The intermediate metabolism of the fat bodies leads to the production and the secretion of lipids and proteins into the hemolymph where it is then utilized by organs and tissues of the insect. Diapause is an energetically demanding process that requires the acquisition and sequestration of large amounts of energy molecules in the form of TAG in the fat body and SPs in the lymph.

* The first step is to quantify and qualify the physiological differences between the diapausing and non diapausing phenotypes of ECB UZ and BE races.
* Qualify and quantify neutral lipid and storage protein content.
  + Whole insect then localized composition in fat body and lymph
  + 4th instar (20?day)🡪 5th instar (35 days)🡪 [Diapause every 7 days]🡪 Pupa
* The next step would be to add some diagnostic information about the levels of AKH in the whole insect
  + Excise glandular lobe of corporum cardiaca
  + Test lobe and insect for AKH

The second phase of this study would involve hormone presentation. The insect would be presented with diet supplements that “burn TAG”

* 4th instar (20?day)🡪 5th instar (35 days)🡪 [Diapause every 7 days]🡪 Pupa
* Excise glandular lobe of corporum cardiaca
* Test lobe and insect for AKH
* Test larva for TAG and SP levels

Doing this would help establish a causal link between AKH and SP production, and further, a link between its role in intermediary metabolism and hormone signals.

SO I would like to start my project off by looking into the distribution of proteins and lipids and how those components change over time. During the life history of the *O. nubilias* there is an accumulation of lipids in the fat body and an accumulation of proteins in the hemolymph. I want to quantify the accumulation of both of these energy storage components. Understanding how these stored components change over time will allow me to make some predictions about the energetic needs of these animals over time. *O. nubilias* presents a unique opportunity to investigate these energy components. Available to our study are the following 4 phenotypes: UZ diapausing, UZ nondiapausing, BE diapausing, and BE nondiapausing.

My investigation will take on a couple of phases with the ultimate goal of producing an informed characterization these components over time. To investigate the composition of the fat bodies over time I will be culturing the 4 different phenotypes simultaneously. These colonies will be used as a resivoir of materials.

**My research is looking to ECB for possible means of control**. I like to think of it as having **two phases**.

ECB does X amount of damage to x plant every (season?) They feed on a wide range of plants (robust herbaceous plants with large stems for boring).

The interesting thing about ECB is that it has two distinct host strains. These are different because pheromone associated behaviors and their time spent diapausing.

* ECB, depending on the strain, ECB has one to multiple generations per season.
  + It takes 50 days (hatch to adult) to complete development
  + They diapause as 5th instar larvae
  + Z strain oviposit in July, E strains oviposit in july then again in August (Roelofs et al. 1985)
  + Z and E strains develop at different rates
    - Z strain life history: these tend to pupate longer
      * Only have one generation
      * Adults emerge from May to late June, then females oviposit mid may to mid June.
      * Eggs hatch late June early July. They feed and grow for about three weeks then go into 5th instar diapause (Bohnenblust and Tooker 2016)
    - E strain life history
      * More than one generation
      * 1- Adults emerge from May to late June, then females oviposit mid may to mid June. Eggs hatch late June early July. They feed and grow for about three weeks then go into 5th instar diapause (Bohnenblust and Tooker 2016)
      * 2-the second generation adult emergence happens in late July-end of August. Hatch 7days, then 3 weeks to 5th instar then diapause. Approx September. (Bohnenblust and Tooker 2016)
* The first phase (Master’s) is to figure out some of the physiology that supports this insect’s (physiological ability to).

After we have strong evidence that there is an observable quality represented in the ECB between the two strains we can move towards phase two.

* The second phase (PhD. phase) we will use the knowledge we learned from how this ability functions at the whole insect level and try and figure out how that ability works at a molecular/metabolic level.

The goal of all of this is to hopefully find a mechanism that we can take advantage of for the control of this insect and maybe even apply some of this knowledge to other insects with this same ability.

**Problem: What is the big deal? What is this insect and why does it matter:**

* Agricultural influence
* Economical influence
* How does ECB effect
* ECB is a pest on corn and is able to overwinter as a larvae
* ECB has two strains
* ECB diapauses as larvae
* Does ECB maintain any metabolic activity during diapauses

**Project Focus: What am I doing as it relates to the problem:**

* Why does this insect diapause?
* Is there a difference in energy storage/energy consumption between diapausing and non diapausing larvae
  + If the larva begins down the diapause path does this change the rate of energy storage and does it affect energy consumption
  + Is there a difference in energy storage based on host plant consumption
  + What does ECB use as energy storage
* How is diapause induced and when is diapause induced
  + Is there a specific discrete moment in ECB life history where diapause becomes inevitable?
  + Besides photoperiod and temperature what are the other indicators of diapause?
  + Can diapause be terminated? Can it be induced?
* What happens after the larvae exit diapause?
  + Physically what does the ECB do?
  + Is there another period of energy storage for pupation? Does it differ from the path used during diapause?
* Where does the energy for diapause come from? Fat, muscles, other
  + What does the insect need to eat in order to remain in diapause?
  + How does the insect food correspond to energy storage in the ECB? How much of what is needed? How much does it use during?
* How are the two strains maintained? What keeps them different? Does this difference contribute to their diapausing differences?
  + Phenotypically what are the differences? Size, shape, color, habits, life history?
  + Are there phenotypic differences that keep the strains different
  + Genetically what are the differences? Genes, gene markers, gene expression?
  + Are there genetic differences that keeps the strains apart?

**Impact: Does my project actually help solve the problem**

* How will your project be applied to the problem?
* Are there any other questions that can be solved by using this research
* Where will this project lead, what questions still need to be asked about this project?
* Biological Pesticides

DIapsuse is determined by the length of day

Its prepared for in the early 5th instar, the system continues to feed through the 5th instar then feeding stops in line with a prepupal diapause

* Prepupal is mature non feeding endopterygote

**POSSIBLE PROJECT DIRECTION**

AMPK signal transduction possible focus on the pattern and how to identify the patteren then we can talk about the process

* Cellular energy sensing unit🡪 look it up

Big lean vs big fat:

* How does that effect diapause timing
* rna knockouts of storage proteins, lipid inhibitors, etc
  + wei wah energetic metabolites h. armigera

**THINGS WE WILL LOOK INTO**

Using that paper I found as a base for experimental design….taking the products from the SDS-PAGE we will then look at the proteins using

mass spec

qpcr

antibodies

Today I had a conversation with paul shir. Appearently he thinks thjat I should be looking at some of the more metabolic causes of diapause. Admittedly he says that his knowledge of how diapause works is almost 40 years old, but with that he thinks that there is an opportunity to look into some of the metabolic things that are shutting down.

1. What are the mitochondira doing
2. How does the system achieve the metabolic slow down
3. How does the gut of the insect change or differ between diapausing and non diapausing
4. Could we pull out cell cultures i.e. fat bodies, gut cells, and see how theu are affected during diapause?
   1. If the fat body is sensitive to and is regulated by hormones. Could we use this system to look at how the fat bodies respond to hormonal changes in vitro
   2. This could be used to determine how inducible the fat body is to hormones or if the fat body is acting constitutively

Speaking to John he is eager to get me on the machine. I am reluctant to do that because I am not really sure what that means. For instance, do I need to be maklking a solution of something from a certain time in the life history then uisoing the HPLC to see what if my analyte is in the column?

1. Whaty are te analytes I will be looking at
2. What will be the metric of my system
   1. 3rd larval instar to pupation? To adult?
3. Is there a chemical component that can be tracked through the life history I am concerned with

Finally, yesterday I had a conversation with Dan where he was talking about what it is that my project COULD entail. One interesting idea was the use of lipid inhibitors and diet pills that we could expose to the system and see how that effects diapause.

**My thoughts:**

I think I would like my project to first focus on the fat body development. Looking at how the fat bodies proliferate, synthesize energy, store energy, and secrete energy. The premise of this is as follows:

* Insects must still meet energy demands even during diapause, fat bodies are a major player of energy storage and utilization.
* First we should focus on some of the things that are happening inside the fat bodies…
  + What metabolites are different between the diapausing UZ and BE strains and non diapausing UZ and BE strains

**7/29/16**

So my meeting dan Hahn was interesting today. On one hand I feel like I am getting closer to what it is he is trying to get me to realize. I do not like how he is constantly interchanging the terms process and pattern. However it is incumbent upon me to keep these things in line in my own mind.

As far as my project is concerned, I brought to his attention a couple of things about what I am interested in doing and he seems interested.

* I mentioned this idea of removing the coporum cardiaca from the insect and then looking at the levels of AKH in the body and in the corpora cardiaca.
* Also I was talking to him about the possibility of doing a lipideomics survey of the lipids in the fat body and he advised me to pursue it.

I want to write a methods and then I wanna start with the lipids that are synthesiezed….. I will keep you posted