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# **Phenotypic Evolution of Circadian Rhythms in *Arabidopsis thaliana***



**Stephanie Wakefield**

**Advisors:**

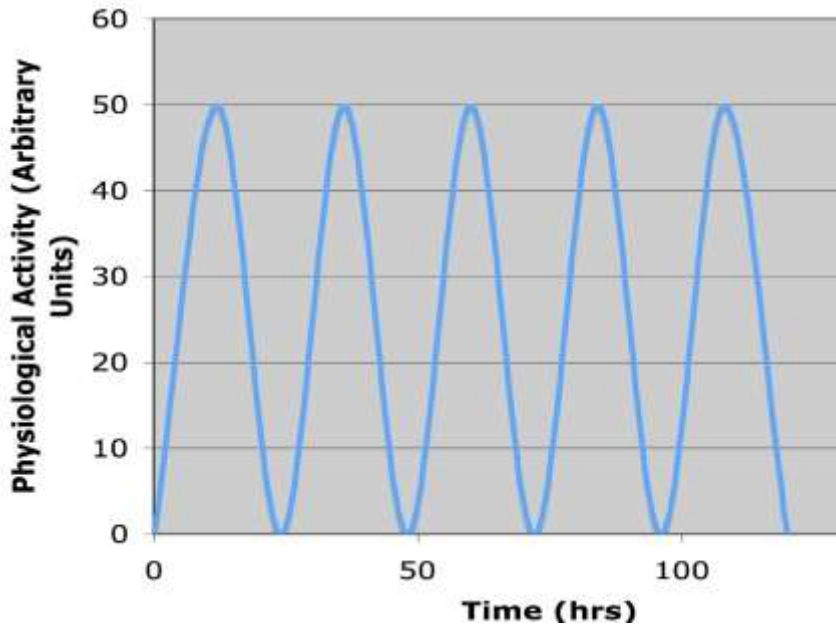
**Cynthia Weinig and Matthew Rubin**

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# Circadian Rhythms

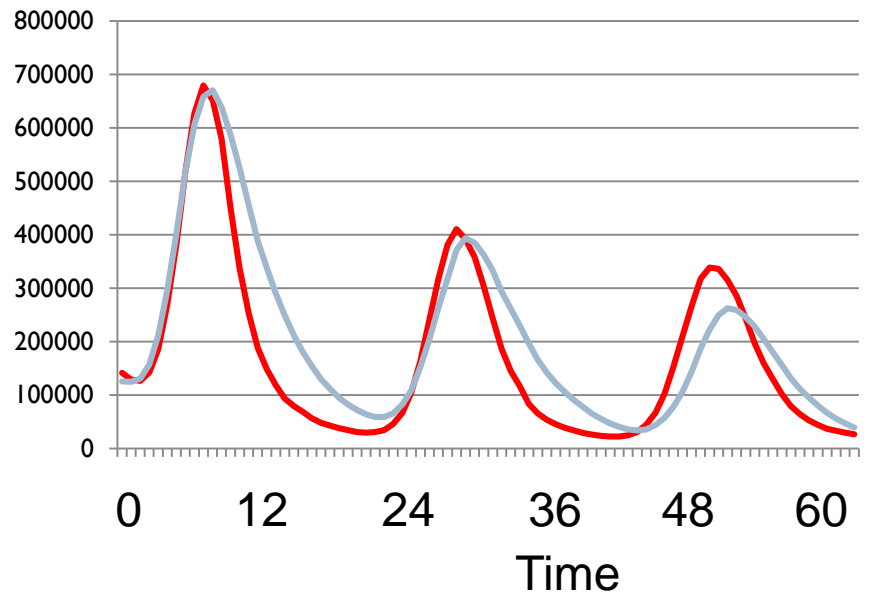
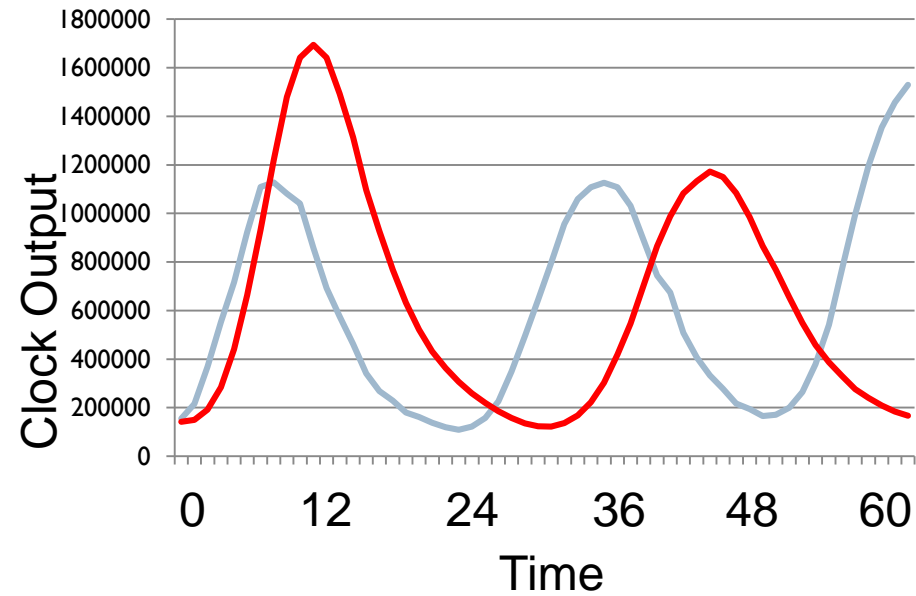
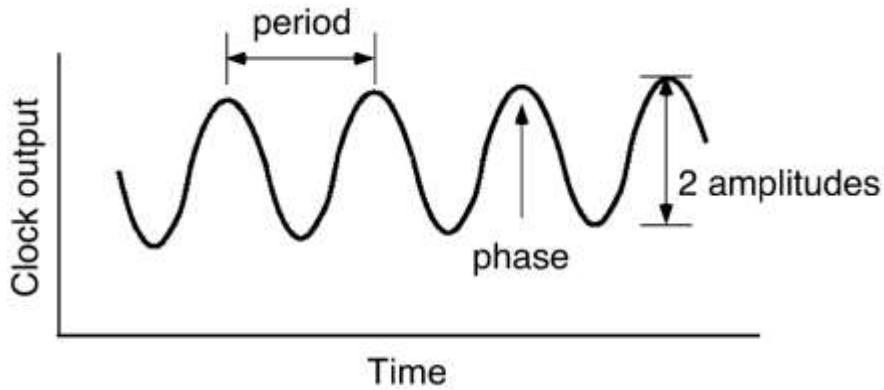
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- The Earth rotates on its axis once every 24 hours
  - ▶ Providing predictable cycles of light and temperature
- Most organisms exhibit circadian rhythms
  - ▶ Circadian rhythms are endogenous repeating rhythms
  - ▶ Circadian rhythms are “set” by external cues
- Many physiological and behavioral characters are controlled by circadian rhythms
  - ▶ Best example in plants is photosynthesis



# Variation in Circadian Rhythms



# Molecular Basis of Circadian Rhythms

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- ▶ Consists of several negative and positive feedback loops
- ▶ 3 main components:
  - ▶ Input
  - ▶ Oscillator
  - ▶ Output
- ▶ Clock characterized by knock-out mutants (i.e. functional vs non-functional alleles at clock genes)
  - ▶ Little known about natural allelic variation



# Research Questions

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1. Does the expression of circadian phenotypes depend on germination conditions (i.e. crowding)?
2. How have circadian phenotypes evolved after five years of selection in the field?
3. Do circadian phenotypes depend on substrate type?



# *Arabidopsis thaliana*: The Real-Live Plant

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- ▶ Member of the mustard family
  - ▶ Related to cabbage, cauliflower, kohlrabi, broccoli, and kale

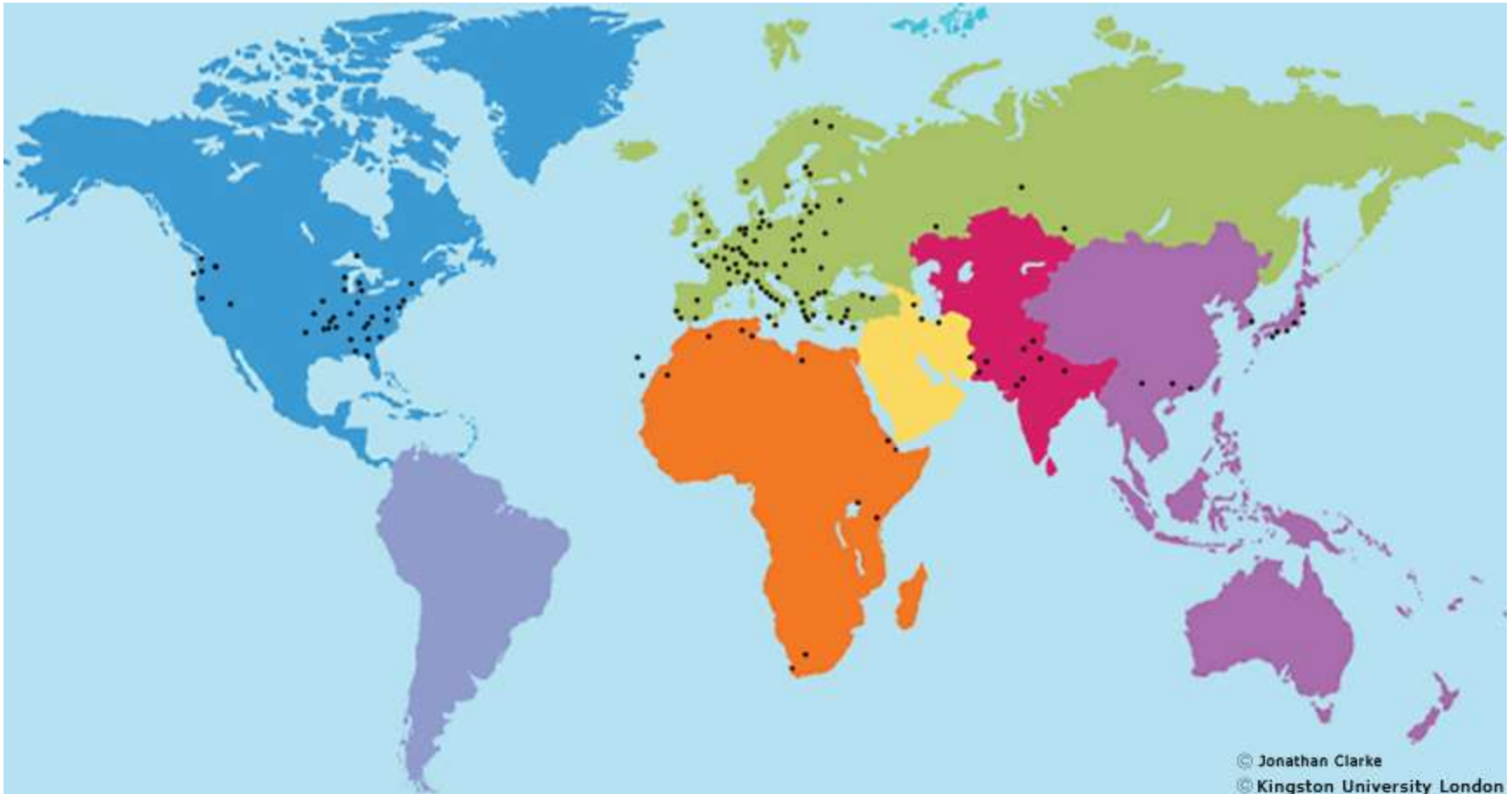
- **Useful for environmental studies:**

- Primarily self-fertilizing
- Naturally highly inbred families
  - Grow replicates of each genotype in each environment.
- Short-lived annual
- Fitness estimated from fruit number

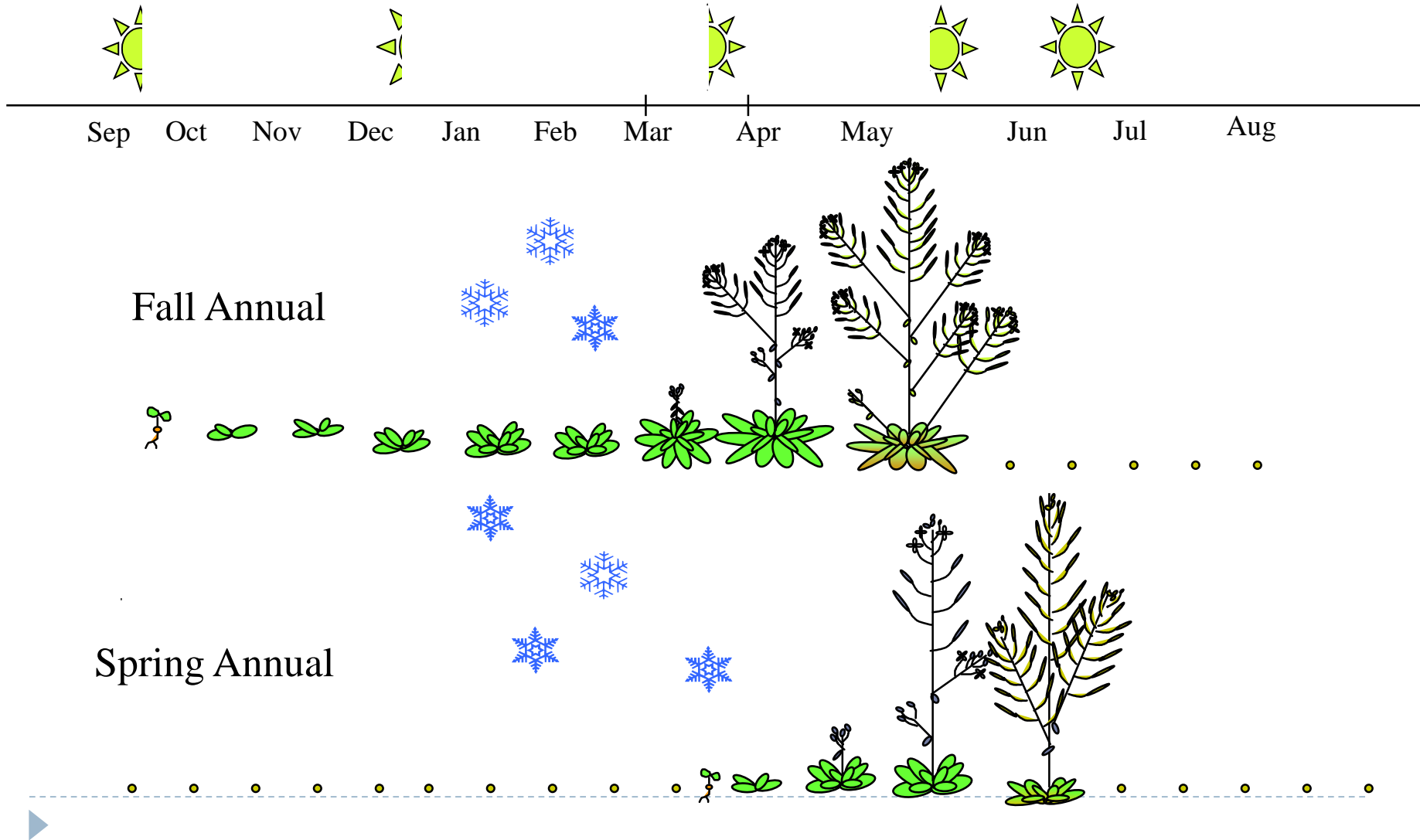


# Distribution of *Arabidopsis thaliana*

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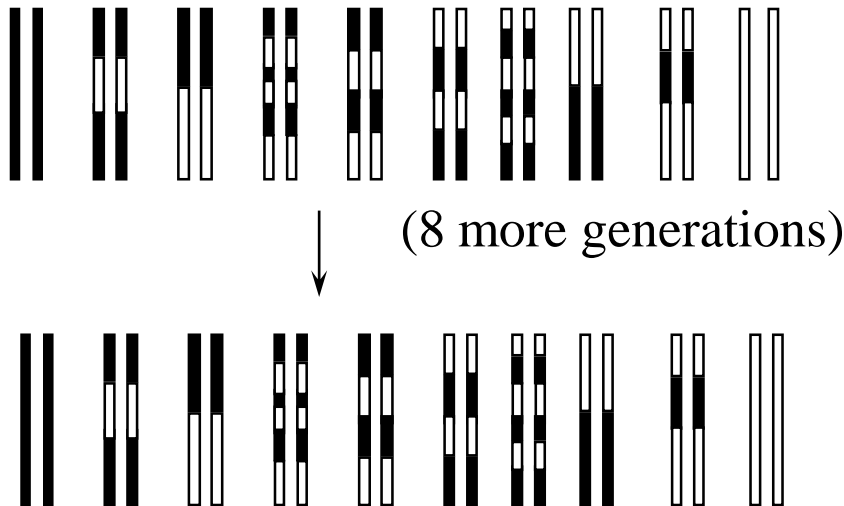
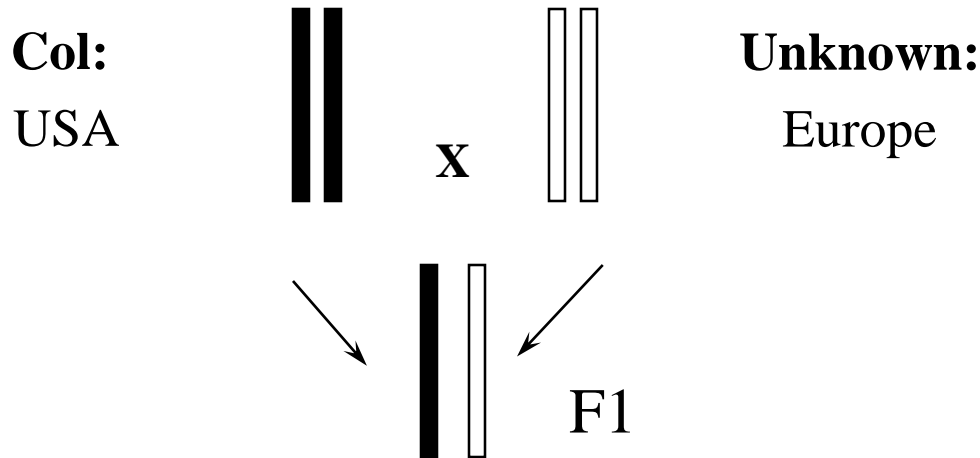


# *Arabidopsis thaliana* Life Cycles





# Recombinant Inbred Lines



- Each RIL is a novel combination of parental alleles



# Growth Chamber Project

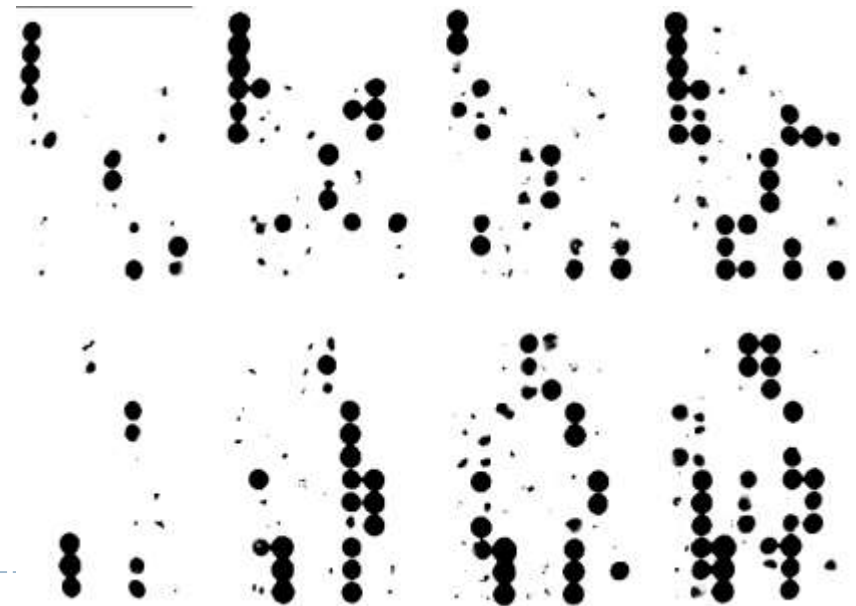
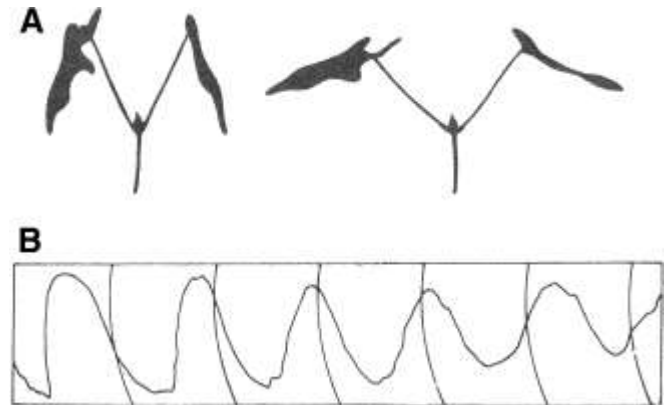
Recombinant Inbred Lines (RILs) of  
*Arabidopsis thaliana*

- 42 lab lines and 52 field-evolved lines
- 16 replicates of 94 RILs per treatment (n=3008)
- Cold-treated seeds for 4 days
- Germinated in growth chamber
- Entrained under artificial spring crowded (PAR= 221.1  $\mu\text{mol/s}^*\text{m}^2$ , R:FR = 0.52) & un-crowded (R:FR = 1.02)



# Measuring Circadian Phenotypes

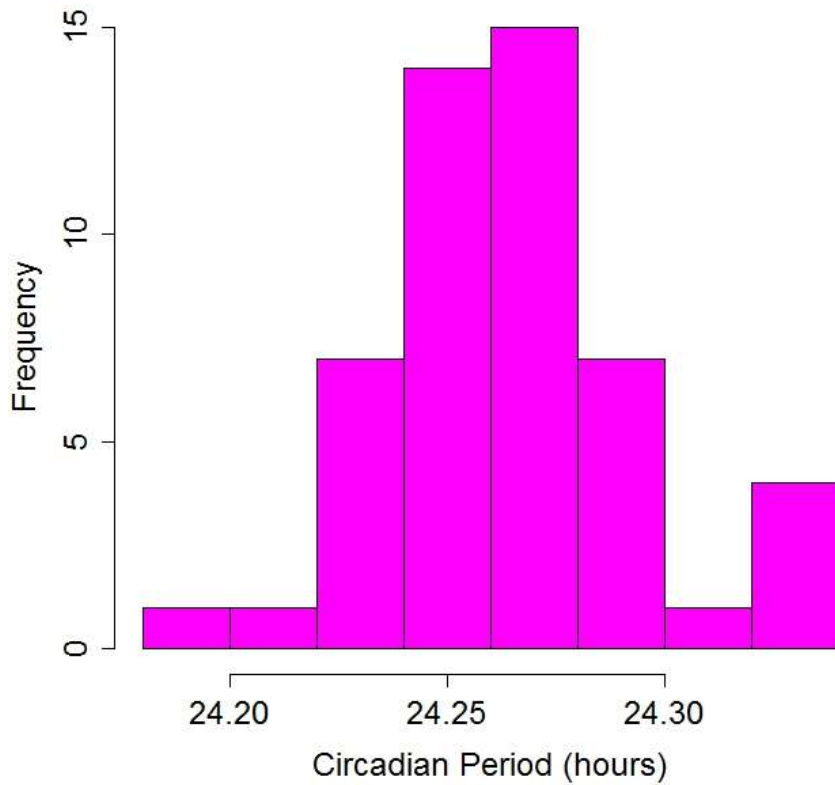
- ▶ RILs contain a reporter construct
  - ▶ Gene *LUCIFERASE* (bioluminescence production in fireflies) fused to *CCR2* (output to the clock)
  - ▶ Image plant for 30 minutes every hour for 100 hours
  - ▶ Determine circadian phenotypes



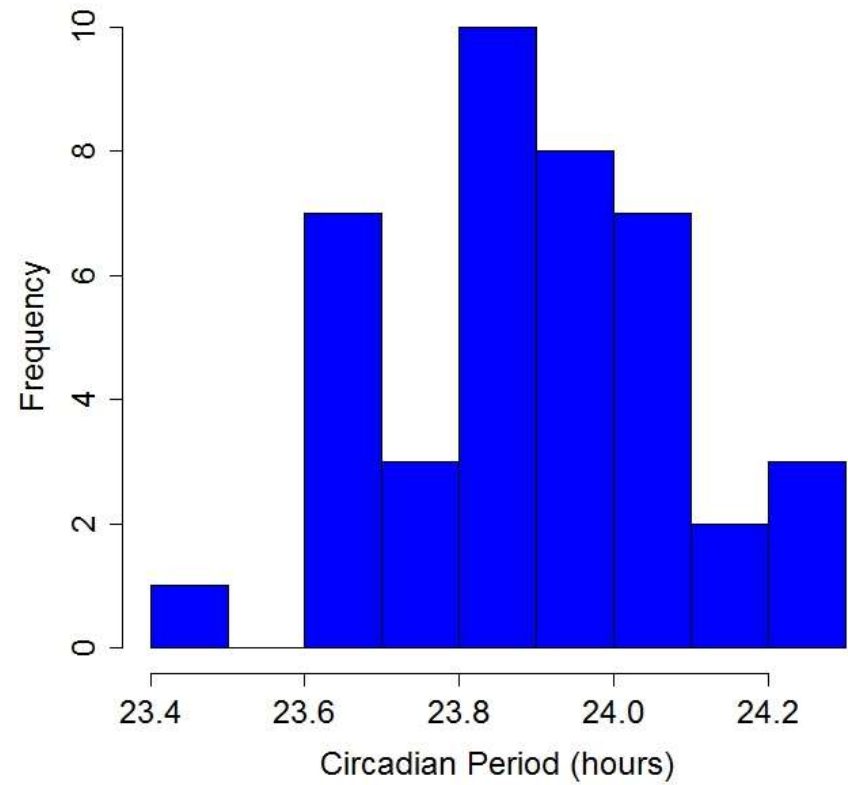
# Period differs in Lab lines

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**Field**



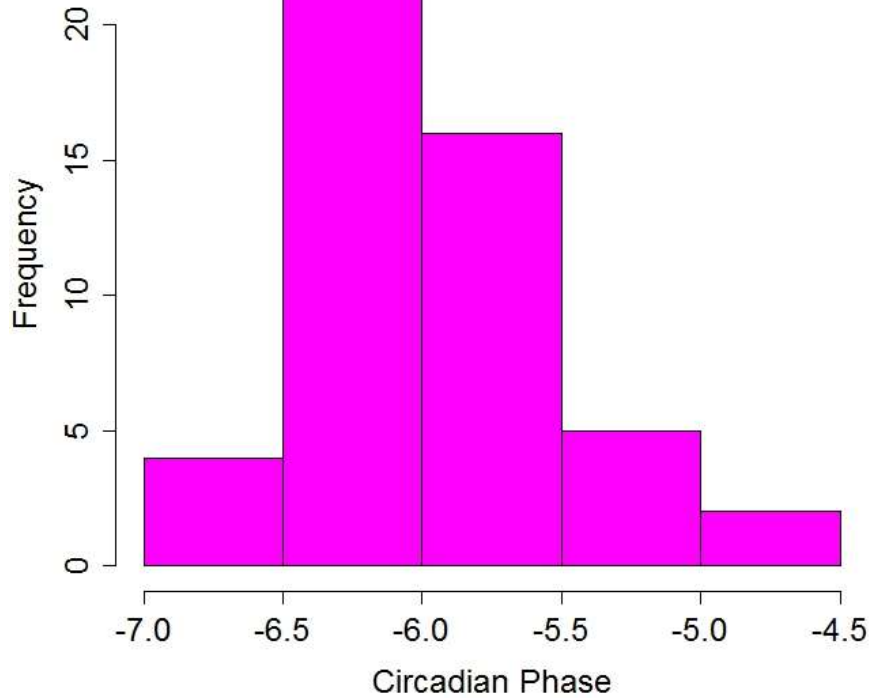
**Lab**



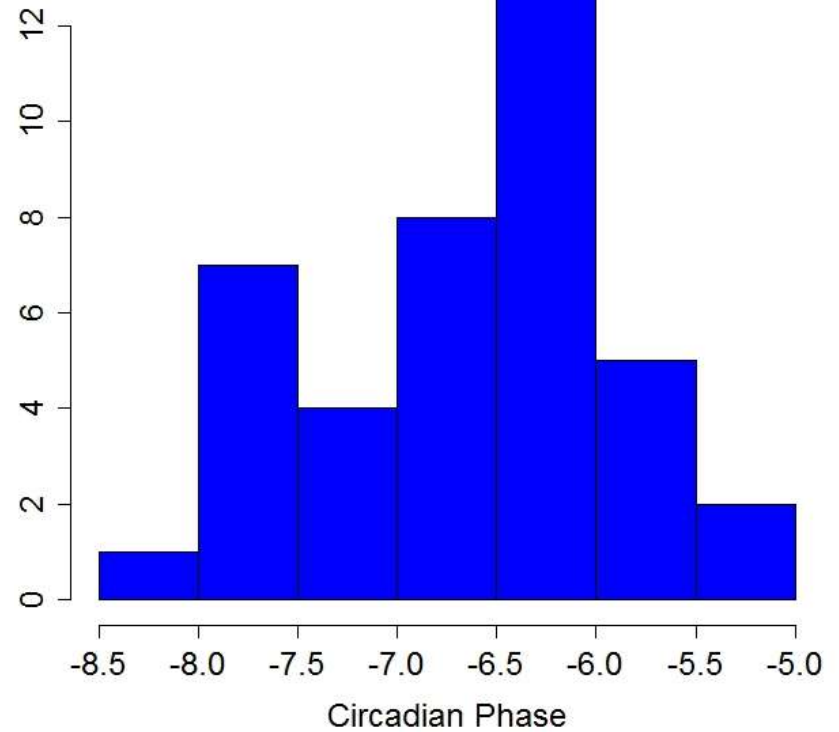
# Phase differs in both sets of lines

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**Field**



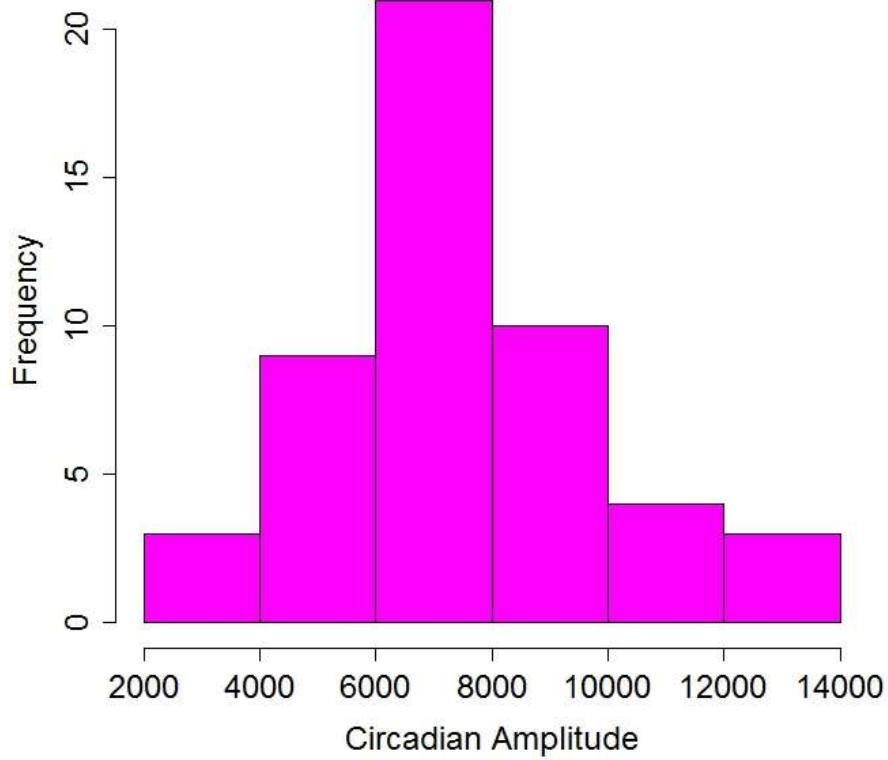
**Lab**



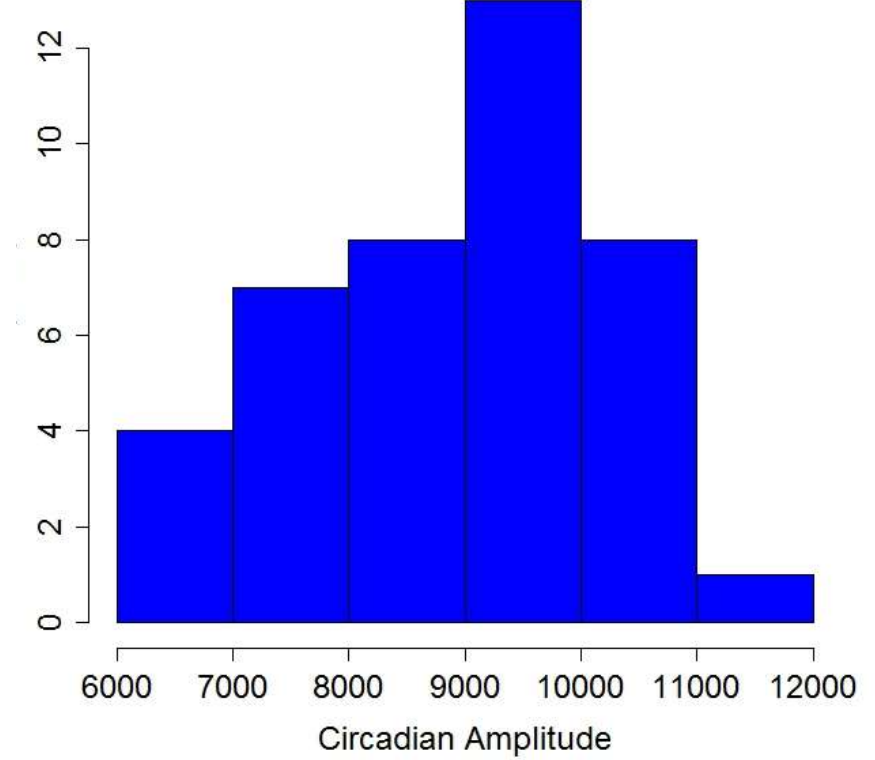
# Amplitude differs in both sets of lines

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**Field**

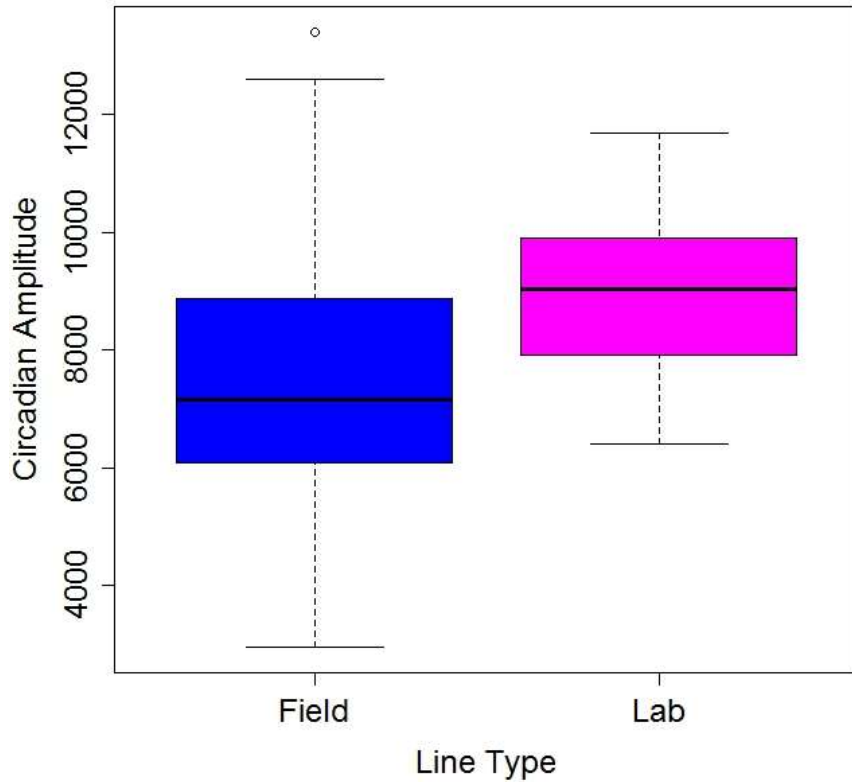


**Lab**

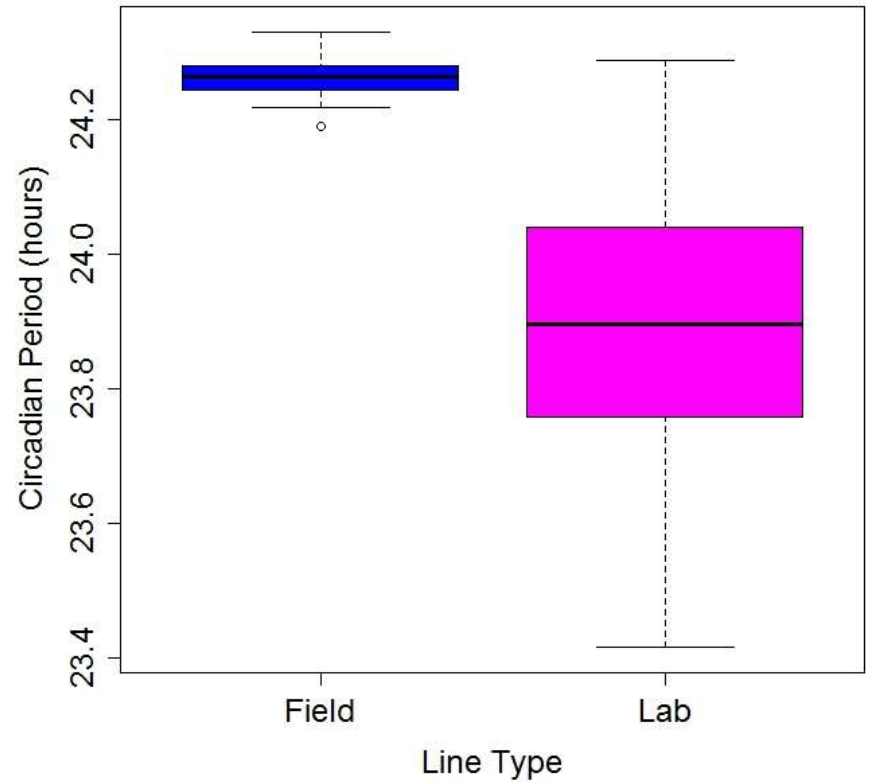


# Amplitude differs between sets

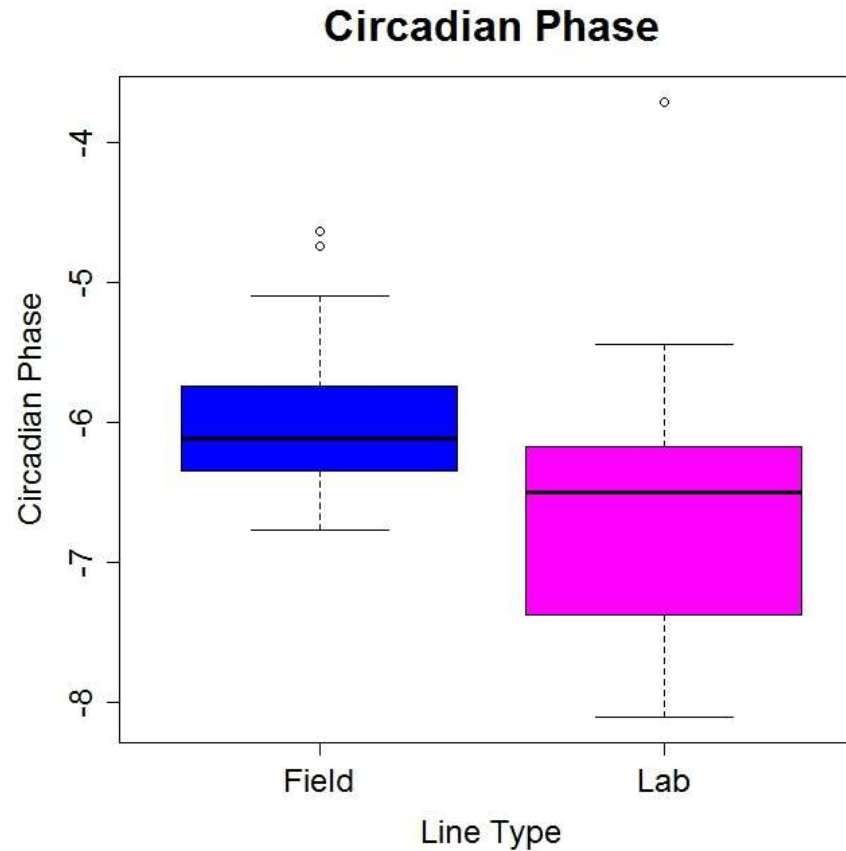
**Circadian Amplitude**



**Circadian Period**



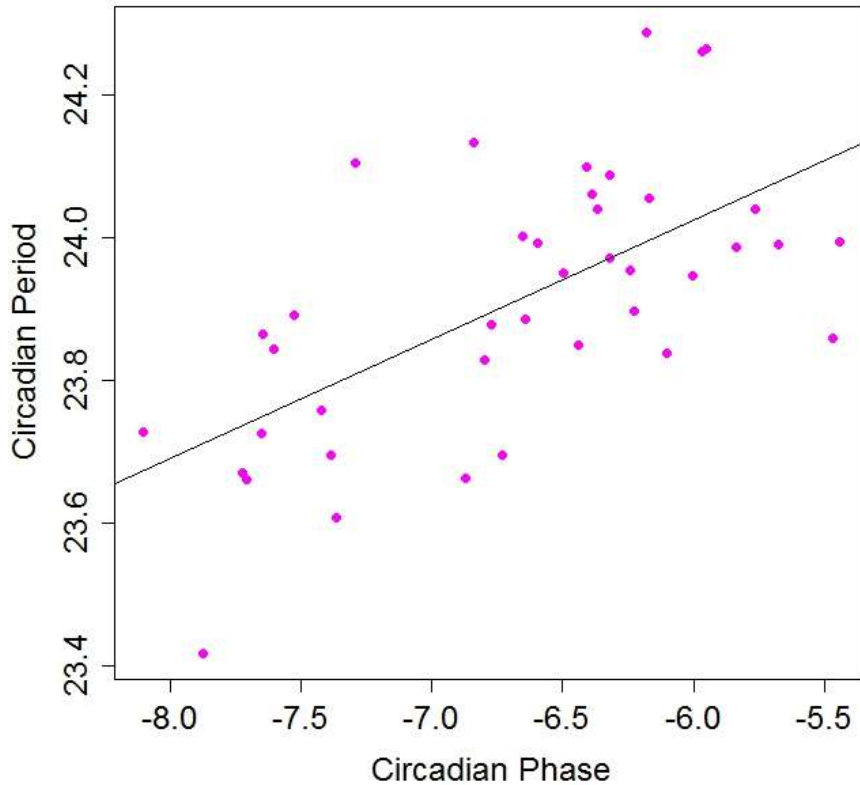
# Circadian Phase does not differ across sets



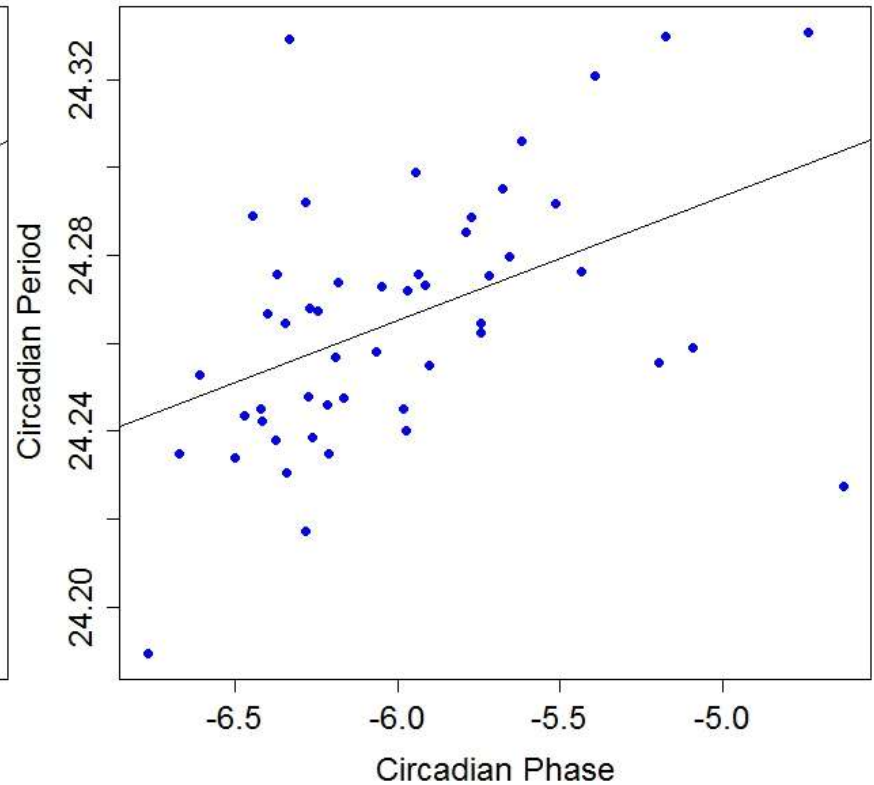


# Longer Period = Later Phase

Lab



Field



# Research Questions

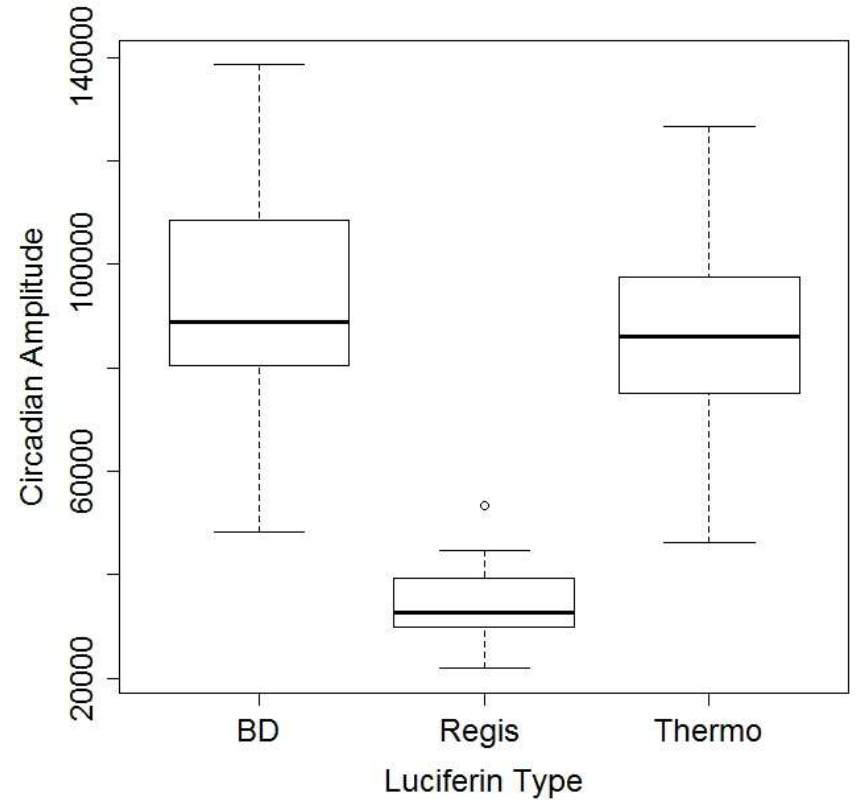
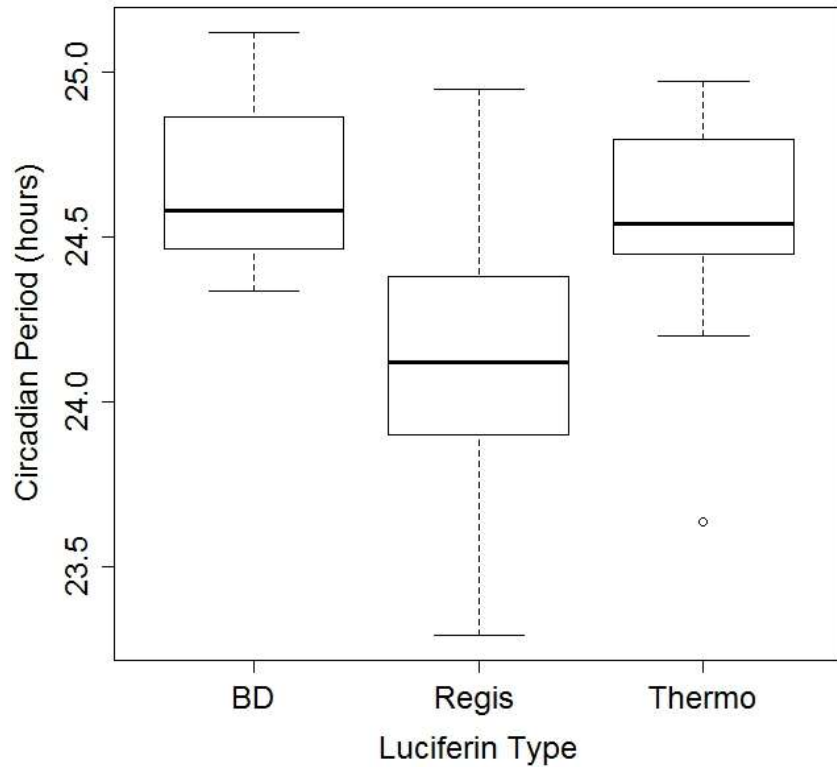
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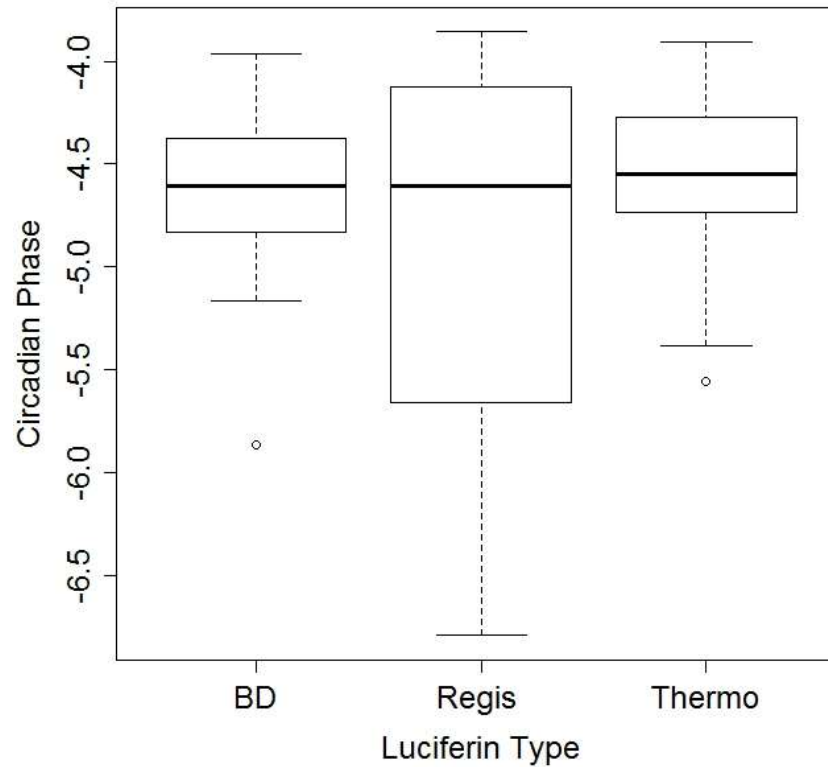
# Period and Amplitude depend on substrate type

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# Phase does not depend on substrate type

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# Conclusions

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- Circadian Phase and Amplitude show significant genetic variation within both lab and field-evolved lines
- Circadian Period only differs in Lab lines
  - High levels of phenotypic variation present in lab lines due to new allelic combinations present as a result of RIL creation
  - Reduced variation in field line possibly due to elimination of unfit genotypes
- Amplitude differs across line types



# Future Research

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- Increase replication
  - Phenotype circadian traits under uncrowded conditions
    - Compare UN to CR



# Acknowledgements

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