Summer 2024

Protein Turnover Rate and Life History as an Explanation of the Extreme Difference in Cost of Biosynthesis in Cockroach Nymphs and Painted Lady Butterfly Larva

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PROTEIN TURNOVER RATE AND LIFE HISTORY AS AN EXPLANATION OF THE EXTREME DIFFERENCE IN COST OF BIOSYNTHESIS IN COCKROACH NYMPHS AND PAINTED LADY BUTTERFLY LARVA

by

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A THESIS

Presented to the Graduate Faculty of the

MISSOURI UNIVERSITY OF SCIENCE AND TECHNOLOGY

In Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE IN BIOLOGICAL SCIENCES

2024

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ABSTRACT

The cost of biosynthesis in painted lady caterpillars is far lower than that of a red runner cockroach. A promising explanation for this phenomenon is the protein turnover rate for the animal during biosynthesis and tissue maintenance. Previous research has shown that, per gram of body weight, the painted lady butterfly can synthesize a biomass using up to 15x less energy and resources than the cockroach, even under similar conditions. The resulting ratios, especially in respect to growth rate, show a wide disparity between species in such a manner that does support a protein synthesis and degradation rates as a partial explanation for the extreme difference in the cost of biomass.

ACKNOWLEDGMENTS

Thank you to Dr. Chen Hou, for all the assistance, Dr. Julie Semon, Dr. Yue-Wern Huang, and Dr. Matt Thimgan, my committee members, for your guidance.

Thank you to my fellow graduate students in the Biological Sciences Department at Missouri University of Science and Technology for your help and companionship.

Thank you to my mother and grandmother for providing the motivation I needed to complete my college education.

Thank you to my father for facilitating me.

Thank you to my siblings, for inspiring me and remaining by my side throughout.

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1. INTRODUCTION

Aging is a well-known phenomenon with many unknown mechanisms, as it holds a multitude of variables. There has been extensive debate on the energy allocation of organisms for their lifetime energy budget, as well as how this affects their lifetime. Life history does affect this energy budget, and thus aging; however, the severity of this tie is unknown. Due to this unknown, it can be assumed that the equation regarding energy consumption is flawed or otherwise inaccurate for lacking these considerations (Sterner & Elser 2002).

The focus of this work is to determine the maintenance portion of the budget in relation to the biosynthesis of protein within two severely different life histories. The main difference is metamorphosis, which is a major biological process that includes reworking all the structures during the pupation period. It is hypothesized that less maintenance is required before metamorphosis, as the organism would be more interested in accumulating biomass for later transformation than the quality of the mass (Elser, J et al 2003). The animal without the option of reworking the tissues would have a much higher interest in maintaining healthy and well-functioning tissues. Thus, one holometabolous (complete metamorphosis) and one hemimetabolous (incomplete metamorphosis) insect species are chosen and assessed in their larval stage.

1.1. HYPOTHESIS

The extreme difference in the cost of biosynthesis between the cockroach and the caterpillar is due, in part, to protein turnover rate, which is directly affected by the life history of the organism.

1.2. OBJECTIVES

Calculate and assess the protein:RNA ratio in comparison to growth and the animal's life history to account for difference in energy expenditure between species.

2. BACKGROUND

2.1. DIFFERENCE IN BIOSYNTHESIS COST

The cost of biosynthesis is described as the amount of energy required to create and deposit a new unit of biomass. This includes not just the energy put into the actual mass itself, but rather the energy lost through heat and other means during manufacture and deposition. The cost of biosynthesis can be calculated with the parent metabolic rate equation, where B is metabolic rate, Em is cost of synthesis, G is growth, a is activity, M is mass and b is maintenance (Figure 2.1). By taking the cost of deposition (Em), multiplying it by the rate of growth (G), and then dividing that total by the total metabolic rate (B), the resulting value is the cost of biosynthesis is equal to the cost of synthesis multiplied by the cost of growth, divided by total metabolic cost. Activity (a), mass (M), and maintenance (b) are held constant and thus are not required.

$$B = E_{\mathrm{m}} G + aM^{b}$$

Figure 2.1. Metabolic Equation

Of the variables in that equation, the metabolic rate is the most debated. Though it has long been correlated with size, there are many outliers to that correlation. This begs the question, why do those outliers exist and what makes them this way? One such outlier is holometabolous, or fully metamorphosing animals. Holometabolous animals have a much

higher growth rate with a lower comparative metabolic rate for their body mass (Figure 2.2).

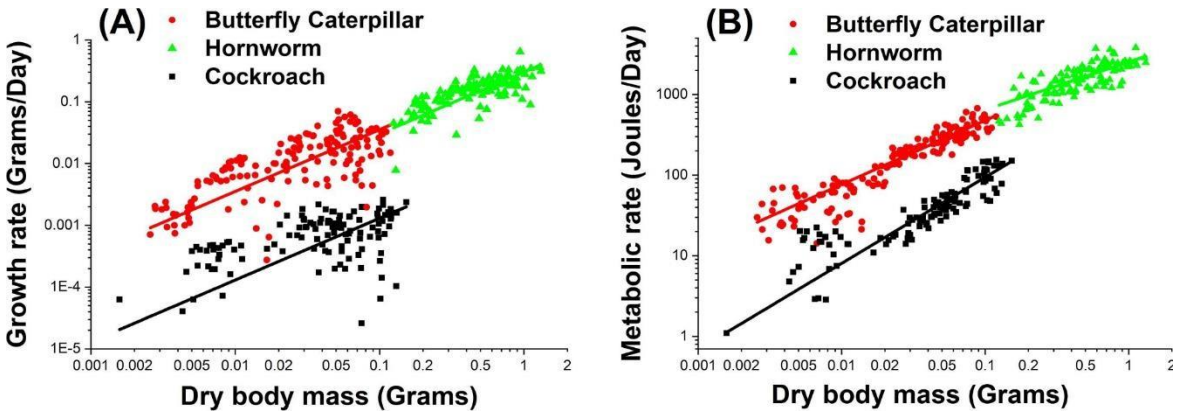

Figure 2.2. Metabolics and Growth Rate

Figure 2.2 displays growth rate (grams per day) compared to dry body mass(grams), and metabolic rate (Joules/day) compared to dry body mass(grams) in caterpillars, hornworms, and cockroaches. It can be clearly observed that while cockroaches have a lower growth curve, they have a steeper metabolic rate slope, and a larger relative metabolic rate per growth rate.

When compared side by side, the cost of the cockroach synthesizing and depositing a unit of biomass is 27x larger, even though the size of the unit of biomass is the same and the overall metabolic rate and growth rate of the cockroach is lower (Table 2.1). On average, cockroaches allocate 17.9 ± 14.4%, while caterpillars allocate 3.1 ± 2.5% of their energy to biosynthesis (N. Ferral, et al. 2020). The high variation the cockroach's energy allocation is due to the variability in their lifespan and the slowness of their growth, it allows for more modification as needed in the energy budget, while the caterpillar is forced to remain on course, with only minor energy reallocations recorded,

likely due to the tight schedule and prioritization of the accumulation of biomass for metamorphosis.

Table 2.1. Comparison of Cost Synthesis (Em), Growth (G), Maintenance and Activity (BMA) and Total Metabolic Rate (B) in Cockroaches and Caterpillars

	E_m (Joules/gdbm)	G (grams/day)	$B_{M,A}$ (Joules/day)	B (Joules/day)
Cockroach	6905.4	$G = 0.0130M$	$B_{M,A} = 1261.0M^{1.165}$	$B = 1101.9M^{1.068}$
Caterpillar	335.6	$G = 0.354M$	$B_{M,A} = 2982.4M^{0.804}$	$B = 2976.5M^{0.794}$
Ratio of caterpillar to cockroach	0.049	27.2	7.06 ± 3.06 (M varies from 0.002 to 0.15 g)	6.1 ± 1.87 (M varies from 0.002 to 0.15 g)

2.2. LIFE HISTORY

The life history of an organism in this context is based on its evolutionary history rather than its personal history, as how an animal would budget its energy would be based on how it evolved to survive (T. Sousa, et al. 2006). Life history refers to the history of the species and the adaptations developed, while personal history refers to the life and adaptations of that singular individual. For example, a caterpillar would have a strong desire to accumulate biomass in order to facilitate metamorphosis, but the quality of this tissue doesn't have to be superb as it will be torn down and rebuilt. The quality of the tissue referencing the resistance to stressors, and tolerance for error within the protein folding. However, a cockroach, which has no pupation stage, would be interested in maintaining a

higher quality tissue as there will be no tear down or rebuild phase for the tissue to erase any errors made in the production of the proteins. (N. Ferral, et al. 2020).

Life history is a major component of this project. Here we can explore the specifics of the difference between the life history of the Turkestan Red Runner Cockroach (S. lateralis) and the Painted Lady Butterfly (V. cardui). We will be focusing on the nymphal stages, as the adult forms occur after metamorphosis.

The Painted Lady has a wildly different approach than the Cockroach. While the Cockroach is hemimetabolous, meaning it does not metamorphosize fully, the Painted Lady is holometabolous, or fully metamorphic. The entirety of the caterpillar's body breaks down, save for the cell disks that migrate and direct the rest of the tissue slurry into rebuilding. The Cockroach does not undergo this process, though its nymphal stages do shed their exoskeleton over time with sexual maturation of the organ systems upon the last shed into an adult. There is no overarching breakdown and rebuild as with the Painted Lady.

Thus, it is reasonable to assume there would be a lesser tolerance for protein synthesis errors in the tissues overall, as the errors would accumulate over time and negatively impact the organism's overall fitness over time.

As can be seen in Figure 2.3 A and B, the painted lady caterpillar has an overall lower resistance to general cell death and specifically apoptosis when placed under oxidative stress (Iromini, Taiwo Bolanle 2020).

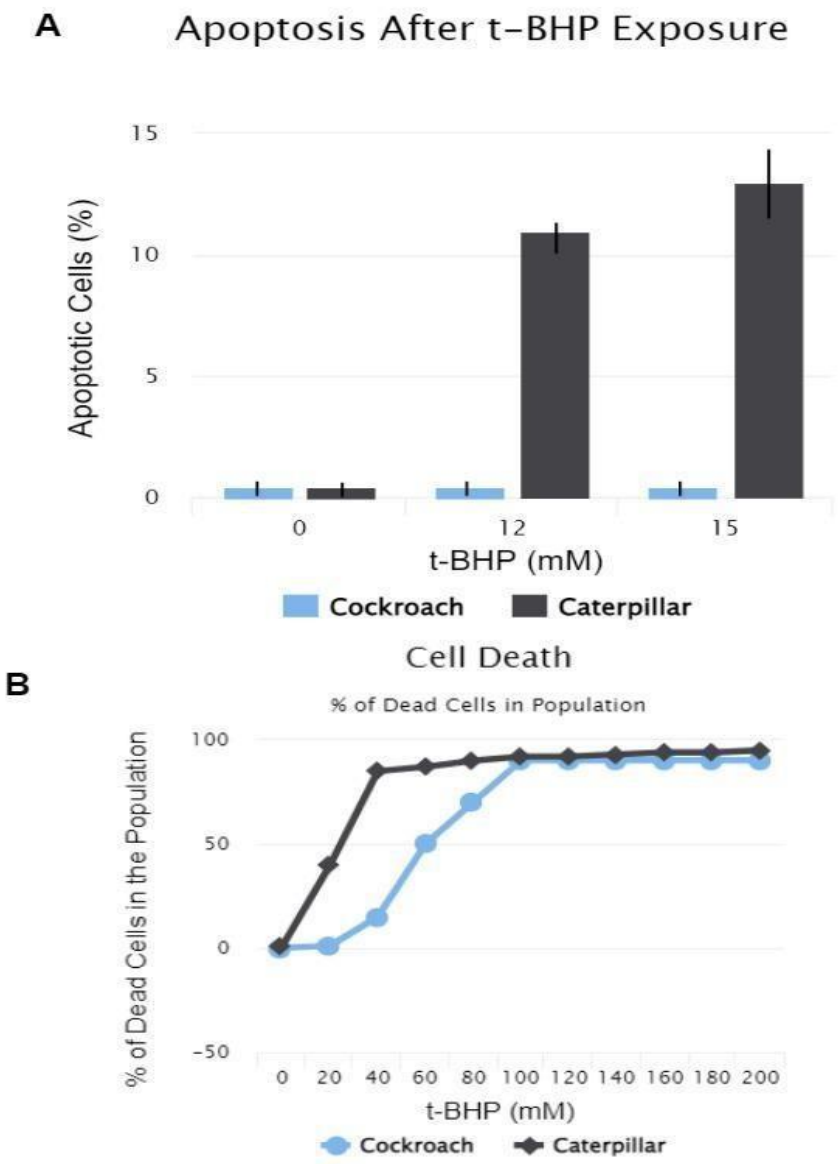

Figure 2.3. Apoptosis and Cell Death in a Population after Exposure to t-BHP

This implies a hardier cell that's more resistant to these stressors. This could in fact be due to a more rigorous synthesis and degradation cycle, and thus a lower tolerance for protein synthesis error. The excess energy cockroaches seem to spend could very well be spent on correcting errors while caterpillars allow for them to accumulate, only to be corrected upon pupation (Worm et al. 2002).

2.3. PROTEIN ERROR THEORY

The Protein Error Theory of Aging describes two modes of behavior within the system. One mode exists in which there is little to no correction, until errors pile up and the organism succumbs to error catastrophe. Error catastrophe occurs when whatever value of error is catastrophic for cell survival. The seconds mode theorizes that the error frequency will stabilize and may or may not be toxic to life (Edelmann P. and Gallant J 1977). Both modes have been viewed in laboratory settings, however mode II is much more compatible with life and appears to be the most common mode as of now. As it has been found no single variable is responsible for organism aging, it is much more likely it is a single factor rather than the catastrophic viewpoint.

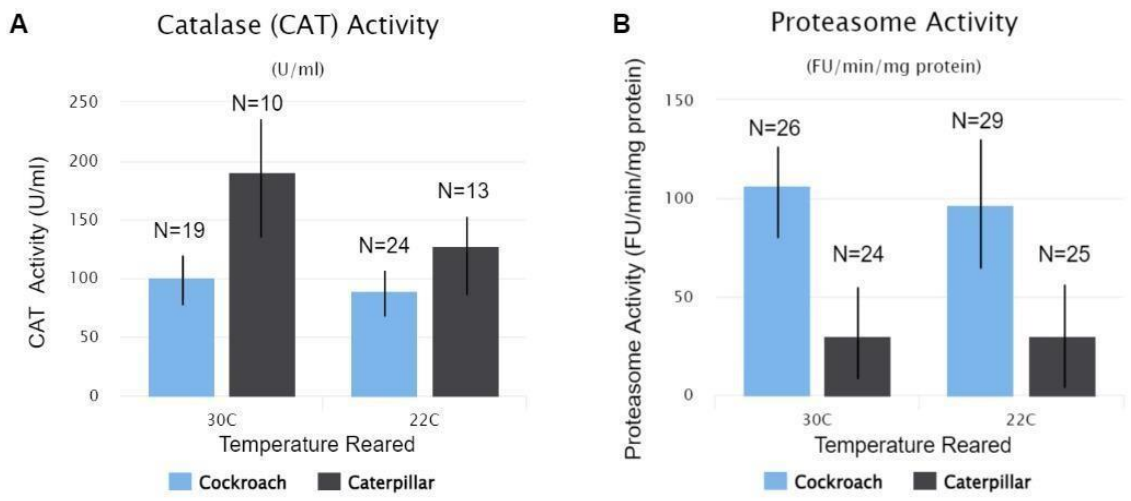

Figure 2.4. Proteasome and Catalase Activity in the Cockroach and Caterpillar at 22 C and 30 C

As can be seen in Figure 2.4 A and B, the data supports the protein error theory, as cockroaches have a much higher proteasome activity, while caterpillars are shown to have a much higher catalase activity. This supports more preventative measures and hardier

cells on the cockroach's part, and lower quality protein and a much higher cell sensitivity to oxidative damage in the midgut.

2.4. QUALITY OF PROTEIN

The amount of protein found in a sample is the result of synthesis and degradation. Protein synthesis rates have been proven to be regulated by RNA, and degradation of misfolded or miscoded proteins by the pathways of proteasome activity or lysosomal proteolysis (Sinauer A. 2000). Thus, it stands to reason that if mistakes are less tolerated, these low-quality proteins being imperfect and not the most efficient but still functional, there will be less RNA as fewer proteins will be created. However, if there is a high amount of RNA with a low number of present proteins, it stands to reason that the proteins are being created, but the rate of degradation is high.

When describing the quality of the protein, the function, fold, and longevity of the protein must be considered, as well as the production of proteins. However, the characterizing of individual proteins and their quality can quickly become expensive and tedious. Rather the previous RNA-protein relationship knowledge to evaluate the possible quality of the protein relative to other organisms. Based on previous studies, the more RNA present, the more protein would be expected (Kristen, A. R. 2013). However, not all proteins are created equal. If a protein is mis-made or misfolded in an intolerable way to the system, the protein will be broken down and remade. If there is a higher presence of RNA than protein, relative to the organisms in question, then it can be assumed there is a higher amount of protein degradation and resynthesis, due to a lower tolerance of error. If there appears to be quite a large amount of protein in comparison to the RNA, relative to

the organisms, it stands to reason that more error is tolerated and more mistakes remain present.

2.5. PROTEOSTASIS

Proteostasis is the process with which protein is regulated within a cell in order to maintain the health of the cell, and thus the health of the organism. In this specific case, we utilize the data from Figure 2.4. The proteasome activity denotes some measure of quality control, as a higher rate of activity correlates with more errors being corrected as more protein is degraded and resynthesized. The catalase activity relays how sensitive the organism is to oxidative stress.

While these give us a window into proteostasis, it does not directly relay the rate of synthesis occurring, rather it only relays how much damage is being repaired and/or how many mistakes are being corrected. To calculate the rate of synthesis, an equation is used.

$$\omega_{\mathrm{p(av)}} = \theta \bullet \left[\boldsymbol{m}_{\mathrm{RNA(av)}} / \boldsymbol{m}_{\mathrm{p(av)}} \right]^3$$

Figure 2.5. Protein Synthesis Equation

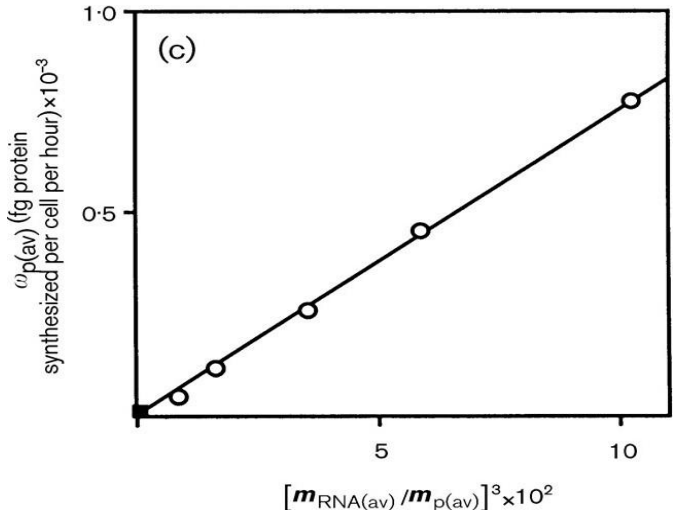

Figure 2.6. Protein Synthesis Equation Chart

In this equation, the specific protein synthesis rate in fg of protein synthesized per cell per hour ($\omega_{p(av)}$ is calculated by multiplying a constant 7.5*10^3 (Θ) by a ratio of average mass of RNA in fg per cell ($m_{RNA(av)}$) and average mass in fg of protein per cell ($m_{p(av)}$) (Cox, et al. 2003) (Karpinets, et al. 2006). This equation is included for further evidence for the RNA/protein ratio as a valid form of describing protein synthesis. Adding growth rate to this ratio will account for it for a more accurate comparison in the results.

3. MATERIALS AND METHODS

3.1. PARTICIPANTS

3.1.1. B. lateralis. B. lateralis was chosen due to its quick growth rate and hemimetabolous traits. The object of study is the difference in cost being due to the difference in strategy, as well as providing continuity for previous works. B. lateralis is also a tropical insect, and thus will have the same seasonal strategy, which simplifies the complex variables at play. B. lateralis is also well studied, readily available, and an expected range of protein. Selection of subjects is based upon age and mass in relative capacities to the growth pattern of the species. As such, B. lateralis samples were chosen based on body mass, as shedding can be difficult to detect and highly unpredictable. Only nymphs are eligible, as the adults no longer grow and thus are beyond the scope of study. Thus, the samples were chosen based on two criteria: 1) body size, and thus probable ease of midgut extraction and 2) body ranges similar in relative size and variety to that of the other participant, V. cardui. Due to their differences a direct sameness is not achievable, thus relative sameness based on that species growth rate and body size range is used.

3.1.2. V. cardui. V. carui was chosen due to its incredibly fast growth rate and holometabolous nature, as full metamorphosis is key to the study. V. cardui is a seasonal insect whereas the larva does not encounter freezing temperatures or hibernations, like B. lateralis. This insect is easy to care for as well as dissect. Selection of subjects is based upon instar and mass, rather than just mass. This is due to the fact the instars are extremely well documented, very predictable, as well as easy to detect. V. cardui were up for selection in instars 4 and 5, as they were still growing but large enough to easily dissect.

Any signs of pupation in 5th instar samples were discarded. Any samples that appeared ill, deformed, or injured were discarded. No adults or pupa were included in this study.

3.2. DISSECTION

The tissue chosen for tissue is the midgut. This is due to the similar anatomy between each species, ease of acquisition, delicacy of the tissue, volume of the tissue, and tissue responsiveness to stress and damage. This is also in the interest of consistency, as the midgut is the tissue that was selected to test in previous studies.

The insects are cleaned via submersion in a set of three surface sterilizing solutions for 2 minutes each. The cleaning solutions include a methyl-4 detergent solution, a 20% septisol solution, and a 1% bleach solution, to remove any contaminants clinging to the insects themselves. The insects are then taken to a sterile hood, where their midguts are removed, rinsed, and placed into pre-weighed centrifuge tubes. Upon successful acquisition of the organ, it was immediately placed on ice. It was then weighed and partitioned into portions for extractions. Any spare tissue is frozen and stored at -80 C.

3.2.1. B. lateralis. B. lateralis was dissected as displayed by Figure 3.1, with the midgut, which was removed and utilized, highlighted as green. Once the organ was removed it was split down the middle and cut into several sections, each weighing between 3-5 mg. These pieces were then vortexed in PBS to remove food contaminants.

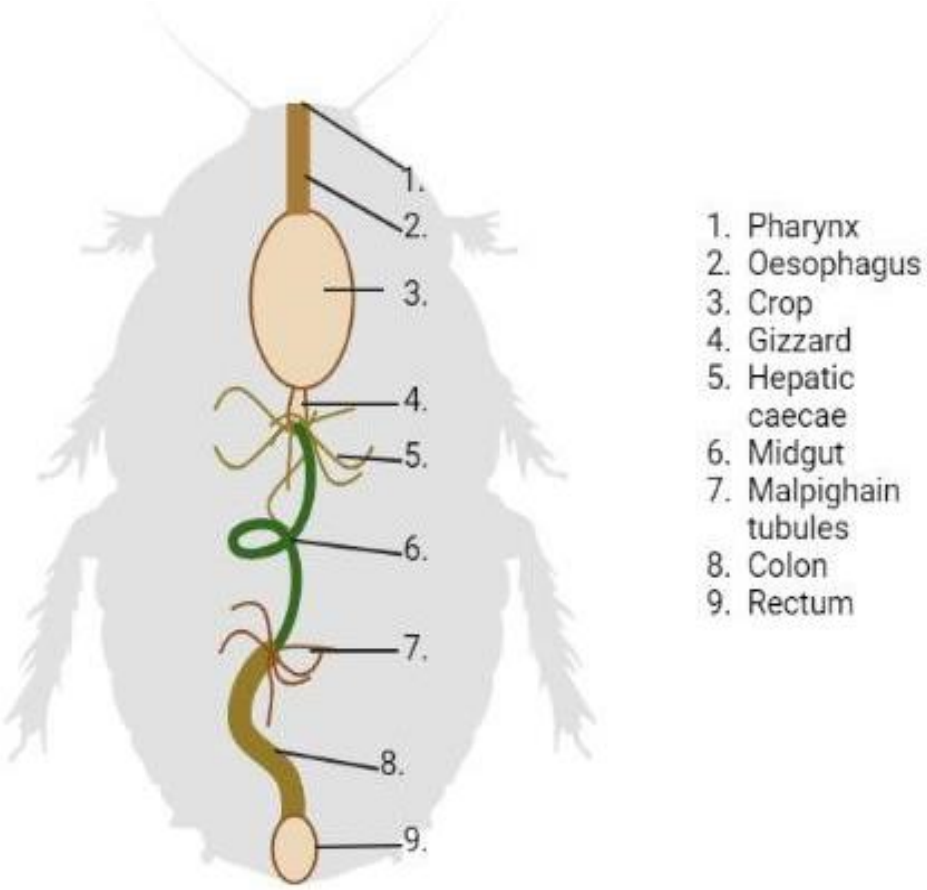

Figure 3.1. Cockroach Anatomy

3.2.2. V. cardui. V. cardui was dissected as displayed by 3.2, with the midgut, which was removed and utilized labeled and highlighted as green. Once the organ was removed the tube was split open to form a midgut sheet, which was then rinsed in PBS to remove any remaining food contaminants.

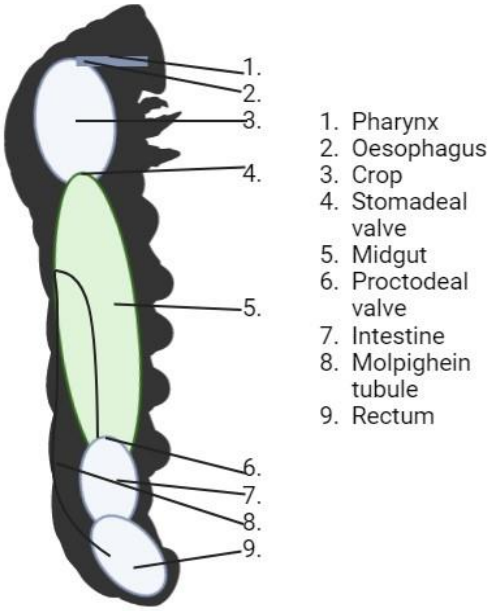

Figure 3.2. Caterpillar Anatomy

3.3. PROTEIN

3.3.1. Extraction. 1.0-2.5 mg of tissue will be used in I-PER, an insect cellular protein extraction solution, to extract the protein from the samples. A protease inhibitor and EDTA were included to stabilize the solution and prevent protein degradation or precipitation. This solution would then need to incubate on ice for 10 minutes, and then another 15 minutes on the centrifuge at 15,000 RCF. This will separate any leftover solids from the fully solubilized protein, as any solids or bubbles can affect the absorbance reading. The supernatant will be pipetted into a separate container. This entire procedure will also need to be performed on ice or in otherwise nearly freezing conditions to preserve the protein and further prevent degradation. Samples will all be taken at the same time and tested at the same time to control for decay or changes induced by the age of the sample.

3.3.2. Assay. The Bradford Protein Assay Quant-Ti kit utilizes a colorimetric dye that binds to protein and the use of a plate reader for the color spectrum to determine protein content of a sample, based on standards tested in equivalent conditions. Equivalent conditions being at the same time, in the same extraction solution, at the same temperature. Each sample is placed 3 times on the 96-well plates used, and each plate is read twice, for averaging and accuracy. Each standard is placed 6 times for the same reason. All the samples and standards are placed into the well first. Dye is then added, first on two sets of standards, and then on one set of samples, until all wells are completed. This allows for a smaller discrepancy in time incubating between samples and standards. They can either be directly linked to one another based on when they were pipetted, or an average can be taken. These values are then taken, and using the standards, run through the slope-intercept equation, where the actual protein content, rather than just the absorbance. At this point all the data will be placed in the table alongside the standard curve. These values are then translated into the quantity of protein in the sample. Divided by the mass of the tissue used, a protein content can be calculated. The Coomassie dye does have several inferring molecules that are ingredients in the IPER extraction solution, however it is stated that the test can tolerate set amounts of these detergents. All interfering molecules listed that are also contained in the I-PER extraction solution are in tolerable amounts for the test.

3.4. RNA

3.4.1. Extraction. A specific portion of tissue will be utilized for this procedure, being the midgut of the insects. Throughout this procedure all surfaces and tools will have to be cleaned with Rnase-zap in order to prevent RNA degradation. The Purelink RNA

Extraction kit from Thermofischer was utilized for the extraction of the RNA, resulting in total RNA from the sample suspended in 30 ul of RNase free water.

3.4.2. Assay. Quant-iT™ RNA Assay Kit was utilized for this procedure. Samples were plated and read in a plate reader. RNA sample solution will be plated alongside the standards to maintain equivalent conditions. Equivalent conditions being at the same time, in the same extraction solution, at the same temperature. Each sample is placed 4 times on the 96-well plates used, and each plate is read twice, for averaging and thus accuracy. Standards are given double the plating and reading for accuracy. Data acquired is then averaged for each specific sample. This value is then taken, and using the standards, ran through the slope-intercept equation, where the actual protein content, rather than just the absorbance. At this point all the data will be placed in the table alongside the standard curve, as severe abnormalities are searched for. Any abnormalities could lead to a refinement in the process. For this assay, considerations for interactions between components of the assay are not required, as the Invitrogen Broad Range Assay kit and the PureLink Mini RNA Extraction kit from Thermo Fisher were made to operate together. The only outside item in use is the Rnase inhibitor used on surfaces and tools before use, and this item does not interfere.

3.5. STATISTICS

G.Power 3.1 software was utilized for statistical analysis. ANOVA and t-tests pot hoc were performed where N = 30. P values are listed below figures.

4. RESULTS

To address the mismatch cost of variables in the metabolic equation, growth rate, metabolic rate, protein abundance, and RNA presence were all tested in order to determine the protein turnover and at least partially explain the extreme differences in biosynthesis cost.

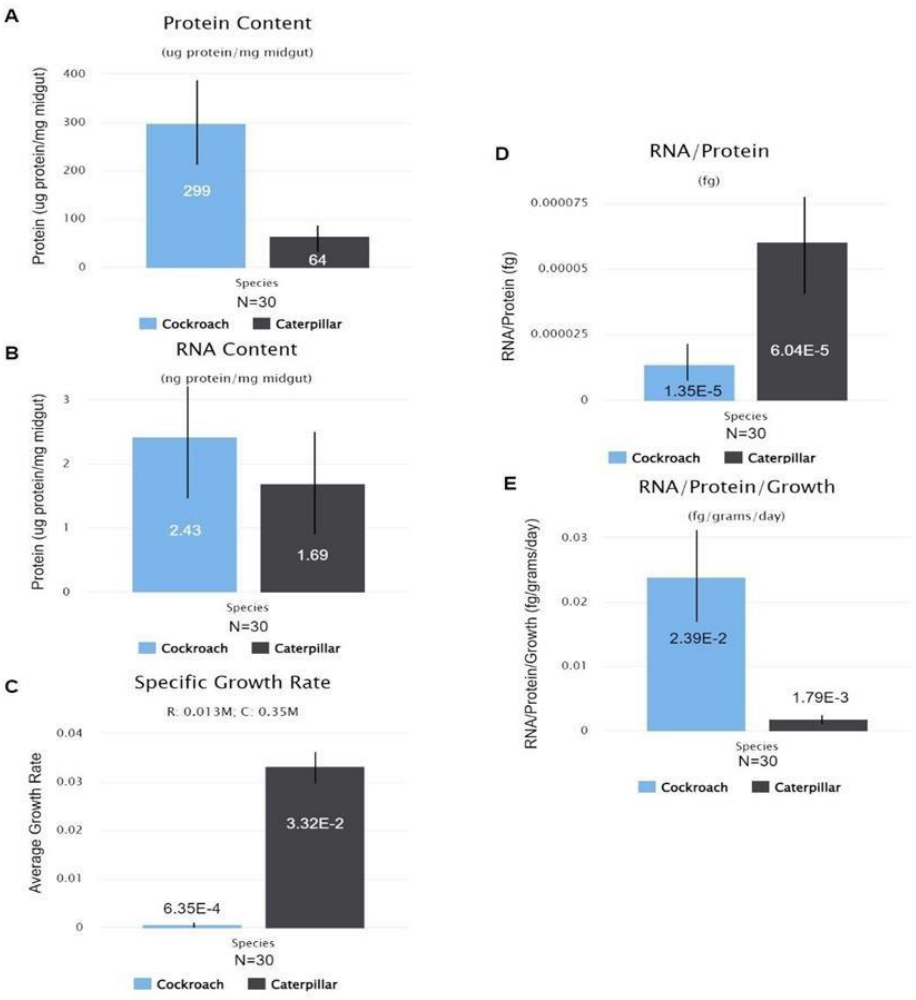

Figure 4.1. Protein Content, RNA Content, Specific Growth Rate, RNA:Protein Ratio, and RNA:Protein:Growth Ratio

In the midgut, the cockroach had 4.7x more protein (ug/mg) than the painted lady caterpillar. $p<0.00011$(A) more RNA (fg/mg), though not significantly, $p<0.77$ (B) However the growth rate (mass adjusted) of the painted lady was three times more than the cockroach, $p<0.00$, which also correlates to the RNA:protein ratios, $p>0.090$ (D). When the RNA:protein ratio is compared to the growth rate, the cockroach is three times higher, $p>0.017$. Data expressed as a mean of 30 per group, ± 0.0306 (E)

The results display a higher protein content in the cockroaches than the caterpillar per milligram of midgut tissue extracted. Simultaneously, the RNA content of the cockroach is also higher per milligram of midgut tissue. Together the ratio displays less RNA and more protein on the part of the painted lady caterpillar, and the reverse on the part of the cockroaches, by nearly five-fold, as displayed by Part A, B, and D of Figure 4.1.

This could be indicative of a larger amount of RNA required for synthesis of proteins in the cockroach, and the reverse being true in the caterpillar. Placed in the context of their growth rate, the caterpillar requires a far smaller ratio of RNA to protein to produce biomass for growth compared to the cockroach, as is displayed by Parts B and C in Figure 4.1. This may suggest that more protein is degraded and resynthesized often due to a smaller tolerance for error and misfolding compared to that of the painted lady, thus a larger presence of protein and a much larger presence of RNA would be required for this quick synthesis rate, even with a slower growth rate.

5. DISCUSSION

The preliminary data shows that proteasome and metabolic levels are higher in hemimetabolous than in holometabolous, while growth rate and catalase activity are the opposite. The cockroach grows at a much slower rate than the caterpillars, as well as spending more energy per their growth rate on new tissues. The cockroaches have also exhibited signs of a higher tolerance for stressors as shown by the proteasome and catalase activity levels, which indicate a lower threshold for protein synthesis error and a better resistance to oxidative stress.

This disparity in synthesis and growth rate could partially explain the extreme energy difference, in relation to the life history of the organisms. The pupation period of the holometabolous animals would provide ample time to repair as they restructure. The hemimetabolous animal has no such advantage, and thus must maintain integrity of their biomass throughout the entirety of their life. The caterpillar has the goal to grow while the cockroach has the goal to survive long term.

The results imply that while protein turnover does play a role in the extreme energy difference in growth and cost of that growth in caterpillars and cockroaches, it is unknown how strong this connection is, and it is certainly not the entirety of the explanation. The proteostasis displayed by the cockroach and the painted lady were vastly different, but only by ~3-fold. The difference in expenditure of energy is ~27-fold.

Other costly mechanisms must be at play to make up the difference between the two. Future studies could do well in examining the synthesis rate more directly, such as through examination of cellular production of protein and RNA overtime, while also monitoring proteasome and catalase activity over the same period within the same group of

cells. A further examination of cost disparity during the larval stage would also be helpful, as examining the excretions such as feces, silk, and milk; and other major events in the larval stage, such as molting, hibernation, and migration. These are all costly endeavors that would factor into life history and thus energy allocation. This study was limited in scope by tissue and animal life history. Future studies would do well to further examine the life history of the animal, as well as including a larger variety of species with life history considered, such as pupation, hibernation, and migration.

6. SUMMARY

The extremely different cost of biosynthesis between holo- and hemimetabolous animals has gone unexplained, while maintenance and activity have been disregarded as vital and fluctuating variables in the metabolic equation. This study has found cockroaches have a higher rate of synthesis and a larger amount of RNA/Protein when compared to growth than the caterpillars, which supports the hypothesis in these two animals. This study is limited by the ability to measure variables directly, and thus in the future more direct measurements should be investigated further, as well as a larger range of animal species including more variables such as stressors. These findings help refine the equation and further explain and demonstrate evolutionary adaptation within the energy budget.

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She will forever cherish the living jar of pond water and muck she collected from the nearby pond.