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Characterizing Genetic Mechanisms Involved in Measuring Day-length in *Drosophila melanogaster*

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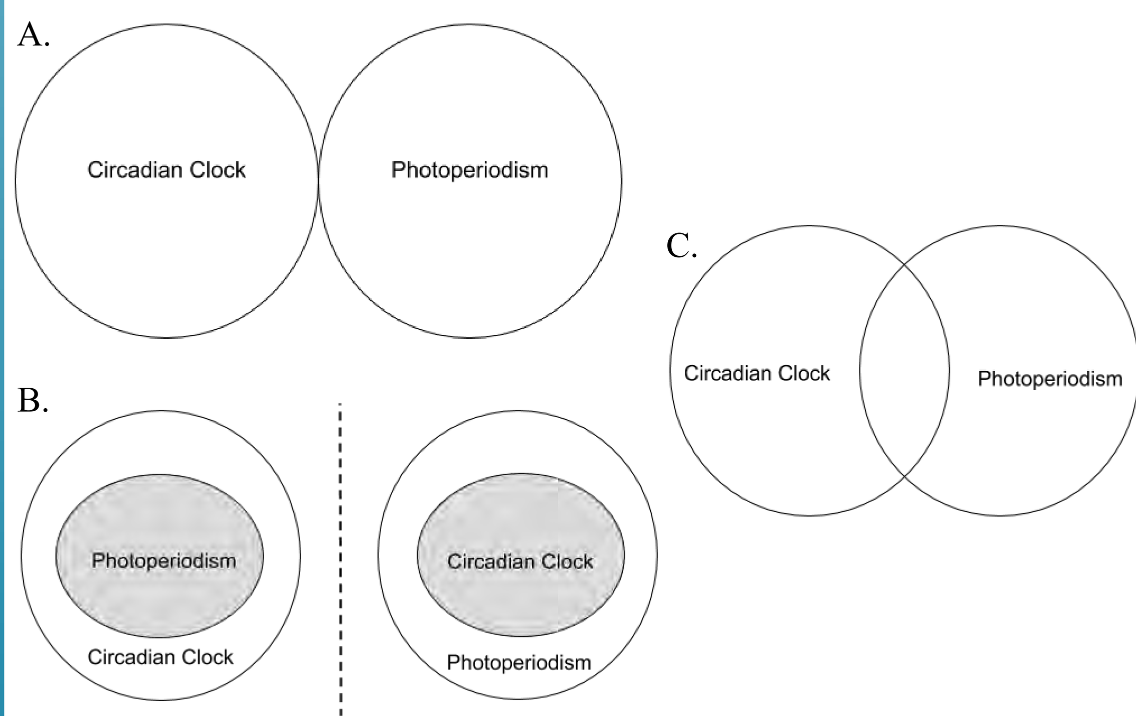
Abstract

Many organisms are known to respond to seasonal day-length changes, this phenomenon is called photoperiodism. Photoperiodism is known to regulate seasonal behavior and physiological processes in many organisms. Since the seminal work by Bünning in 1936, there are many reports supporting the view that an organism can measure the day length through an endogenous 24-hour cellular clock, called the circadian clock (1). However, little is known about the genetic and molecular mechanisms about how the circadian clock and photoperiodism interact to interrupt day-length changes. To understand the underlying mechanisms of photoperiodism, we decide to identify the genetic components of photoperiodism in the model organism *Drosophila melanogaster*. For this purpose, we performed a Genome-Wide Association Study (GWAS) on photoperiodism using the *Drosophila* Genetic Reference Panel (DGRP) which consists of fully sequenced inbred lines created from a natural population (2). For the photoperiodic response trait, we measured the difference of each genotype's Chill Coma Recovery (CCR) time which is an established trait that reflects photoperiodic specific effect (3). The GWAS analysis suggested four candidate genes. Three knockout strains of the four candidate genes showed a significant alteration in their photoperiodic responses in comparison to that of the wild type. We also hypothesized that circadian clock genes might play a role in photoperiodism directly or indirectly. The knockout strains of the known circadian clock genes showed a significant alternation in photoperiodic responses. Thus, we have identified several photoperiodic genes that might provide us a novel insight in the genetic mechanisms of photoperiodism.

Research Objective(s)

1) We aim to better understand the genetic mechanisms of photoperiodism using the *Drosophila* Genetic Reference Panel.
   - We hypothesis that by preforming a Genome wide association study we will identify genes involved in photoperiodic regulations.
2) We also aim to understand how the circadian clock and photoperiodism interact with each other to interpret day-length changes.

Possible interactions between the Circadian clock and Photoperiodism

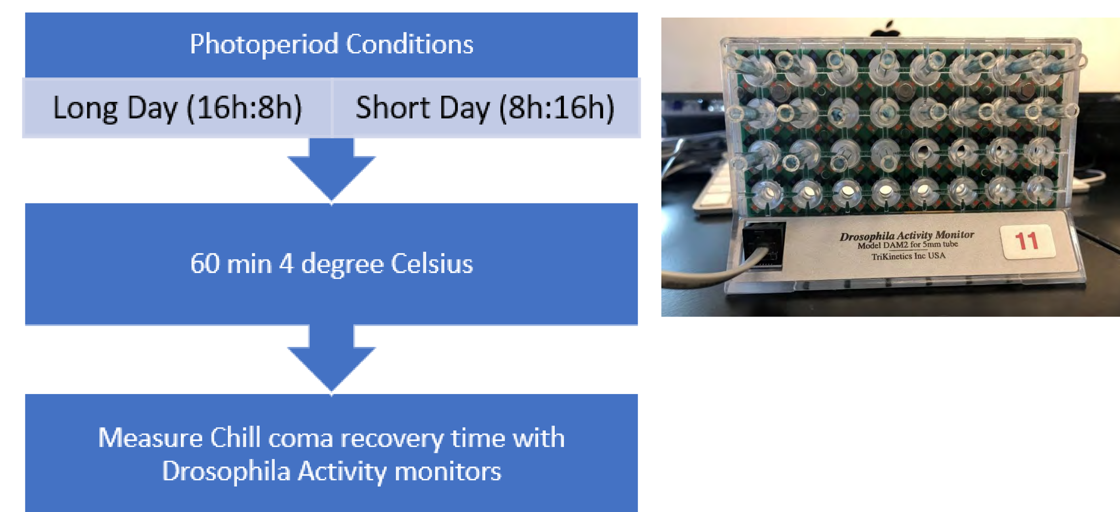


Chill Coma Recovery Assay and *Drosophila* Genetic Reference Panel

Chill Coma Recovery (CCR) assay was used to assess the photoperiod phenotype in the *D.melanogaster.* Chill coma recovery (CCR) is the time it takes an organism to recover from a paralysis like state after being kept under extremely low temperatures. It has been clearly demonstrated that flies raised under short day (SD) photoperiod conditions compared to long day (LD) photoperiod conditions have an advanced (or shorter) recovery time (3).

Drosophila Genetic Reference Panel (DGRP) is a community resource of 205 inbred lines from 40 wild-type parents. DGRP can be used for the analysis of population genomics and for whole-genome association mapping of quantitative trait loci (2). The DGRP population is useful for performing the GWAS where we can link the desired phenotype to the genotype.

Experimental Design



Results

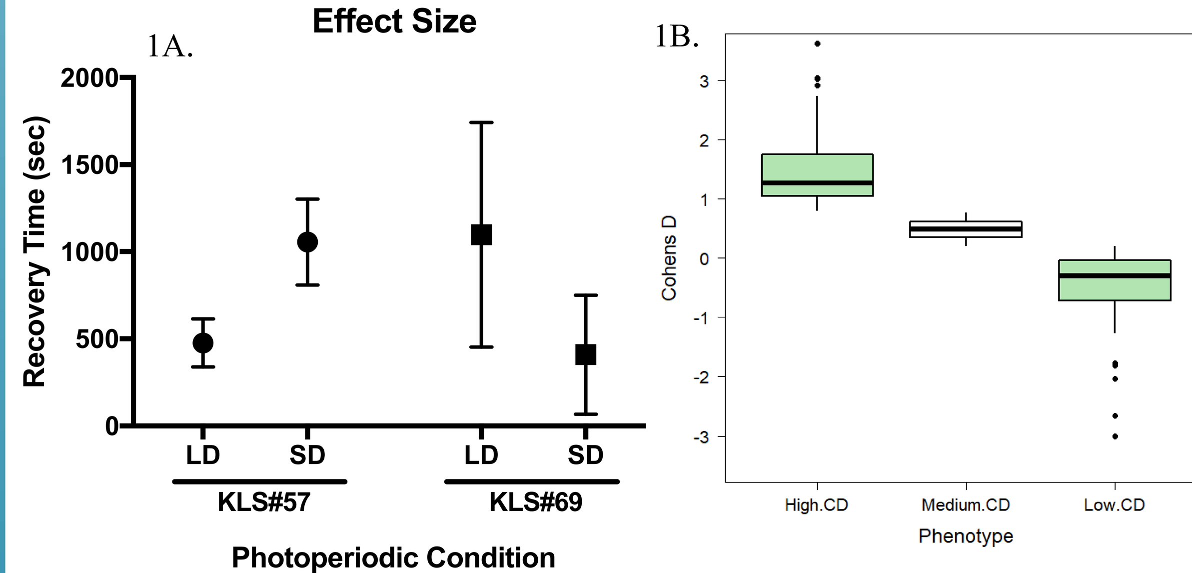


Figure 1. A. Averages of the two genotypes KLS#57 and KLS#69. For KLS#57 in reference to LD, the SD photoperiod has a delay in recovery time. While for KLS#69 in reference to LD, the SD photoperiod has an earlier recovery time. B. The Cohen's D values of all genotypes which shows the differential photoperiod response of each genotype. Values separated by effect size, high (>0.8), medium (0.5) and small (<0.2) in response to LD and SD photoperiod.

Results

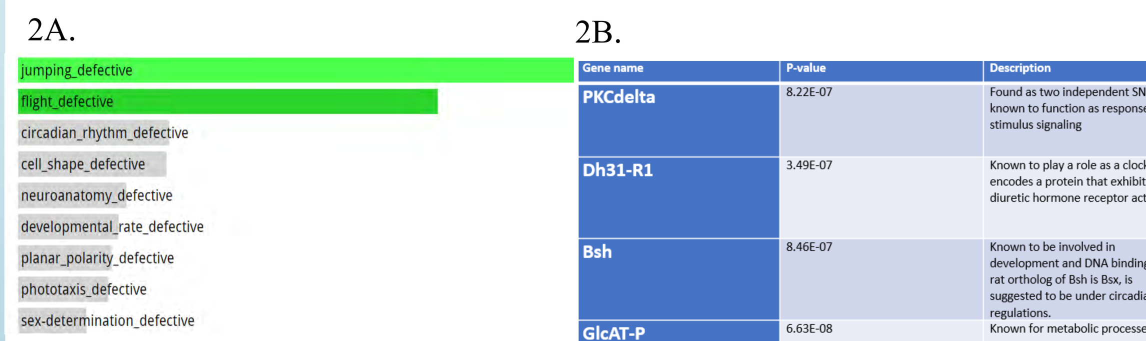
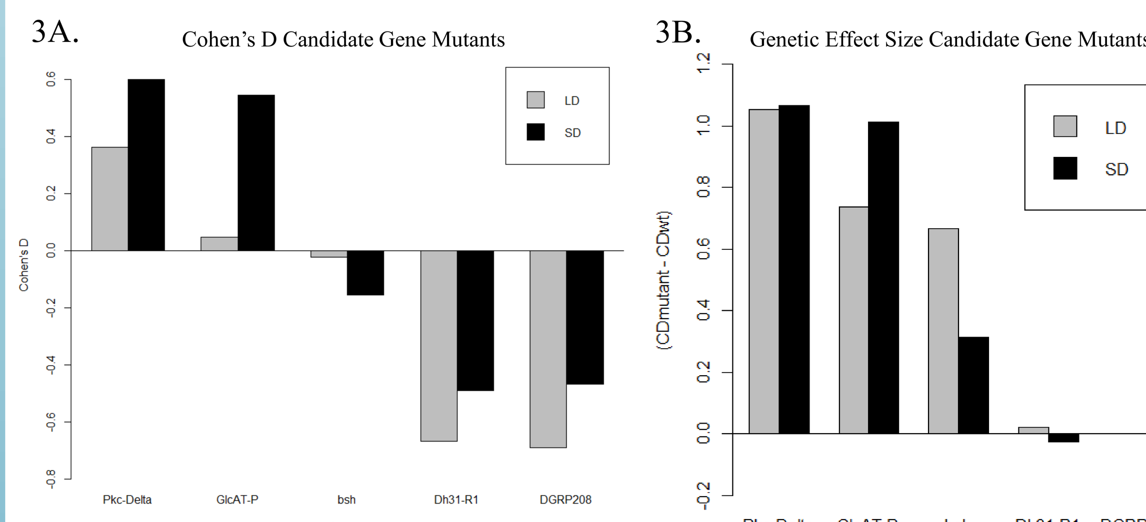


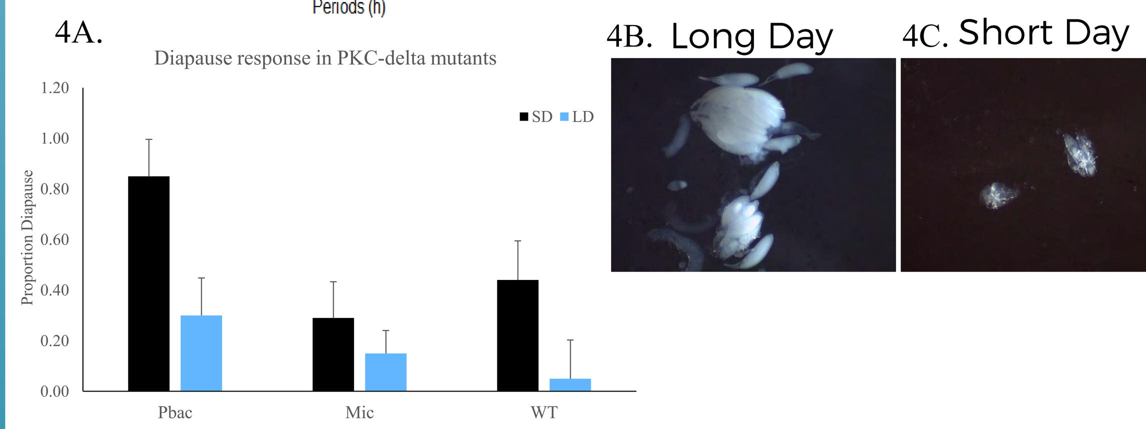
Figure 2. A. Gene enrichment analysis of top 40 hits of GWAS analysis using ModEnrichr. B. Candidate genes from tops 40 hits of GWAS analysis chosen by significant P values.



3C. Period of Candidate gene mutants

DGRP_208, Bsh, GlcAT-P, Dh31-R1, PKCdelta — Periods (h)

Figure 3. A. Cohen's D values of four candidate gene knockout mutants compared to the WT DGRP208 strain, after CCR assay in LD and SD photoperiod conditions. B. Genetic effect size (CDmutant – CDwt) of the candidate genes compared to WT DGRP208. PKCdelta, GlcAT-P and Bsh, show significantly different photoperiod response compare to WT DGRP208. C. Period of the candidate genes and DGRP208 WT. GlcAT-P has a significantly longer period compared to WT (T-test: p<0.05 (p=1.16E-13)).



4B. Long Day 4C. Short Day

Figure 4. A. The diapause response of candidate gene PKCdelta mutants compared to WT in Long day vs Short Day photoperiod. There is a significant difference in Pbac mutant diapause response, compared to WT in both photoperiods (Z-test: LD&SD P values: 0.0014, 0.010). MIC diapause response compared to WT in both photoperiod is not significantly different (Z-test: LD&SD P values: 0.27, 0.30). However, there is a significant difference in Pbac LD and SD diapause response (Z-test P value: 0.002) and in WT(Z-test P value: 0.007), but not in MIC (Z-test P value: 0.29). B. Picture of fly ovaries in LD. C. Picture of fly ovaries in SD undergoing diapause.

Results

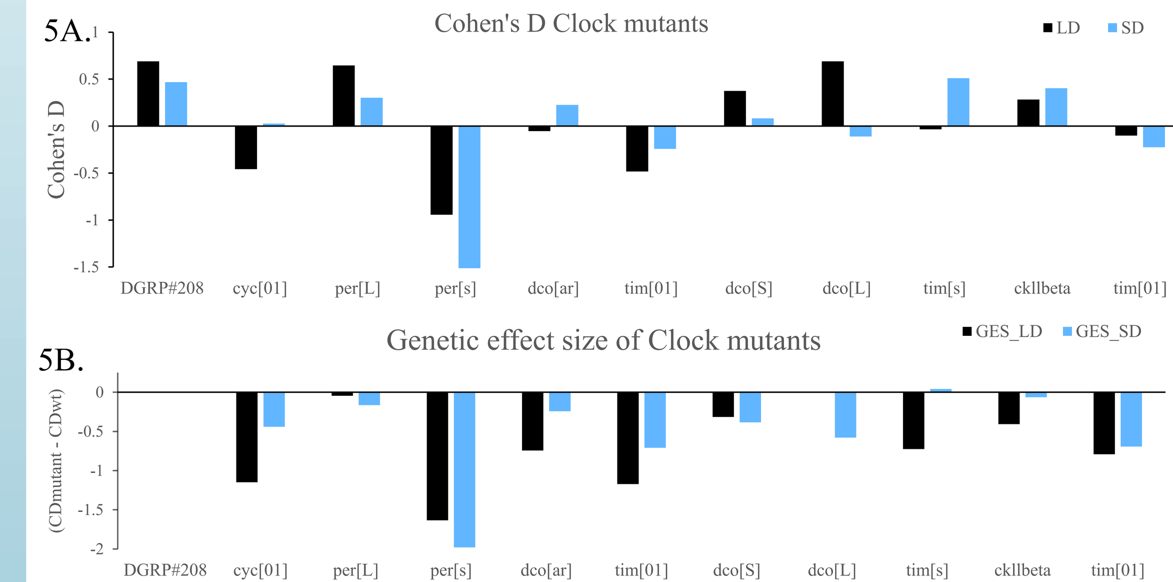


Figure 5. A. The Cohen's D values of circadian clock mutants after preforming the CCR assay. B. The Genetic effect size (CDmutant-CDwt) values of the clock mutants, along with the DGRP208 WT. Per[s] shows significantly differential photoperiod response compared to the WT.

Summary

- There is a variation in the CCR time of DGRP.
- GWAS show that certain genes are enriched, 4 candidate are chosen from top 40 hits of GWAS.
- PKCdelta candidate gene show significantly differential photoperiod response compared to WT.
- One of the photoperiod candidate gene has altered period, GlcAT-P.
- Diapause assay in the PKCdelta mutants and the WT strains confirm the CCR results.
- Pbac, PKCdelta mutant show significantly different photoperiod response from the WT.
- Clock gene mutants show photoperiod specific response.

Conclusion & Future Directions

By preforming the GWAS analysis were able to determine the photoperiod candidate genes. Our results also show that one of these candidate gene(GlcAT-P) might play a role in the circadian clock regulations and the clock gene mutants also show photoperiod specific responses. Indicating the hypothesis that there are shared regulations between the clock and photoperiodism. Moving forward we plan to further study PKC-delta as the top candidate photoperiod gene. We want to look at the RNA expression level of PKCdelta in DGRP strains that show extreme photoperiodic responses. We also plan to created Advance Intercross Population of DGRP strain with extreme photoperiod response to independently confirms the results of GWAS by preforming a QTL analysis.

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References

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