperiod responses in plants? *Journal of Experimental Botany* 60: 2501-2515.

Shimizu, M., Ichikawa, K., and Aoki, S. 2004. Photoperiod-regulated expression of the *PpCOL1* gene encoding a homolog of CO/COL proteins in the moss *Physcomitrella patens. BBRC* 324: 1296-1301.

Zobell, O., Coupland, G. and Reiss, B. 2005. The family of *CONSTANS-like* genes in *Physcomitrella patens. Plant Biol. (Stuttg.)* 7:266-75.