Effects of Royal Jelly and Juvenile Hormone on Growth and Immunity in *Gromphadorhina portentosa* (and using our findings to support science outreach in the New River Valley)

Background

Invertebrate endocrinology is far too commonly overlooked and under appreciated, in part because the complex web of hormone interactions and energy investment strategies is not clearly comparable to that of a vertebrate system. Humans are vertebrates, therefore learning more about other vertebrate endocrine system seems to be the most beneficial and profitable allocation of research efforts. Here, I will explain why we need to stop ignoring what is right in front of us, and further investigate the multidimensional strategies that invertebrates depend on for survival and how we can use this information to better interpret the evolved world around us.

Hormones and energy allocation

Growth hormones play a key role in not only development but also in immune function; investment in reproduction and sematic growth may also come at the cost of immune function. Previous research has focused on endocrine modulation of immune function on vertebrates, rather than the interactions of investment patterns and immune growth and reproduction in insects. Royal jelly (RJ), a modulator of reproduction and growth in honeybees, has been shown to induce similar effects in fruit flies (*Drosophila melanogaster*) (Kamakura, 2011). Specifically, flies reared with RJ experienced hastened turnover time from youth to functioning adult and increased ovary size. Similar effects have been observed in Madagascar hissing cockroaches (*Gromphadorhina portentosa*) exposed to RJ.

Juvenile growth hormone (JH) maintains adolescent morphology and physiology in juveniles and increases reproductive output (via vitellogenesis) in adults in a wide variety of invertebrate taxa (Riddiford 2010). However, studies in our lab have shown inconsistencies of the effects of JH exposure on hissing cockroaches and fruit flies specifically, in mortality and reproduction. JH given in combination with RJ produced distinct differences in both sex traits and reproductive patterns across species. Mortality and reproduction in these species, in addition to specific impacts on immune functions, as measured by lytic and coagulatory activity, are newly observed modulatory effects of JH and RJ on growth. There are implications of these findings for both our understand of hormonal control in invertebrates and consequences of variable energy strategies across systems and taxa.

Functioning in an energy deficit

Royal jelly in combination with juvenile hormone in adult Madagascar hissing cockroaches produces, what we have deemed, the Roachzilla. Our Roachzillas produce thickened exoskeletons, enhanced secondary sex trait structures, and wider body plans- all responses to compensate for exogenous hormone treatments of RJ in combination with JH (Truman 1973). By creating a giant cockroach, we are forcing the body to be more selective about where energetic resources are moved to. While RJ and JH both encourage reproductive energy input in adults, the cockroaches reach a point where energy is stretched too thin and they must begin omitting unnecessary systems (Moret 2000). Researching more in depth about resource placement in the body is crucial because we have evidence that suggest that the immune system may be compromised as a result of limited availably energy under RJ + JH conditions. This leads to the question of how much energy is too much energy to take away from self-healing processes and instead reinvest more into producing offspring (Traniello 2002).

Hypothesis

Royal jelly and juvenile hormone treated Madagascar hissing cockroaches will compromise immunity to maximize investment in reproductive efforts.

Protocol

Treatment groups

Control	Royal jelly	Juvenile hormone	Royal jelly and juvenile hormone

*exogenous hormone treatments began January 2018

Bleeding procedure for MAdagascarr hissing cockroaches

In order to determine immunity in cockroaches, the composition and function capacity of hemolymph needs to be examined. Instead of blood traveling throughout the closed circulatory system of a body, invertebrates have hemolymph distributed thought their bodies in an open circulatory system. For this reason, cockroaches from each treatment group will be chilled (to slow down metabolic rate) and pricked with a needle to release hemolymph into a capillary tube. Hemolymph coagulates rapidly, so the capillary tube of hemolymph must be measured rapidly, then mouth pipetted out of the tube and placed in a vial with buffer solution. We have discovered that feeding the cockroach 24 hours prior to bleeding helps to encourage hemolymph release. Nourishment alters hormones to invest in more than emergent bodily functions, which could explain increased hemolymph post feed. The hemolymph is then centrifuged and incubated to be used for testing,

Custom assays

Three concentrations of hemolymph in a rabbit blood solution are used to understand immune reaction through lysis rate coagulation patters. Rabbit blood acts as the invading species that the hemolymph attacks, therefor the more lysis that occurs, the higher functioning the immune response is. Then, three custom in house assays are used to determine immune response across the treatment groups.

1. Visual test: After the hemolymph and rabbit blood have incubated in well plates, and image of each plate is obtained using a dissection microscope. The image is taken so that coagulation patterns can be measured and color can be recorded. A consistent visual color recording method needs to be established in order to compare data across multiple trials.

2. Spectrophotometer: Light absorbance is used to understand the amount of coagulation occurring per well.

3. Blood slide: The hemolymph and blood solution is pipetted onto a blood slide to observe for cell lysis within small parameters of the sample.

Timeline

January: RJ and JH will be ordered to continue treatments, cockroaches will be measured and examined for sex trait variation and observed for reproductive activity and production.

February, March, and April: Blood samples will be obtained every two weeks from different members of the treatment groups to allow regeneration of hemolymph. Data will be collected and organized

May: Data collected will be organized and prepared for a presentation at the Society of Integrative and Comparative Biology conference of 2020.

May, June, and July: Outreach months used to teach people of all ages about our studies in venues such as schools, universities, camp programs, day cares, museums, science celebrations, farmer's markets, and more.

Outreach

While peruse a better understanding of energy investment strategies between reproduction and immunity in Madagascar hissing cockroaches, I have developed a program to educate the New River Valley about invertebrates versus vertebrates and how altering various hormones can impact the way an animal invests its energy. Our research serves as an important outreach tool for our lab and for Radford's department of biology because we have a local following with people of all ages that regularly attend our events at science fairs because we make our research relatable to the general public, while still teaching them about cutting edge research. I have written a children's book to explain our previous work with fruit flies and how to understand the same concept in other invertebrates, I have developed many outreach tools such as "Dr. Roach" to explain the immune system to children, and I have crafted research pitches that target specific age groups and venue specific learning. I would like to use this project to both increase our understanding of these hormones investment strategy and to increase public knowledge of insects and endocrine physiology.

Budget

	Quantity	Individual Price	Total Price
Video Camera	1	$109	$109
Terarrium	8	$32	$256
External hard drive	1	$75	$75
Juvenile Hormone	3x Methoprene 100MG	$140	$420
Royal Jelly	2x Stakich fresh royal jelly 1KG	$139	$278
Clip on microphone	1	$40	$40
Outreach supplies	T-shirts, buttons, stickers, poster, silicon bracelets (~$250)		$250

References

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