Bundling up for the Winter:
Effects of Photoperiod on Thermotolerance and Circadian Rhythms in *Drosophila melanogaster*

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Is a change in the photoperiod sufficient to cause fruit flies to sense that the season has changed?

Figure 1. The fruit fly, *Drosophila melanogaster*, was an ideal test subject because it changes its circadian rhythm quickly and is easy to breed. *Source: Why Evolution is True*

In this experiment, a change in the perception of season was measured through

a) Thermotolerance: Changes in chill-coma recovery times

b) Circadian rhythms: Changes in PER (a core clock protein) levels

Previous research has demonstrated that fruit flies raised under shorter, winter-like photoperiods have significantly shorter chill-coma recovery times and that PER levels peak at dawn during the winter and slightly after dawn during the summer (Pegoraro et al., 2014; Menegazzi et al., 2013).

Hypothesis: Flies raised in shorter, winter-like photoperiods will have increased cold tolerance and greater PER levels at dawn than flies raised in longer, summer-like photoperiods.

Experimental Design:

Flies were raised under three photoperiods for a minimum of 5 days in a temperature-controlled incubator:

1. 12 hours of light and 12 hours of darkness (intermediate)
2. 16 hours of light and 8 hours of darkness (summer)
3. 8 hours of light and 16 hours of darkness (winter)

Chill-coma recovery: Flies were placed on ice and their chill-coma recovery time was recorded.

-Placed 10 flies in 5 fly tubes (for each photoperiod)
-Placed flies on ice for 3 hours
-Removed flies and measured time taken for each fly to stand up

Figure 2. Microdissection setup with forceps and dissection plate under a dissecting microscope.

Figure 3. Cold assay setup with fly tubes (containing 10 flies each) placed into an ice bucket.

PER Levels: Flies were microdissected and their brains were imaged using immunohistochemistry.

-Collected flies approximately 30 minutes before "dawn" and placed them on ice
-Microdissected flies, isolating the brains, then fixed brains in 4% paraformaldehyde
-Blocked brains in 5% NGS to limit nonspecific binding
-Incubated brains in primary rabbit anti-PER antibody
-Incubated brains in fluorescent secondary goat anti-rabbit antibody (Alexa Fluor 458)
-Placed brains in 70% glycerol for ease of imaging
-Mounted brains
-Imaged brains using fluorescence microscopy - counted stained cells and qualitatively assessed brightness

Results and Data Analysis:

Both data sets were analyzed using ANOVA in JMP 11.

No statistically significant effect of photoperiod on chill-coma recovery time.

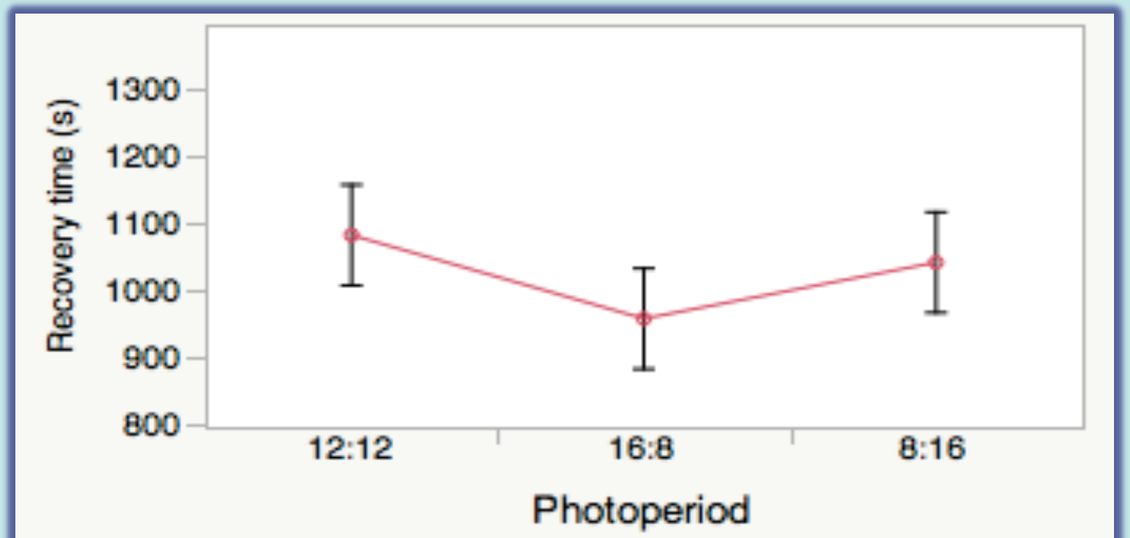

Figure 4. ANOVA results for dependence of chill-coma recovery time on the photoperiod. No obviously statistically significant effect found (p=0.0638).

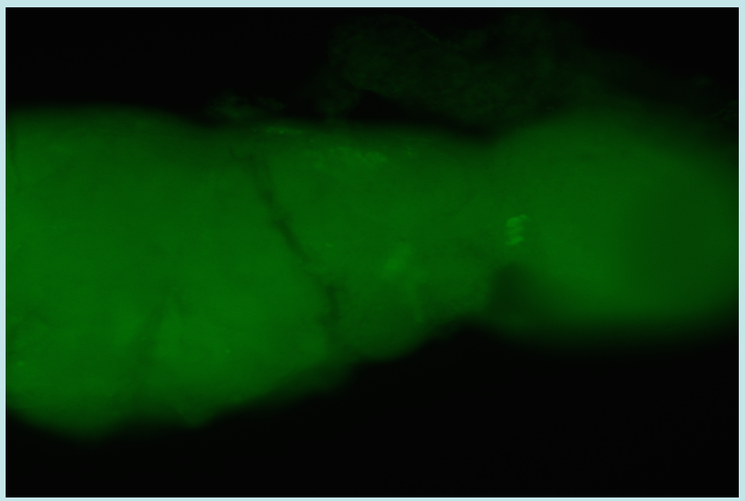

Figure 6. Example of a 12:12 brain stained with anti-PER antibody. Three bright PER-stained cells can be seen on the right half of the brain.

Statistically significant effect of photoperiod on the brightness of cells in fly brains labeled for PER. Winter-like photoperiod, 8:16, had brighter labeling (indicating greater PER levels) than summer-like photoperiod, 16:8.

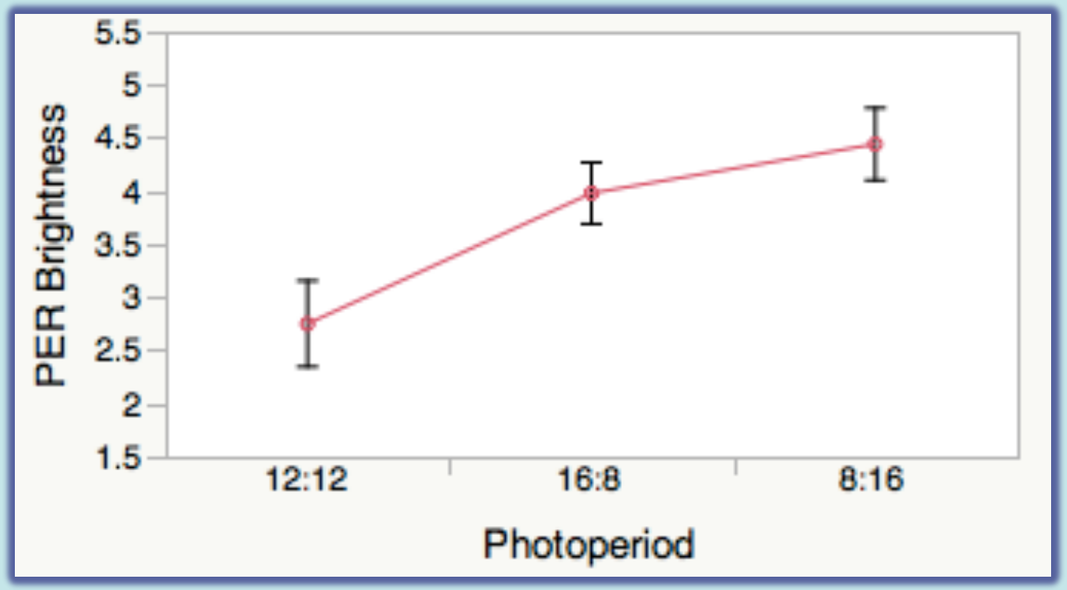

Figure 5. ANOVA results for dependence of brightness of PER labeling (indicating greater or lower PER levels) on photoperiod. Each photoperiod produced statistically significantly different PER brightness levels ($p < 0.0001$).

Decreased PER labeling in 12:12 brains relative to 16:8 or 8:16, but the sample was confounded by being the first attempt at the protocol.

Conclusions:

While a change in photoperiod can shift circadian rhythms,
it is not a sufficient signal to induce a change in thermotolerance.

Future Directions:

A change in photoperiod alone is an insufficient signal to cause a change in thermotolerance. So, what *is* necessary to trigger that change? Does a fly need to be raised in colder temperatures to become significantly more cold tolerant, or does there need to be some combination of a shorter photoperiod and lower temperatures to induce greater cold tolerance? Future experiments could explore the effects of the environmental temperature on thermotolerance and test whether or not there is an interaction effect between a change in temperature and a change in photoperiod on thermotolerance. Subsequent studies could also research whether or not a change in temperature affects a fly's circadian rhythm.

References:

(1) Menegazzi, P., et al. (2013). "Drosophila Clock Neurons under Natural Conditions." Journal of Biological Rhythms **28**(1): 3-14.
(2) Pegoraro, M., et al. (2014). "Role for Circadian Clock Genes in Seasonal Timing: Testing the Bünning Hypothesis." PLoS Genet **10**(9): e1004603.
(3) Wu, J. S. and L. Luo (2006). "A protocol for dissecting Drosophila melanogaster brains for live imaging or immunostaining." Nat Protoc **1**(4): 2110-2115.

Images:

http://whyevolutionistrue.wordpress.com/2011/03/20/whos-related-to-fruit-flies/

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