

The Negative Impact of Increased Carbohydrates and Suboptimum Temperatures on Caterpillars

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Abstract

Caterpillars prefer warmer temperatures to maintain their normal physiology because of their need to thermoregulate. Thermoregulation is a process in which caterpillars maintain their internal body temperature. Because thermoregulation is an energy expensive process, warmer temperatures make it easier for them to maintain this optimum internal body temperature. Caterpillars mostly rely on carbohydrate-based diets for energy. To gain insight on the effect of added carbohydrates on a caterpillar's diet, we tested a hypothesis that a diet high in carbohydrates may influence the survival of caterpillars in various suboptimum temperatures. In the current study, we focused on traits that are related to the fitness of the organism; weight gain and number of days till formation of the chrysalis. It has been found that the larger the caterpillar, the more capable it is of surviving lower temperatures (Nielsen and Papaj, 2015). Previous research states that carbohydrates cause an increase in body mass in caterpillars (Wills et al., 2015). Therefore, we expected to see an increase in body weight that would cause an increase in survival at suboptimum temperatures.

To our surprise, our data suggested a negative impact of added sucrose on the fitness of caterpillars in optimum and suboptimum environmental temperatures. The groups fed sucrose at 16°C and 22°C gained significantly less weight than their counterpart that was not fed sucrose (t-test, $p = 0.03, 1E-05$ respectively). The groups fed sucrose at 16°C and 22°C also took significantly more time to form their chrysalis than their counterpart without sucrose (t-test, $p = 0.001, 0.0003$ respectively). Our results suggest that sucrose has a negative impact on caterpillars when they are confined to a small space.

Introduction

For insects, such as caterpillars, temperature is the main environmental factor that determines their survival success (Karl et al., 2011). Temperature is important to caterpillars because they are ectotherms, which means they rely on outside sources to help regulate their temperature (Nielsen and Papaj, 2015). In order to maintain optimum internal temperature, caterpillars must thermoregulate (Karl et al., 2011). Thermoregulation allows the caterpillar to maintain

a certain internal temperature even when their surroundings are a different temperature (Altimier, 2012). Caterpillars are able to thermoregulate by changing their posture, their coloration, and blood flow (Nielsen and Papaj, 2015). Some of these methods of thermoregulation require energy, such as changing position and changing blood flow. For this reason, caterpillars prefer warmer temperatures that allow them to minimize the amount of energy they have to spend to maintain their body temperature (Karl et al., 2011).

In order to save energy, insects enter hibernation in sub-optimum temperatures. Certain ranges of below optimum temperature have vastly different effects on different species of insects and most of them are locked in the lethargy of hibernation. During hibernation most nonessential bodily activities suspend without harming or killing the insect. Once the temperature rises, the insect returns to its normal lifecycle (Aron, 1904).

When the environmental temperature is too low, caterpillars require more energy to regulate internal temperature, leading to compromises in other metabolic functions to save energy for thermoregulation (Karl et al., 2011). These adaptations to rapid changes of environmental temperature include different behavioral changes in different insects. For example, going into resting condition instead of flying, in which the wings are not required to work as much, results in decreased energy use (Heinrich, 1995).

Carbohydrates and proteins are essential organic compounds for survival of most living organisms. In general, carbohydrates are the main energy producing molecules in caterpillars as they depend mostly on leaves, which contain the carbohydrate cellulose among others. Researchers found that carbohydrates have a positive influence on the lifespan of insects by increasing energy production, and with the help of protein also increasing body mass (Wills et al., 2015). The larger the caterpillar, the better it is able to maintain optimum body temperature because it is less affected by convection (Nielsen and Papaj, 2015). Since larger caterpillars are able to maintain a higher temperature, ectotherms, including caterpillars, are usually larger when raised in cooler

temperature, a phenomenon known as the temperature size rule (TSR) (Lee et al., 2015). The nutrients the caterpillar obtains have a major impact on TSR (Lee et al., 2015).

Due to changing environmental factors, such as temperature, caterpillars may not have access to food sources with optimum nutrient content. A caterpillar's food source is plants, whose survival and chemical composition are highly susceptible to changes in temperature. The plasma membrane and cytoplasmic membrane of plants are composed of lipids and proteins. Evidence shows that the lipid component can change due to environmental conditions, such as temperature (Quinn, 1988). This change can completely kill off the plant. Whether the main plant the caterpillar consumes changes its nutrient content or becomes unavailable due to environmental changes, the caterpillar must rely on a different source of food. The caterpillar could therefore face a decrease in carbohydrates. This decrease could result in a decrease in the energy available for thermoregulation hindering their ability to survive (Nielsen and Papaj, 2015).

Since carbohydrates have a positive influence on body size, we hypothesized that access to abundant carbohydrates will lead to the caterpillars having warmer internal body temperatures making them more capable of surviving at suboptimum temperatures. We explored the effect of added carbohydrates in caterpillar diets and their physical changes in response to temperature stress because of the importance of carbohydrates for thermoregulation. The carbohydrates should also provide a source of energy for the caterpillar to thermoregulate by changing their posture and blood flow. However, the results of this experiment show that sucrose has a significantly negative effect on caterpillars.

Materials and Methods

Grouping

Before the Painted Lady caterpillars were separated into different groups they were all housed in 22°C and given the food that was provided by the supplier, The Nature Gift Store (Bremerton, WA). This was to allow the caterpillars to adjust and start off from the same point. In this experiment, three temperature groups were used, 10°C, 16°C, 22°C. Each temperature group had two subgroups of with or without carbohydrate supplement, resulting in 6 groups, each with 10 caterpillars. The temperature was monitored with a HOBO Pendant® Temperature/Light 8K Data Logger (Part # UA-002-08; Bourne, MA), a device that records the temperature and light intensity in the chambers, to ensure that experimental conditions were maintained throughout the duration of the experiment. The food provided by the supplier was wheat and soy based with sugars, vitamins, and food grade preservatives. The experimental groups with carbohydrates were supplemented with 10% (w/w) sucrose added to their food source. Each group was exposed to a 12:12 light dark cycle, similar to that in their natural habitat. A paintbrush was used to pick up the caterpillar, as they would stick to it and it was gentle enough not to harm them. The first temperature group was at 10°C, the second was at 16°C, and the third group was at 22°C. During the first two days all 10 caterpillars in

each subgroup were housed in the same container. Due to an inability to distinguish between them as their paint markings rubbed off, and possible cannibalism between the caterpillars; after the second day each caterpillar was separated into its own container.

Weighing caterpillars

Baseline weights were recorded as individuals were given separate housing containers. Approximately every other day the new weights of the caterpillars were recorded and observed. This was done using a Mettler Toledo PB303 scale that was accurate up to three decimal places. Each caterpillar was removed from their cup and placed in a tared weigh dish. Changes in weight were recorded.

Formation of chrysalis

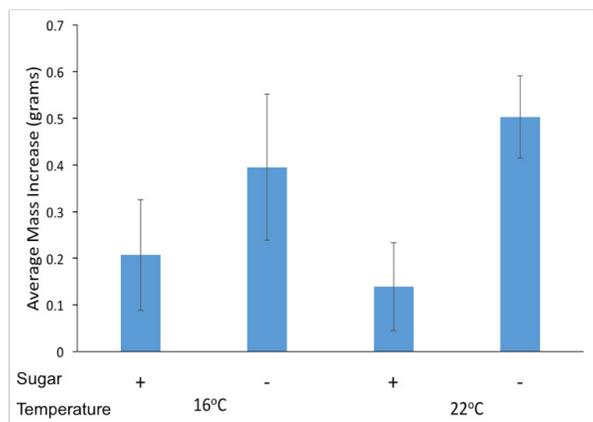
The formation of the chrysalis was determined by observing their body position and if a visible chrysalis was formed. If the caterpillar was hanging from the top in a J-shape, with its head at the bottom with no sign of movement, it was starting to form the chrysalis. Approximately 48 hours after chrysalis formation the caterpillars were transferred from their cups into a butterfly net cage at room temperature.

Results

Weights

Most of the caterpillars gained weight steadily throughout the experiment. There were some caterpillars that lost weight in the process, however, it was observed that in most of these cases, the caterpillars were deceased by the next weight recording. While this was true for some, others had lost weight and then gained some back the next day and continued on upward. The group with the highest average gain in weight was at 22°C without sucrose (Fig. 1). The group with the lowest average gain in weight was at 10°C without sucrose. The average gain in weight was lower in the group that received sucrose than the group that did not for both those at 16°C and 22°C (Fig. 1).

A



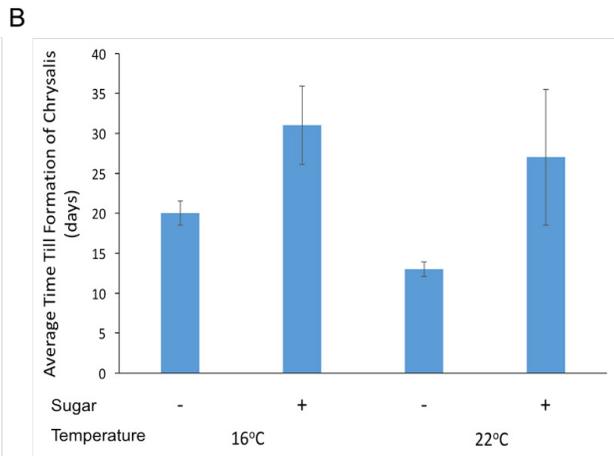


Figure 1. Average mass increase for each group (A). Average time until formation of chrysalis for each group (B).

Graph A compares the change in weight of the caterpillars at each of the temperatures with and without sucrose, error bars are standard error. A significant difference was seen between the groups with and without sucrose at 16°C and 22°C (p-value: 0.03 and 1E-05 respectively). Graph B depicts the average number of days each group took to form a chrysalis with standard deviation error bars. The groups at 10°C were not included because no caterpillars had formed their chrysalis. A significant difference was seen between the groups with and without sucrose at 16°C and 22°C (p-value: 0.001 and 0.0003 respectively). T-Tests were used to determine if there was a significance between these groups.

Formation of chrysalis

The first group to have all caterpillars in the chrysalis stage was at 22°C without sucrose (Fig. 1). Both groups at 10°C had no caterpillars enter the chrysalis stage and the data not shown in figure 1.

Survival

The majority of deaths occurred early in the experiment with only a few outliers. The group at 16°C with sucrose had the most deaths. The group at 22°C with sucrose had the most caterpillars go missing due to possible cannibalism.

Other observations

In the first few days it was observed that some of the caterpillars had different coloring. While this could just be a normal occurrence among caterpillars of the same species, it could also indicate that different species were received. This could also explain the cannibalism observed after the first day. However, some of the caterpillars at suboptimum temperatures were observed to be paler than those at 22°C. A blind color test before the caterpillars started to form their chrysalis was not performed. It was also observed that the groups fed sucrose had increased physical activity, such as increased movement around the container compared to their counterparts that were not fed sucrose. A motility test could not be performed.

Discussion

Based on the results we concluded that sucrose has a negative effect to caterpillars at both optimum and suboptimum temperatures. For both the groups at 16°C and 22°C, caterpillars that were given sucrose gained significantly less weight than their counterparts not given sucrose. For both of these temperature groups a significant negative influence of sucrose on the progression to the chrysalis stage was also observed. Survival was neither positively nor negatively influenced by feeding the caterpillars sucrose. Therefore, sucrose has negative effects on Painted Lady Caterpillars without any observed positive effects to outweigh the negative ones in terms of fitness.

Further research is required to fully answer the question of how sucrose affects caterpillars in suboptimum temperatures. In this study it was observed that caterpillars in suboptimum temperatures were paler than those at 22°C. This color change could be estimated by a double-blind test. We also observed increased motility among sucrose-fed caterpillars in comparison to the control group. It would be interesting to study if the increased mobility among the caterpillars fed the sucrose-supplemented food could partially explain the changes in weight and in time to form chrysalis. Another potential explanation for the observed data is the possible diet preference by caterpillars. We cannot rule out the possibility that the added sucrose in food may make it less appealing to caterpillars. It could explain why the experimental group did not gain weight.

Our results in the current study bring questions about the conclusions of previous studies. Several previous studies used sucrose as a carbohydrate source (Lee et al., 2015)(Mason et al., 2014). However, based on our data, using sucrose as a carbohydrate food source require more careful interpretation of the data. The results of this study also seem to contradict previous research done on the Temperature Size Rule (Lee et al., 2015). Previous research states that caterpillars will be larger in lower temperatures, but our results show the opposite. One possible explanation is the combination of the suboptimum temperature, small housing containers, and specific components of the food provided by the supplier. What we discovered about caterpillars survival at suboptimum temperatures is also important because it helps us understand the impact of global warming on caterpillars. We observed that the caterpillars survived the sudden drop in temperature to 10°C and 16°C could survive the remainder of time till formation of their chrysalis. Global warming can cause random temperature fluctuations that could be unusual during certain times of the year. Therefore, the sudden drops in temperature that occur during global warming will cause a sudden drop in caterpillar numbers, but those that survive the sudden drop are likely to survive to the chrysalis stage.

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