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Nutritional Requirement for Protein Production from Trehalose Synthesis in the Male Accessory Gland of Tribolium castaneum

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Nutritional Requirement for Protein Production from Trehalose Synthesis in the Male Accessory Gland of Tribolium castaneum

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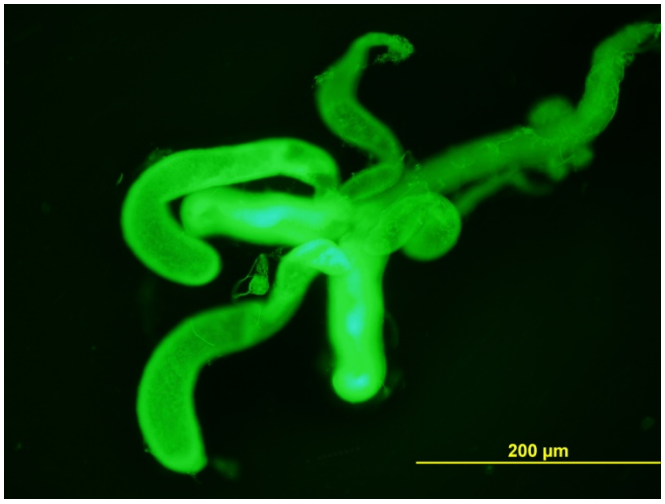
1. **Nutrition facilitates male reproduction in *Tribolium castaneum***

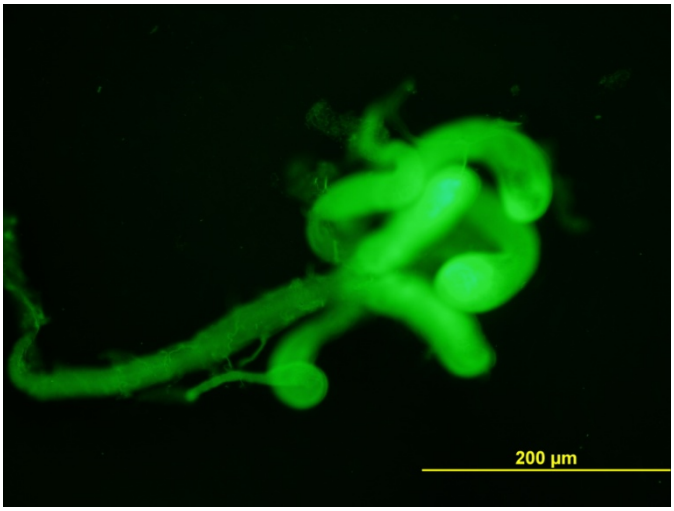
In my previous research, I found that there is no nutritional requirement for spermatogenesis to occur in the adult *Tribolium castaneum.* This conclusion came from the initial question, is nutrition required for male reproduction in the *Tribolium?* Indeed, there is a nutritional requirement for female reproduction (Sheng et al., 2011). Thus, females will not reproduce if they are not fed because they will instead save their energy for survival. Males, however, will still produce sperm because my research found that spermatogenesis begins in the pupae stage, so feeding or nutritional intake in the adult stage did not show a significant effect on the production of sperm. However, there are two important processes that play a role in male reproduction; sperm production and the production of ACP protein, which is part of the seminal fluid that protects the sperm until it reaches the egg. Although sperm production is not affected by the amount of nutrition available, the production of ACP protein, produced in the male accessory gland, is clearly affected when the *Tribolium* are starved (Fig.1A&B). As shown below, the male accessory gland from the fed male is visibly larger in size than that of the starved male.

Fig.1A



feeding

Fig.1B



starved

Over the summer I also continued to conduct research regarding the still unknown functions of the insulin like peptides present in *Tribolium.* However, I focused my insulin research further on the topic of trehalose because knowing the pathways of trehalose will help clarify the crosstalk between juvenile hormones (JH) and the insulin-insulin like signaling (IIS) pathways. Trehalose is the major source of glucose energy within *Tribolium,* and is regulated by

both juvenile hormones as well as insulin-insulin like signaling pathways (Riddiford, 1994). However, the mechanism for the regulation of trehalose synthesis metabolization—by either JH or insulin signaling—is still unknown. Therefore, I have been conducting experiments regarding JH, IIS, and the trehalose transporter in order to gain insight into the crosstalk between JH and IIS through trehalose homeostasis.

To combine the projects I have been working on—male nutrition vs. reproduction and trehalose synthesis—I have been examining the role of trehalose particularly in the production of the male accessory gland protein. This combination of my projects was in fact my focus for research this summer. My hypothesis drawn from the figures above is testing if there is in fact a nutritional requirement in order to produce trehalose as well as ACP proteins in the male accessory glands. My hypothesis will be tested through several molecular biological experiments that highlight the trehalose transporter G00121, because G00121 is a specific transporter of trehalose that is only found within the *Tribolium* males' male accessory glands. Thus, G00121 is vital to this work because without it, the trehalose cannot be transported from the haemolymph to the male accessory gland.

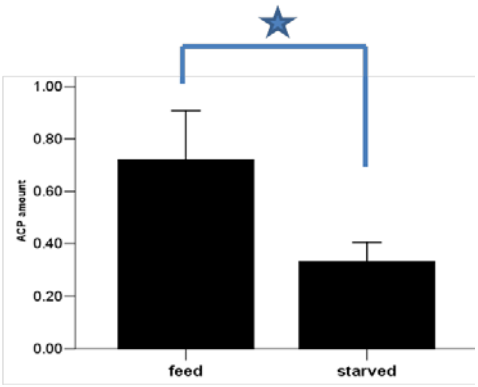
During this past summer, I have learned a great deal about how to conduct molecular biological experiments using the *Tribolium castaneum* and how to utilize these experiments in order to further my research into the comparison of nutrient requirements, trehalose transport, and male reproduction. I have been trained to dissect both the male accessory glands and testis of *Tribolium* males, isolate RNA from the dissected male accessory glands, synthesize double stranded RNA from the isolated RNA, purify the double stranded RNA that I synthesized, check how well the dsRNA was synthesized using gel electrophoresis, and to either convert the dsRNA into cDNA to check specific male accessory gland protein gene expression using real time polymerase chain reaction, or using the double stranded RNA to inject into the *Tribolium* in order to check other genes. Lastly, I have learned how to analyze all of my data using the Microsoft Excel spreadsheets.

Trebolium castaneum in their natural habitat are major pests, specific to stored grains. Unfortunately, the rapid reproduction of these pests have been increasingly hard to control because they have developed resistance to most insecticides. Thus, the main purpose of conducting these experiments is to gain a more complete insight into the biological processes of the *Tribolium castaneum.* The newly acclaimed data would allow us to be able to synthesize new forms of pest controlling substances that directly target the genes of the *Tribolium*, thus not affecting the productivity of the crop nor harming the people consuming the food.

2. Nutrition is required for ACP Protein Production in *Tribolium*

In regards to methodology, thus far, I have been conducting several experiments to gain data for my research. Most of the experiments require the collection of adult male *Tribolium* and dividing them into two groups—one group fed with the standard diet and the other being starved—in order to compare the male reproductive organs of the beetles who lack nourishment and those who do not. After four days of either feeding or starving, the beetles were collected and the following experiments were conducted.

Fig.2

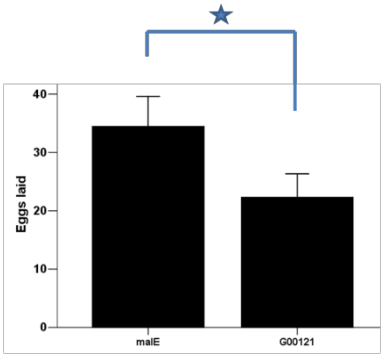


The first experiment was to check the total protein level in the male accessory glands. This is done by first dissecting the male accessory gland under the microscope, because it is the current male reproductive organ of interest, followed by crushing the male accessory glands in 50 microliters of a PBS solution, and using either Bradford's reagent or the Nanodrop machine to determine the total ACP protein level. The results are consistent with the morphological size, which is pictured in Fig.1 above, and the data for the ACP protein amount is given in Fig.2 above. As you can see, the amount of ACP protein is significantly higher in the fed males; however it is important to note that the starved males still produce some ACP protein as well.

3. Trehalose is required for the secretion of male accessory gland protein

The next experiment was conducted in order to confirm that trehalose is required for male reproduction. This experiment involved the injection of G00121 dsRNA, which is the trehalose transporter, and the control, malE dsRNA, into newly emerged adult male *Tribolium.* These males were then mated with normal females after 5 days post injection.

Fig.3



From the results gained, it can be concluded that trehalose is indeed required for male reproduction. This is drawn from the fact that the secretion of ACP proteins in the male requires trehalose to be present within the male accessory gland in order to secrete viable protein. As illustrated in Fig.3 to the left, when the trehalose transporter, G00121, is knocked down in the *Tribolium* there is a significant decrease in the amount of eggs laid by the females they mated with, in comparison to the control. The transporter G00121 is specifically used to transport trehalose from the haemolymph to the male accessory gland. The data suggests that G00121 must be present in order to transport trehalose to the male accessory gland, thus maximizing the amount of viable ACP proteins secreted. With a sufficient amount of ACP proteins present within the male accessory gland, the sperm will in turn be protected and nourished long enough to successfully fertilize the females' eggs.

4. Trehalose transporter is induced by both nutritional signaling and juvenile hormone

The next experiment required five days of either feeding or starving before the male accessory glands were dissected, and then the molecular biological experiments outlined in the previous paragraphs above were used to check expression levels with the real time polymerase chain reaction. In another experiment, double stranded RNA of JHAMT, Met, and insulin like peptide 2 (ILP2) were used to inject into the beetles, to obtain whole body samples to trace the trehalose amounts after injection.

Thus far, it has been determined that the transporter G00121 is expressed from the dissection of the male accessory glands. From day 1 to day 4 of a normal male *Tribolium* the levels of G00121 increase (Fig.4A). Thus, G00121 is in fact directly involved in transporting

trehalose from the haemolymph to the male accessory gland. This statement is true for both fed and starved beetles (Fig.4B).

Fig.4A

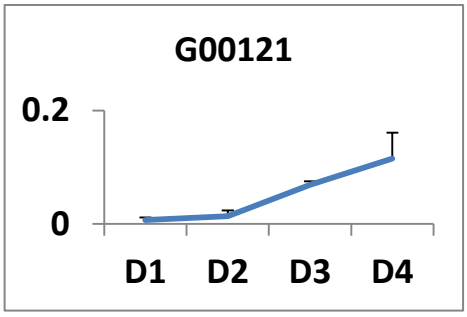


Fig.4B

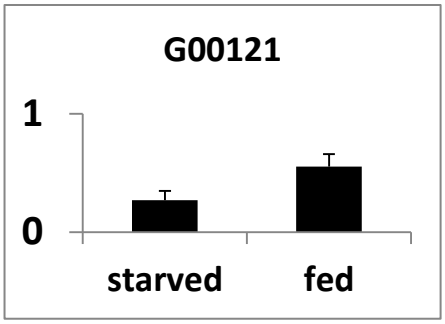
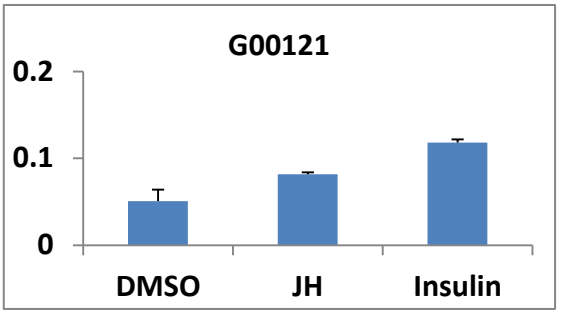


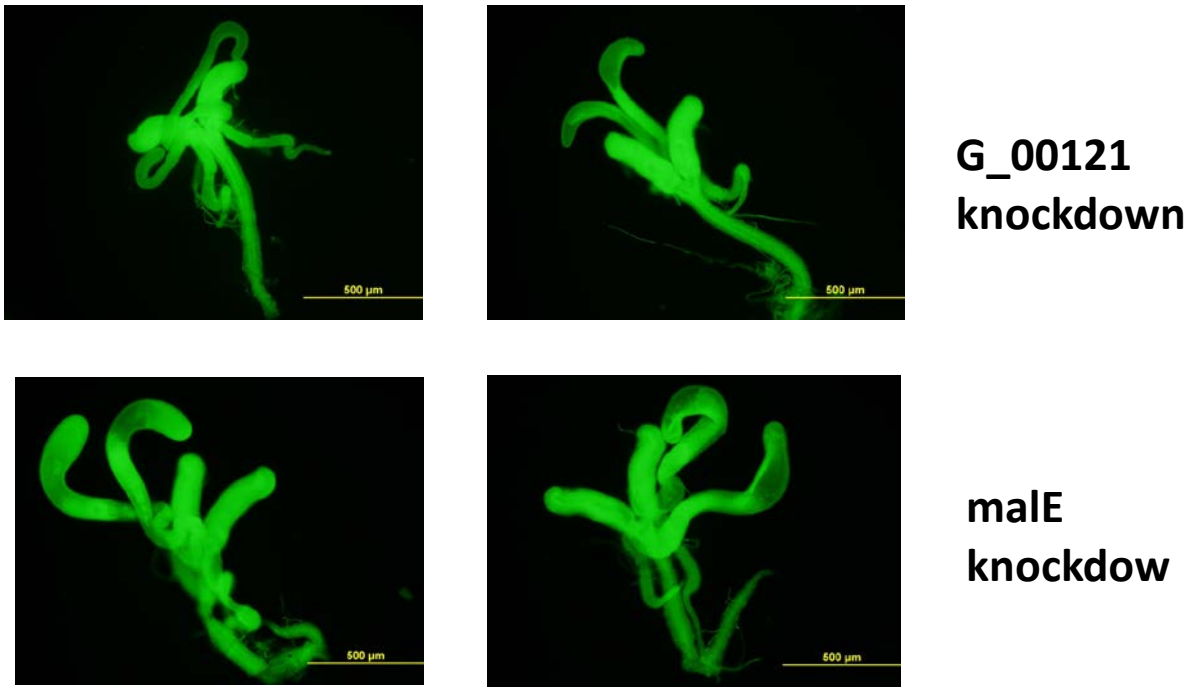
Fig.4C



5. **<u>Knock down in the expression of gene coding for G00121 led to less amount of ACPs</u>**

In order to confirm that the knock down in genes coding for G00121 would decrease the amount of ACPs, G00121 dsRNA as well malE dsRNA were first injected *Tribolium* males. After three days, the males' male accessory glands were dissected in order to take pictures under the microscope, shown in Fig. 5A. It is obvious that after knocking down the genes coding for G00121, that the male accessory glands were visibly smaller than the control. This simple fact confirms that when G00121 is not present, that the concentration of ACPs is also less. Thus, the trehalose transporter, G00121, plays an important role in synthesizing the ACP proteins in the male accessory gland.

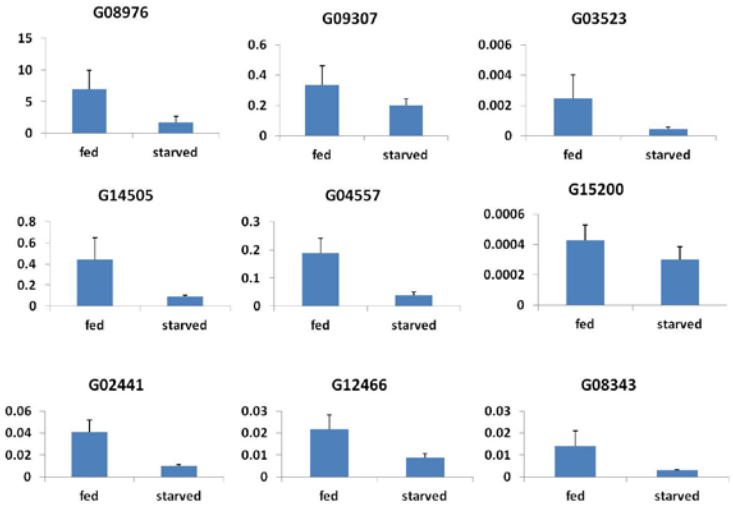
<u>Fig. 5A</u>



6. **Trehalose transporter promotes the secretion of feeding-induced ACPs**

To check if the trehalose transporter would promote the secretion of feeding-induced ACPs, the *Tribolium* males were again split into two groups, either feeding or starving the males for five days. After five days the male accessory glands were dissected to isolate the RNA present. The isolated RNA was then converted into cDNA in order to run a real time PCR to check the mRNA expression of ACP. The data from this experiment is shown in Fig.6A. As you can see there are 9 different ACP genes that showed a higher expression in fed males than those in starved males, confirming our hypothesis, both the MAG size and the ACP amount.

Fig.6A



In conclusion, nutrition is required for ACP secretion in the male accessory gland, which is vital to sufficient male reproduction. The size of the MAG is directly proportional to the production of G00121 trehalose transporter. With nutrition, the Juvenile Hormone induces the G00121 transporter, which then transports trehalose to the MAG, ultimately regulating ACP production. Thus, trehalose synthesis in the MAG depends on the production of G00121 which in turn depends on the amount of nutrition available, verifying the initial hypothesis.

Citiations:

- Riddiford, L.M. "Cellular and molecular actions of juvenile hormone." *Advanced Insect Physiology*. 47.9 (1994): 24, 213-227.
- Sheng, Z., Xu, J., Bai, H., Zhu, F., and Palli, S.R. (2011). Juvenile hormone regulates vitellogenin gene expression through insulin-like peptide signaling pathway in the red flour beetle, Tribolium castaneum. The Journal of biological chemistry 286, 41924-41936.