

Cracking the Developmental Code: Juvenile Hormone and its Influence on Growth and Metamorphosis in *Osmia lignaria*

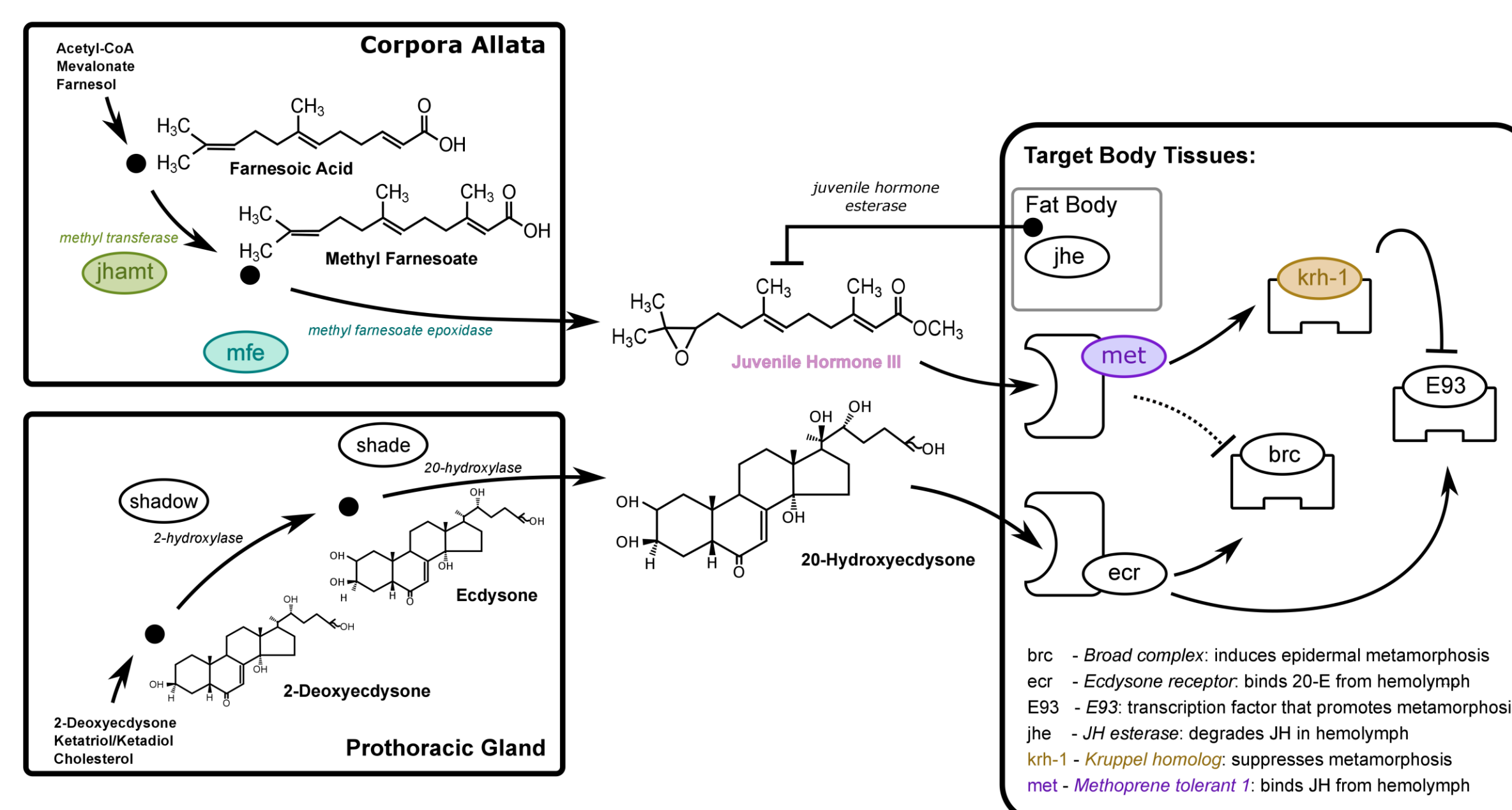
Laurie M. Agosto^a, Bryan R. Helm^b, Vanessa Bentley^c, Jason Holthusen^d, George D. Yocum^e, Kendra J. Greenlee^b, Julia H. Bowsher^b

Abstract

For most insects, the cue for metamorphosis remains a mystery. Physiologically, metamorphosis is regulated by hormones—primarily juvenile hormone and ecdysone which control different aspects of the metamorphic processes¹⁻³. Much of our understanding of metamorphosis is based on studies focusing on just a few model organisms, but none focus on the physiological dynamics and their underlying molecular mechanism¹⁻³. To make this connection, we tested the hypothesis that starvation starts the hormonal cascade associated with the initiation of metamorphosis in the blue orchid bee, *Osmia lignaria*. We removed provisions from the specimens and conducted a time series by collecting hemolymph and extracting RNA at various time points. We measured the hemolymph titer of juvenile hormone III (JHIII) using an established HPLC-MSMS protocol⁴. From these same individuals, we quantified the expression of genes that regulate JHIII synthesis and reception in target tissues. This research explores the conceptual gaps concerning body size and hopes to develop a model for bees.

Aims

1. Measure JHIII concentrations in hemolymph in starved *O. lignaria* for metamorphic commitment.
2. Quantify relative gene expression of genes regulating JHIII synthesis and reception (see figure below).



Methods

1. Extract hemolymph and quantify by HPLC-MSMS

2. Extract mRNA and quantify by qPCR

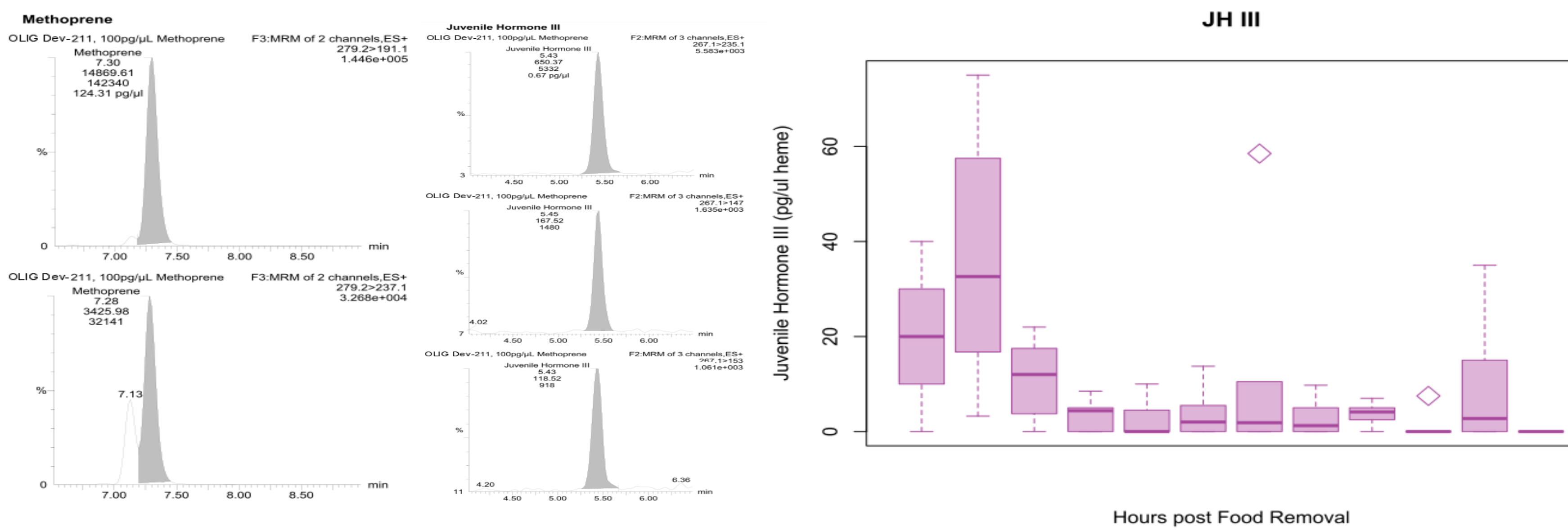
JHIII Synthesis

- jhamt
- mfe

JHIII Receptor

- met
- krh-1

Quantification of JHIII in Hemolymph via HPLC and qPCR After Food Removal



Discussion Points

Quantification of JHIII in hemolymph via HPLC & qPCR

- We successfully quantified gene expression and the pattern follows the expected molecular mechanism trend for metamorphic commitment. JH was also clearly expressed in HPLC thus giving a two-way confirmation.

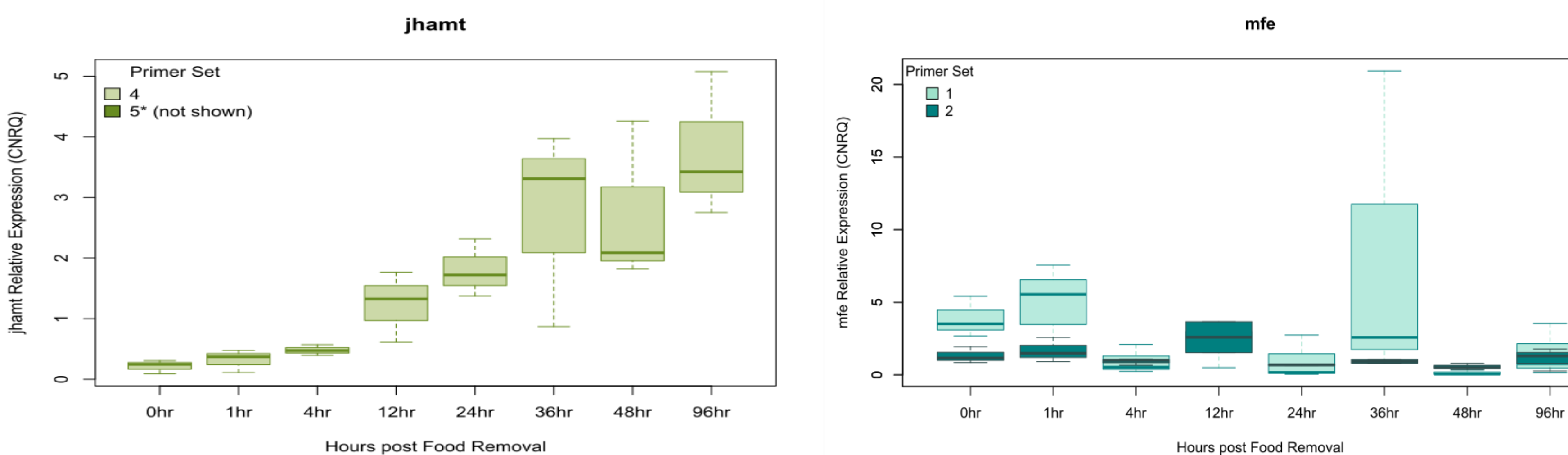
JH III Biosynthesis Genes

- Once committed to the biosynthetic pathway, JH is expected to decline along with corresponding synthesis genes. Instead jhamt increased while mfe remained constant.

JHIII Receptor and Response Genes

- As JH gets synthesized, met has a high affinity to bind with JH and the signal is transduced by krh-1⁵. Instead, these results show met being upregulated 12 hours after food removal and krh-1 decreasing.

JHIII Biosynthesis Gene jhamt Increase and mfe Remains Constant After Food Removal



*jhamt second primer did not accurately quantify

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References

1. Nijhout HF. Insect hormones
2. Shingleton AW. Evolution and the regulation of growth and body size
3. Gäde G, Hoffmann KH, Spring JH. Hormonal regulation in insects: facts, gaps, and future directions.
4. Ares AM, Nozal MJ, Bernal JL, Martín-Hernández R, M. Higes, Bernal J. Liquid chromatography method
5. Belles X, Santos CG. The MEKRE93 pathway in the regulation of insect metamorphosis, and the homology of the pupal stage

Affiliations

- a. Undergraduate, Department of Molecular and Microbiology, University of Central Florida, Orlando FL 32816
- b. Department of Biological Sciences, North Dakota State University, Fargo ND 58108
- c. Undergraduate, Department of Biology, Aurora University, Aurora IL 60506
- d. USDA-ARS Animal Metabolism-Agricultural Chemicals Research, Fargo ND 58102
- e. USDA-ARS Insect Genetics and Biochemistry, Fargo ND 58102

JHIII Receptor Gene met Increase and Response Gene krh-1 Decrease After Food Removal

