

IDENTIFICATION OF A ROLE FOR THE *PERIOD* GENE IN MEDIATING HATCHING IN THE MAYFLY, *HEXAGENIA LIMBATA*

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The aim of this work was to study the role of the gene *period* in mediating hatching in the mayfly *Hexagenia limbata* (Ephemeroptera: Ephemeridae). Clock genes have previously been found to influence hatching in *Drosophila*, and *period* is expressed in the head of fruit flies at high levels soon after sunset, and becomes weak around midnight. Since both *Drosophila* and mayflies share evolutionary history as part of the Insecta clade, *period* in *H. limbata* was also expected to be expressed at a high level in 6PM sampled nymphs, and at lower levels in 9AM sampled nymphs because *H. limbata* hatches into the winged phase at/around twilight each evening in June and July in NH. Samples of black wing pad stage nymphs of *H. limbata* were collected from Sky Pond and Jackson Pond (New Hampton, NH) in the spring and summer of 2012. Samples were preserved in 100% ethanol for DNA extractions, RNA Later solution for RNA extractions and Trizol reagent for protein extractions. The expression of *period* in the head (where the protocerebrum and subesophageal ganglion or brain located), and thorax (to act as negative control) of *H. limbata* nymphs was investigated at three different time points (9AM, 12PM and 6PM) by isolating *period* from the genome of *H. limbata* then conducting Western blotting to characterize protein expression. Six different degenerate primer pairs targeting *period* in *H. limbata* were designed, and false positive bands were returned over numerous amplification attempts. Two different *Drosophila* anti-**PER** primary antibodies were used in Western blots, these blot results showed a single clear band in the *Drosophila* samples (the positive control) but did not return any bands in *H. limbata* samples. Both nucleotide level and amino acid differences between fruitflies and mayflies in the structure of the *period* gene and the corresponding **PER** protein could have resulted in these cumulative results. Further study, and likely a transcriptomics approach, is required to continue to investigate the level of similarity between the proteins in these two species, to resolve if increased **PER** expression relates to hatching in *H. limbata* nymphs.