# Molecular and Evolutionary Assessment of Seasonal Reproduction and Circadian Regulation Using the Bryophyte *Physcomitrella patens*

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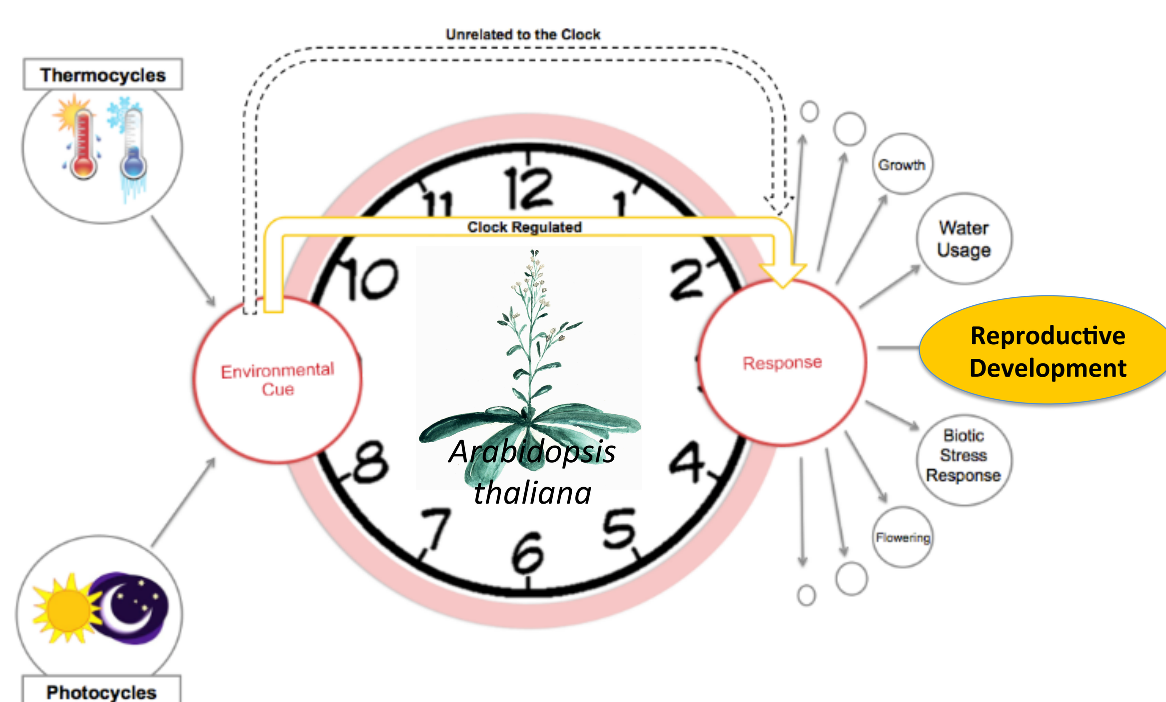
## Abstract

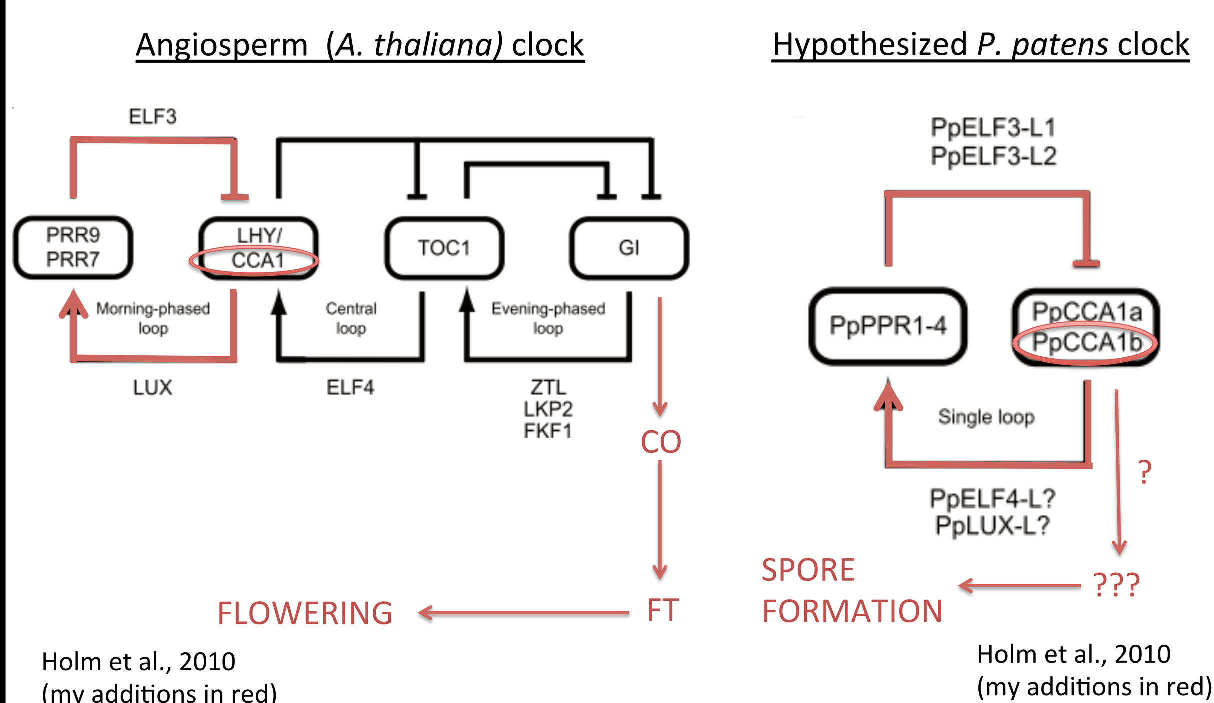
The timing of plant reproductive development in angiosperms (flowering plants) is optimized by circadian anticipation, detection, and consequent response to external seasonal cues. These external cues, such as daily light and temperature cycling, are integrated by an endogenous circadian clock. Under the direction of this internal clock that is harmonized with its environment, plants are able to decisively begin reproductive development at the appropriate time of year, thereby outcompeting those individuals that are not primed for this critical life event. I am particularly interested in how seasonal changes in light and temperature regulate reproductive development in plants and whether this mechanism is unique to angiosperms or if it has been conserved in the plant lineage. Here, I use the moss *Physcomitrella patens*, a bryophyte, to gain an ancestral standpoint on this mechanism. I have begun characterization of the expression of *PpCCA1b* (*circadian clock-associated 1b*), a candidate gene for reproductive development in this moss. I hypothesize that seasonal regulation of reproduction is a conserved pathway among land plants and that my compiled expression profile for this gene and other candidates will indicate participation in the formation of reproductive structures. By comparing the expression pattern of *PpCCA1b* and other *P. patens* candidate genes with their corresponding homologs in angiosperms, I hope to ultimately make hypotheses about the evolution of this essential plant system.


## Background

- Reproductive development is regulated by the circadian clock in angiosperms (Andrés and Coupland, 2012)




- I hypothesize that seasonal regulation of reproductive development is a conserved pathway in bryophytes and angiosperms.




## Goal

Characterize expression of candidate genes in different accessions of *P. patens* cultured under inductive conditions (eight-hour days, 15°C) to identify differential expression patterns.

## Candidate Gene Characterization

**Cultivation:** *P. patens* accessions from western Europe were grown to maturity under long days at 22°C (LD22) on BCD media. Upon transfer to inductive conditions (8-hour days, 15°C (SD15)), tissue samples were collected according to a multi-day or 24-hour time regime.

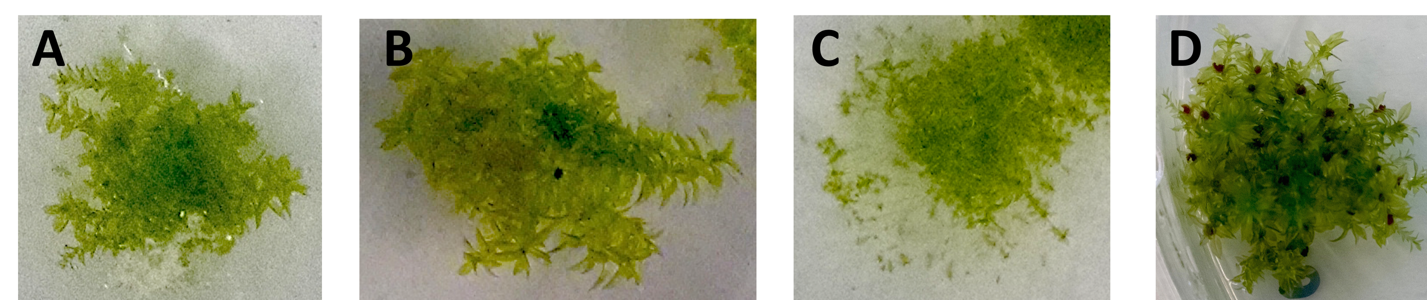



**Figure 1** Selected accessions of *P. patens* four months after germination under LD22. **A, B** are photosensitive accessions Pp1 (Gransden reference strain) and Pp13, respectively. **C** Late-photoinsensitive accession Pp3. **D** Early-photoinsensitive accession Pp20 with mature spore capsules.

**Selection of Candidate Genes:** Candidates were identified by RNA-sequencing using padj < 0.1 (Wald test) with and without a criteria of 2-fold change in transcript levels under SD15 (unpublished data, K. Hicks).

**24-Hour Characterization:** Collection took place five days after transfer to inductive conditions (SD15), at which point reproductive structures should begin forming (Landberg et al., 2013). Tissue was collected every two hours for 24 hours in order to create a high-resolution profile of diurnal expression. Identification of peak expression time helps to plan multi-day collections.

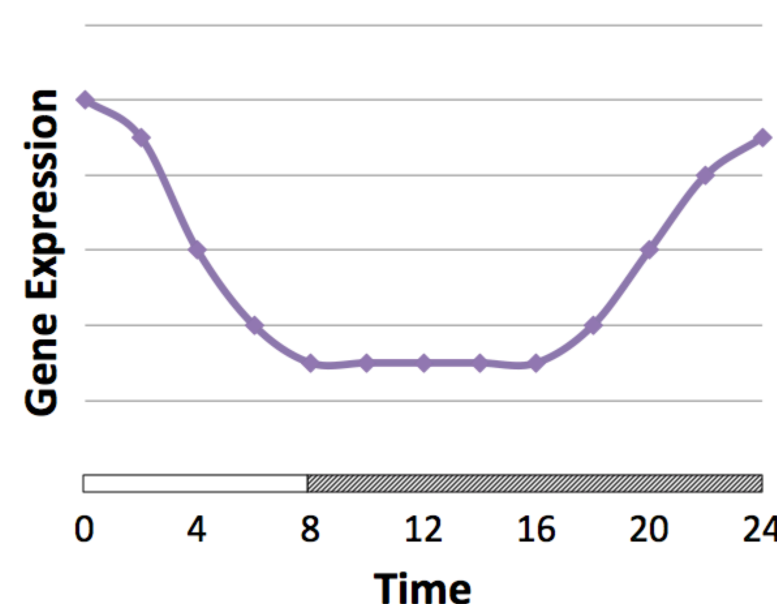



**Figure 2** Putative candidate gene expression profile compiled from 24-hour collection. White and grey-shaded boxes on x-axis indicate the subjective day and night.

**Multi-Day Characterization:** Tissue was collected at the same time each day over a 10-day period after transfer to SD15. The selected collection time reflects the time of day when circadian expression is at a maximum.

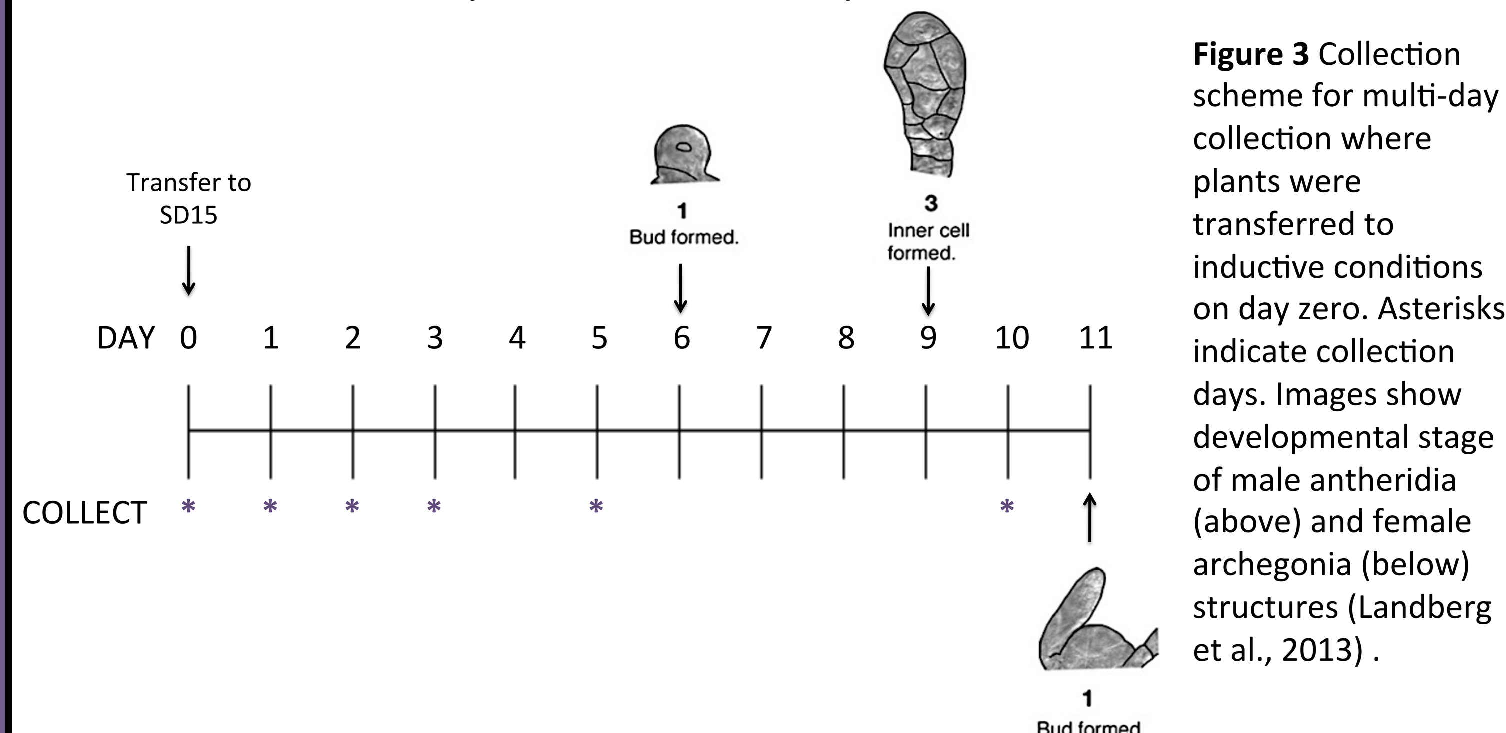



**Figure 3** Collection scheme for multi-day collection where plants were transferred to inductive conditions on day zero. Asterisks indicate collection days. Images show developmental stage of male antheridia (above) and female archegonia (below) structures (Landberg et al., 2013).

**Expression Quantification:** Total RNA was extracted from tissue samples with TRIzol reagent. DNase-treated RNA was reverse transcribed to cDNA using the High-Capacity RT kit. Candidate gene (here, *CCA1b*) and *ACTIN2* (endogenous control) mRNA level were measured by qRT-PCR with SYBR Green reagent.

## qRT-PCR Optimization

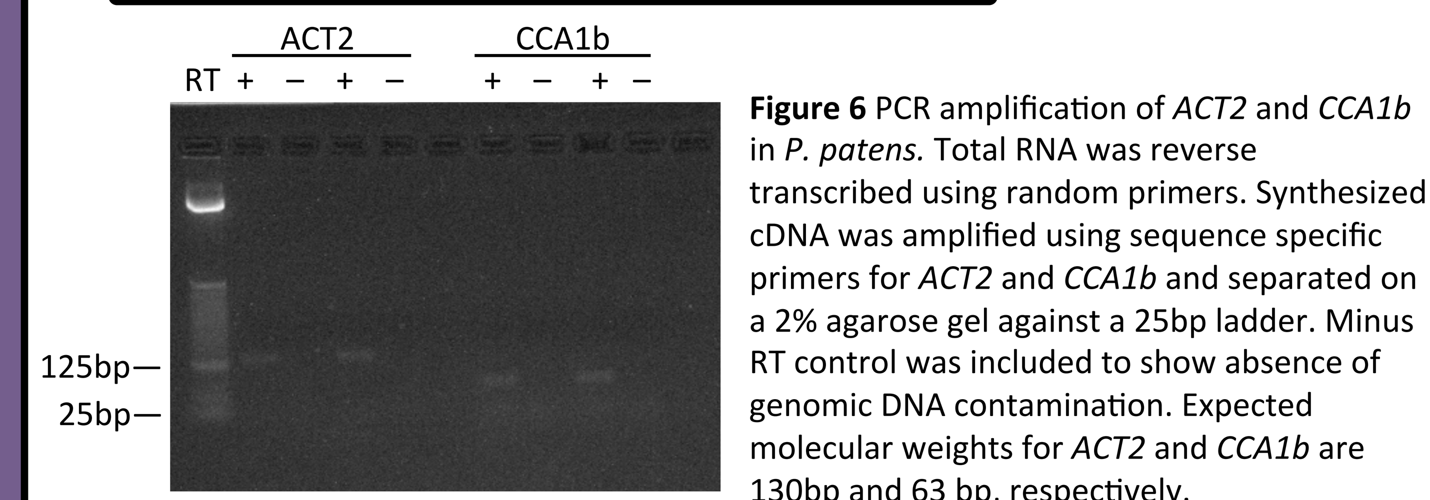



**Figure 6** PCR amplification of *ACT2* and *CCA1b* in *P. patens*. Total RNA was reverse transcribed using random primers. Synthesized cDNA was amplified using sequence specific primers for *ACT2* and *CCA1b* and separated on a 2% agarose gel against a 25bp ladder. Minus RT control was included to show absence of genomic DNA contamination. Expected molecular weights for *ACT2* and *CCA1b* are 130bp and 63 bp, respectively.

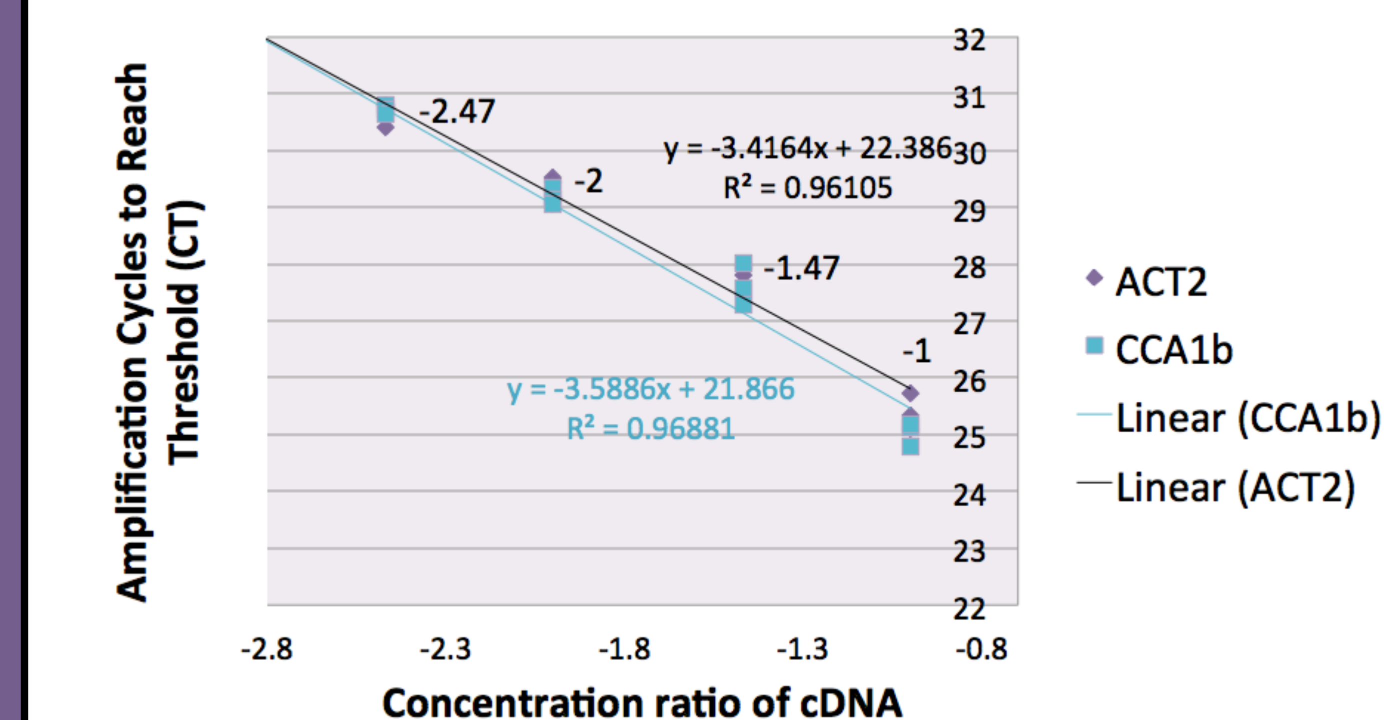



**Figure 5** Optimization of cDNA concentration for quantification of *ACT2* and *CCA1b* by qRTPCR. Cycle threshold (CT) is plotted against cDNA dilution. Dilutions tested were 1:10, 1:30, 1:100, 1:300, and 1:1000. Equation for line of best fit and $R^2$ value are included.

## Future Work

- Ongoing optimization of qRT-PCR procedure will enable accurate quantification of mRNA for the creation of expression profiles.
- Tissue from 24-hour and multi-day assays will be used for the characterization of multiple candidate genes.
  - Next up: cycling DOF factors
- Vary inductive conditions to better qualify which environmental cues dictate the differential expression of which genes in each accession.
- Make hypotheses about the evolution of seasonal regulation of reproductive development.

## References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