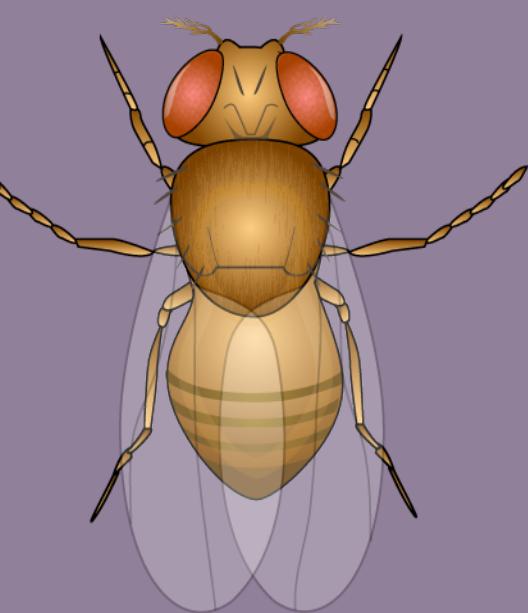


Juvenile Hormone Sensitivity of a *Drosophila melanogaster* Enhancer Region on the Foraging Gene



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ABSTRACT

- The main objectives of this experiment were to determine if:
 - (1) juvenile hormone affects transcription
 - (2) if juvenile hormone regulated enhancers are also regulated by ecdysone.
 - (3) *foraging* is a juvenile hormone target gene
- Results indicate that the enhancer regulates transcription in a temporally, spatially, sex specific, environmentally and ecdysone receptor dependent manner.

- LacZ expression was found in ecdysone regulated locations such as the eye disc morphogenic furrow, imaginal discs, and optic lobes.
- Juvenile hormone INCREASES the transcription of LacZ in males and females.
- Ecdysone receptor (EcR) INHIBITS juvenile hormone sensitivity among both males and females.

- Foraging enhancer is BOTH JH and ecdysone regulated.
- Results support the theory that (1) foraging is a juvenile hormone target gene, (2) the juvenile hormone regulated enhancer is also regulated by ecdysone, (3) juvenile hormone INCREASES transcription of LacZ, (4) EcR binding decreases JH sensitivity.

BACKGROUND: Juvenile Hormone and Ecdysone

- The juvenile hormone and ecdysone are essential hormones for insect development.
- JUVENILE HORMONE:** delays early metamorphosis and regulates the insect's physiological development.
- ECDSONE:** promotes growth, and larval molts in the presence of the juvenile hormone.
- The molecular pathways of the JH response are unknown.
- Scientists speculate that there are hundreds of JH target genes. However, only ten have been confirmed. (Dubrovsky, et. al., 2014)

BACKGROUND: Foraging Gene

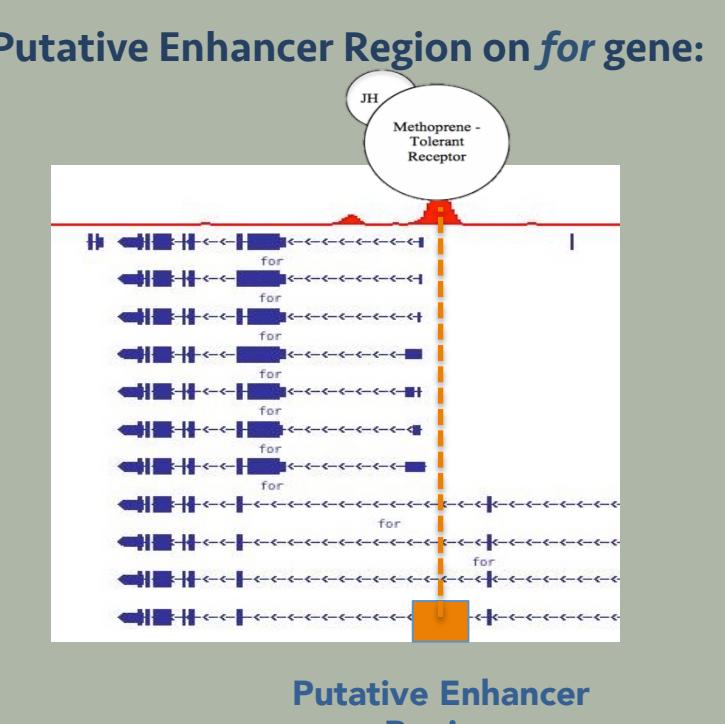
- Possible juvenile hormone target gene, the **FORAGING GENE**, affects:
 - Metabolite
 - Food-leaving behavior
 - Gene expression levels in response to food deprivation
- Responsible for 2 behavioral morphs in *Drosophila melanogaster*:
 - (a) rovers
 - (b) sitters
- Juvenile hormone expression is associated with increased *foraging* expression in other insects:
 - Honey bee: high expression of *for* shifts the role of the honey bee from nursing to foraging for pollen/nectar (Kent CF, et. al., 2009)
 - Ants: high expression of *for* present in harvester ants

HYPOTHESES

- HYPOTHESIS 1:** LacZ expression will change among methoprene treated and ethanol treated W3L, which indicates juvenile hormone sensitivity of the enhancer region on the foraging gene.
- HYPOTHESIS 2:** LacZ expression in mutants will be greater than in the full enhancer flies; this will prove JH regulated enhancers are also ecdysone regulated.

HOW MIGHT JH AND FOR GENE BE RELATED?

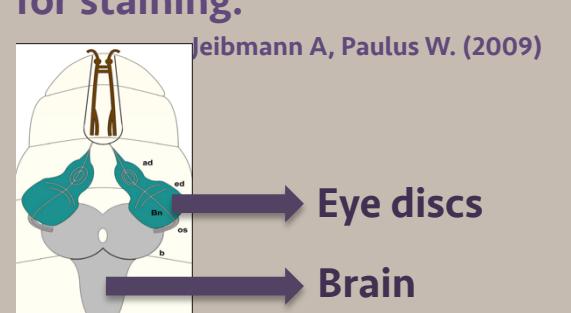
- Methoprene-tolerant binding sites are on the *for* gene.
- Juvenile hormone controls the *for* gene in honey bees.
- Both are heavily involved in the behavioral and nutritional status of flies.



METHODOLOGY

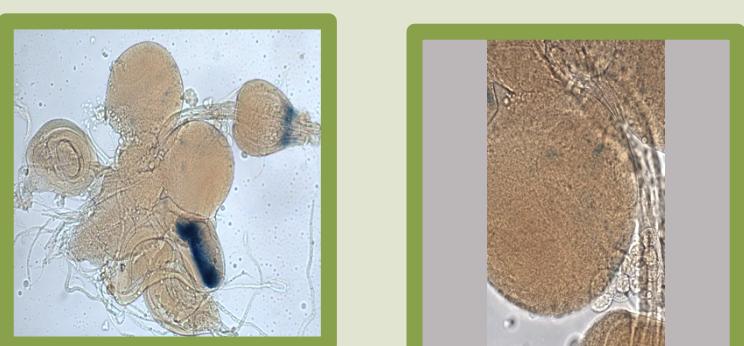
JH Sensitivity of the Enhancer

- for* gene enhancer attached to a reporter gene (*LacZ*).
- Enhancer-reporter construct inserted into control and mutant flies.
- Third instar larvae were treated either with ethanol, or JH mimic: methoprene; they were then dissected and stained.
- The brain and eye discs were examined for staining.



RESULTS

CONSTRUCT



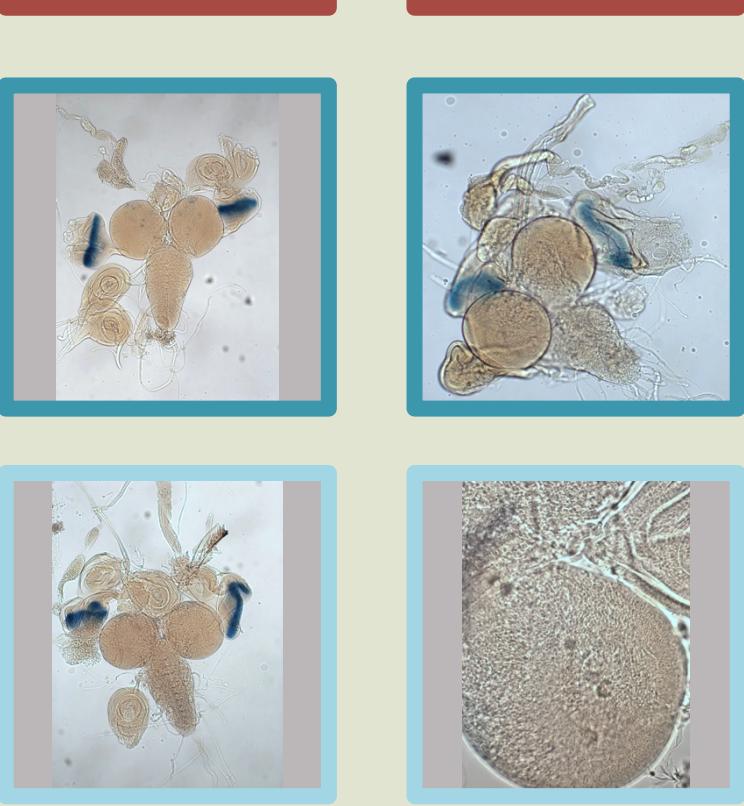
- No LacZ expression in the optic lobes.
- Reduced LacZ expression in the eye discs following methoprene treatment.
- LacZ expression is sensitive to JH in the eye discs.

WILDTYPE FEM. ETH.



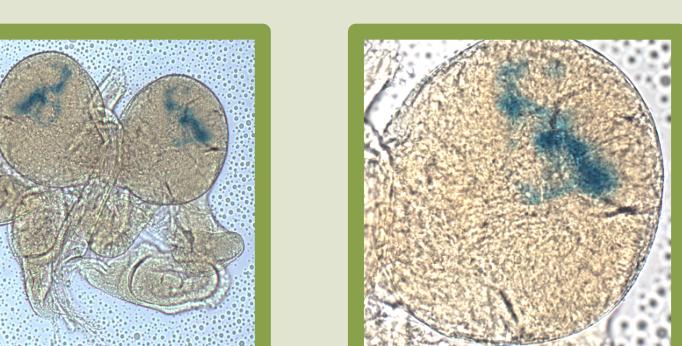
- No LacZ expression seen in the optic lobes.
- No change in LacZ expression within the optic lobes following methoprene treatment.

WILDTYPE MALE ETH.



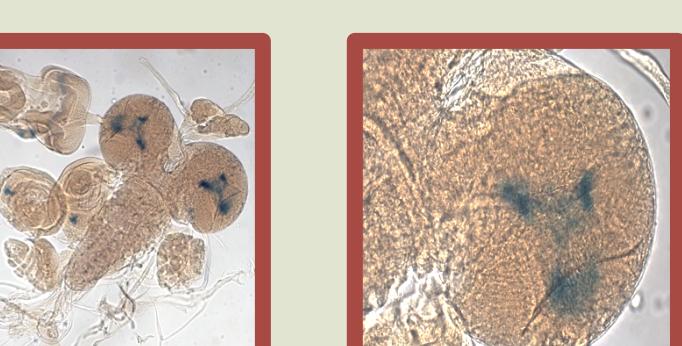
- No LacZ expression seen in the optic lobes.
- No change in LacZ expression within the optic lobes following methoprene treatment.

CONSTRUCT



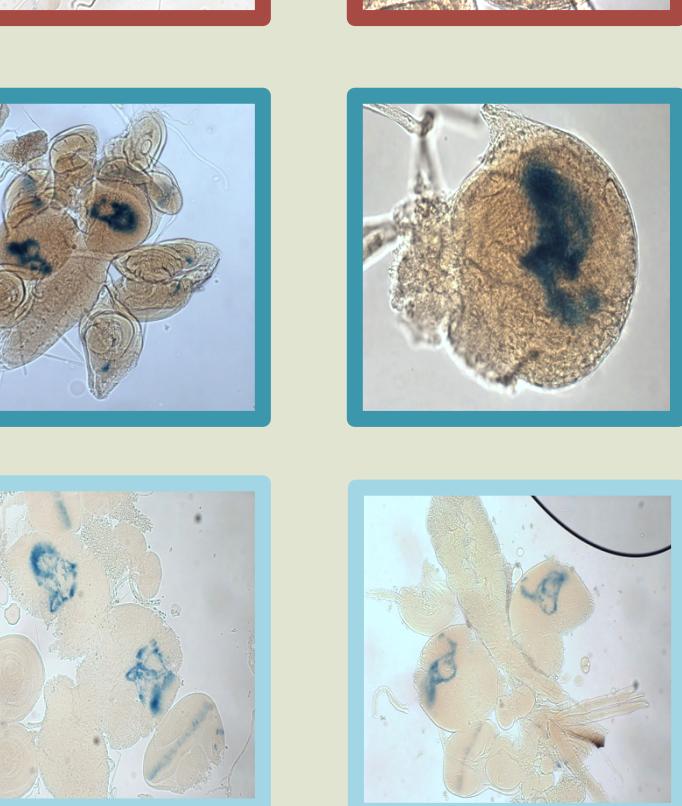
- LacZ expression seen in the optic lobes of the mutants.
- Reduced LacZ expression in the optic lobes following methoprene treatment.

MUT. FEM. ETH.



- Strong LacZ expression seen in the optic lobes.
- Greatly reduced LacZ expression in the optic lobes following the methoprene treatment.

MUT. MALE ETH.

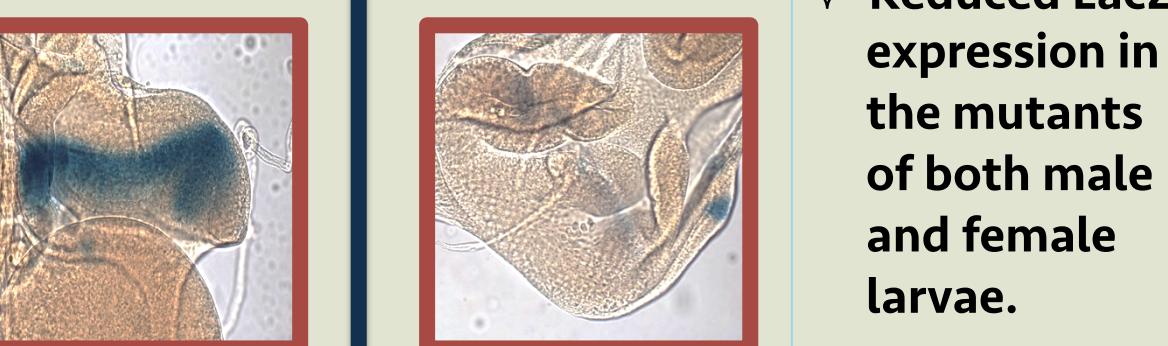


- Strong LacZ expression seen in the optic lobes.
- Greatly reduced LacZ expression in the optic lobes following the methoprene treatment.

MUT. MALE MET



- Reduced LacZ expression in the mutants of both male and female larvae.
- JH does not appear to affect LacZ expression in the eye disc.



DISCUSSION

OPTIC LOBES

- EcR INHIBITS LacZ in the optic lobes.
- The WILDTYPE flies had no LacZ expression, but the mutant flies without EcR exhibited strong expression in the optic lobe.
- JH further reduces the expression of LacZ in the mutant larvae.
- The optic lobe expression is sensitive to BOTH EcR and JH.

EYE DISCS

- EcR is required for normal LacZ expression.
- JH inhibits LacZ expression in the eye discs.
- EcR is not required for JH sensitive response seen in the larvae.

FUTURE DIRECTIONS

- Flies without EcR entirely will be treated and stained to determine the role of EcR in juvenile hormone regulated enhancers.
 - We expect absence of EcR will INCREASE juvenile hormone sensitivity.
- Foraging* gene expression will be examined in tissues that had the most LacZ staining (enhancer activity).
 - This will be done to confirm the target gene activity.
- Test for JH sensitivity in Methoprene Tolerant mutants:
 - Methoprene Tolerant: JH receptor
 - Methoprene Tolerant mutants have a mutated JH receptor.
 - Will allow us to confirm whether JH sensitivity is dependent on JH receptors.

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