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Ovary Staging Analysis of the Female Adult Blow Fly, Lucilia sericata

Ovary Staging Analysis in Female *Lucilia sericata*

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Introduction

Lucilia sericata, the green bottle fly, is a significant organism with forensic applications in determining post-mortem interval (PMI) of deceased organisms and individuals due to the insect's ability to feed on decaying organic matter and human remains (Tomberlin et al. 2011). Insect colonization and the following series of introduction of new species to the decaying system is well understood. It is known that *Lucilia sericata* is one of the first species of insect to colonize and feed on the decaying matter. Adult female *L. sericata* are appealed to decaying matter to fulfill their dietary needs of protein consumption. Protein is required for production of sex pheromones, egg development, and aiding sexual development (Wall et al. 2002).

Laboratory protocol for culturing blowflies typically consist of a dietary treatment containing carbohydartes and protein which is required by females. A dietary treatment with protein is shown to be correlated with the insect's development of ovaries (Rachman, 1980).

The PMI of a deceased individual can be determined based on the developmental life-cycle stages of *Lucilia sericata,* which is well understood. However, these insects are cultured on laboratory methods and little is known regarding the effects of dietary timing and condition on ovary and egg development. In order for a more accurately determined PMI to have forensic applications, the effects of the dietary regime on the eggs and ovary need to be analyzed.

Materials and Methods

Pupa were separated into individual containers and stored until emergence. Upon emergence, the flies were separated according to sex and housed in Bug Dorms. The adult flies were placed into two types of groups: Group #1 included both male and female flies (1:1 ratio) and Group #2 consisted only of females. Both groups received identical dietary treatments consisting of a 1:1 honey:water mixture *ad libitum*, and >5g of liver. Fresh dietary substrate was supplied to each cag every other day throughout the duration of the experiment. Water was given to the flies *ad libitum* via a watering mechanism which used capillary action.

Both groups were observed under controlled conditions of temperature (21-23°C), light (12:12 light:dark cycle), and humidty (25%).

Three females from Group #1 and Group #2 were removed at twelve hour intervals per day (7AM and 7PM). Removed flies were preserved in 70% ethanol prior to dissection. The female flies were dissected under a microscope with the use of tweezers. The exoskeletons of the flies' abdomens were removed imaging of the revealed ovaries were used for analysis.

Ovaries were staged using two methods. The first staged the organs using a known 0-3 scale. In this scale, 0= no egg development and 3= full egg development. The second method of staging uses length and width measurements to calculate and area of the ovaries. The resulting data were averaged for a specific time point and presented with standard deviation.

Figure 1.Ovary Staging vs. Day Post-Ecclosion

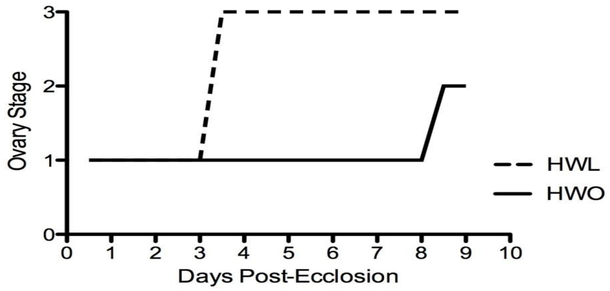

Figure 2. Ovary staging based on non-vitellogenic, pre-vitellogenic, post-vitellogenic at days post-ecclosion.

Figure 3. Ovary Stages

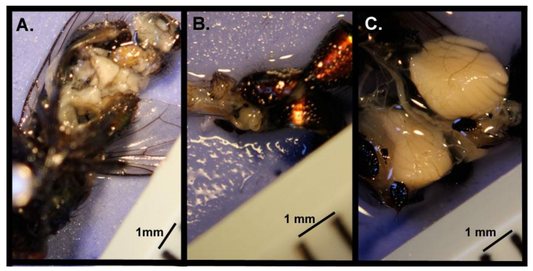

Figure 3. Ovary stages of dissected images: non-vitellogenic (A), pre-vitellogenic (B), vitellogenic (C).

Figure 2. Ovary Area vs Days Post-Ecclosion

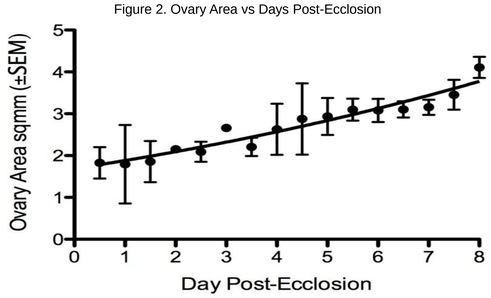

Figure 2. Linear regression analysis of ovary area vs day post-ecclosion Linear Regression (R square 0.9168, Degrees of Freedom 13, P< 0.0001).

Results

Figure 1. contains the results of ovary staging based on non-vitellogenic (1), pre-vitellogonic (2), and vitellogonic (3) analysis of the dissected ovaries. From Day 0 to Day 5 the HWL (Honey Water + Liver) and HWO (Honey Water only) female flies were non-vitellogenic. However a large jump between ovary stages occurs between Days 3 and 4 occurs for the females subject to HWL, whereas the female flies exposed to HWO remained non-vitellogenic at that point in time. For HWO flies, a change from non-vitellogenic to pre-vitellogenic/vitellogenic occurs between Days 8 and 9 (Figure 1.). Figure 2. represents a linear regression between the two variables: day and ovary area. Similar to the observation in Figure 1., the regression analysis indicates an increase in the ovary area between Days 3 and 4 which shows sigmoidal tendencies.

Discussion and Conclusions

Results from this experiment indicate that there is statistically significant difference between ovary area and the day of ecclosion (R^2= 0.91). The data allows us to conclude that a dietary treatment consisting of HW+ L causes female *Lucilia sericata* to reach the vitellogenic state three days after ecclosion (five days earlier than *Lucilia sericata* without a liver as a protein source). Therefore, this information allows us to better understand and easily predict what day the female flies will be sexually active, as well as when their ovaries will be fully development.

Although previous laboratory protocols for breeding *Lucilia sericata* exist, ovary and egg developmental effects are not understood for a dietary treatment containing carbohydrates and protein. Rachman concluded that insect vitellogensis is correlated with a diet containing protein consumption (1980). Results from this study are supporting evidence to Rachman's claim, and also provide a more accurate time frame of when ovary and egg development occurs which has forensic applications.

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