**[](http://ed101.bu.edu/StudentDoc/Archives/ED101fa09/bgibbons/Larva%20Page.html)Time of Metamorphosis as a Result of Milk Thistle Leave Amounts**

October 2, 2013

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UGS 303 FRI Research Methods

TTh 10-11 AM Lecture

**Introduction**

Butterflies are winged insects that start out as caterpillars. Caterpillars undergo metamorphosis to become adult butterflies. Metamorphosis is affected by physiological and ecological changes (Bishop et al. 2013). As caterpillars, they feed mostly on leaves until they pupate, or start their transformation into a butterfly. They stay as caterpillars for about four weeks and then start forming a cocoon in which it will become a butterfly. The Painted Lady Butterfly also travels long distances starting from southern Europe to Northwestern Africa (Stefanescu 2013). Long distance migration causes these butterflies to encounter different environments. There have been studies that show caterpillars adapting to lack of food sources in their environment. Malnutrition or lack of nutrients can lead to stressful conditions in animals (Lee et al. 2012). These stressful conditions can affect the stages of the larval period which either speeds up or slows down metamorphosis. There are a variety of factors that affect the time for metamorphosis. These factors include the butterfly’s environment, the amount of food available to the caterpillar, and the availability of light (Kehl et al. 2012).

Based on previous studies, researchers have used frogs as a way to study other animals that metamorphose. Since the frogs and butterflies have similar stages in metamorphosis, frogs’ metamorphosis is a good example to understand the relationship between butterfly metamorphosis time and survival. Some organisms that undergo metamorphosis include butterfly and frogs. In this experiment, butterflies will be used to determine whether the amount of food fed to them will affect the time for metamorphosis. However, the comparison between the metamorphic stages of frogs and butterflies should be noted. In frogs, the time for metamorphosis is affected by the amount of resources in its environment, including the amount of water available to the tadpole. The frogs that were put in an environment with less water metamorphosed faster (Loman 1999). This is crucial evidence in that it suggests the resources in an organism’s environment can potentially affect the time for metamorphosis.

Organisms that undergo metamorphosis go through distinct stages in which at every stage the organism requires different foods. If the resources in an environment are few, then the organism would adapt by decreasing its time for metamorphosis; therefore increasing its chance for survival (Tigreros 2012). Additionally, there is also evidence that supports the fact that animals can adapt to their environment if resources are not readily available. For example, depending on the frog’s time for metamorphosis, the frog can be bigger or smaller before metamorphosis occurs (Edge et al. 2012). If the time for metamorphosis is earlier, than the frog will be smaller before metamorphosis and the majority of its growth would occur after metamorphosis. In the case of butterflies, parasitoids also pose as a threat to the caterpillars’ survival. A previous study suggests that Painted Lady Butterflies migrate long distances to evade parasitoids that prey on larvae (Stefanescu 2012). In this situation, another possible adaptation would be to metamorphose faster so that they can avoid predators. Since time of metamorphosis reflects an organism’s survival, we are measuring metamorphosis time to access the survivability of the caterpillars. In order to manipulate the resources, we are changing the food amount so that we can see the effects of food abundance in the environment on the individual organism.

**Methods**

Does the amount of food available for caterpillars affect their time of metamorphosis? We address this question by varying the amount of milk thistle leaves for Painted Lady Butterflies. This experiment is designed to reflect the butterflies’ natural environment in order to apply effects of food abundance to the butterfly’s survival in their natural environment. Based on previous experiments, we assume that an increase in food will slow down the caterpillars’ time of metamorphosis.

***Preliminary experiment***

Since the optimal daily amount of food necessary for our caterpillar is unknown, we use this experiment to find the maximum amount a caterpillar will eat in a day. We are ordering 5 caterpillars that have all eaten artificial food prior to the experiment. The specimen jars will be numbered from 1 to 5. By drawing numbers out of a hat, we will assign each caterpillar to a container. Every day, each caterpillar will be given 1 gram of dried milk thistle leaves with 20mL of water or an amount of water that provides a good consistency for the milk thistle leaves paste. The water is added to mimic a live milk thistle plant that is found in the caterpillars’ natural environment. The next day we will measure the mass of food eaten (mass of thistle leaves given – ­mass of leaves left over). Then we will calculate the arithmetic mean of the mass of food eaten by the caterpillars that day. This will continue for approximately four days or until the butterfly eggs hatch. The highest daily mean will be the mass of food given to the control group in our actual experiment. We chose the highest mean to represent the control group because the control group represents the maximum amount of food a caterpillar will eat. This method causes bias because we assume that caterpillars eat the same amount of food after hatching and right before becoming chrysalises. Due to time constraints, we are making this assumption.

***Variables and treatments***

The independent variable is the mass of the food given to the caterpillars. The dependent variable is the time of metamorphosis (from time of hatching to emergence of the adult butterfly). There will be ten caterpillars in each treatment. In order to have independent replicates, each caterpillar will have its own specimen jar. The amount of food given to the control group will be based on the preliminary experiment. The first treatment group will have half the amount of milk thistle leaves as the control group’s milk thistle leaves. The second treatment group will have one-fourth of the control group’s milk thistle leaves.

***Randomization***

We conduct the same randomization technique as in the preliminary experiment to assign each egg to a container. Again, each container is assigned a number from 1-30. In order to randomly assign each caterpillar to a container, numbers are drawn out of a hat. The pieces of paper are returned to the hat after being drawn out. In each drawing, a number will be assigned to a caterpillar. The first ten different numbers drawn out of the hat will be assigned to the control group. The next ten numbers will be assigned to treatment one and the rest will be assigned treatment two.

**Experimental Design**

*Eggs and larvae:*

After assigning a container and a treatment to the butterfly, we record the general observations of the eggs daily. Then, we record the day of hatching which is approximately four days after the eggs arrive. The eggs will have access to food right after the food they hatch. The first dose of food will be placed next to eggs to prevent any harm to eggs due to unnecessary movement of the egg. During the following daily feedings, the caterpillars will be removed from the specimen jar and a layer of milk thistle leaves will be spread out on the bottom of the jar. The thickness of the food layer will depend on the treatment. Then, the caterpillar will be placed back into the jar. The caterpillars are housed on their food source to mimic their life in the wild on a milk thistle leaf.

*Pupae and adult butterfly:*

Within two weeks, the caterpillars will take on their chrysalis form. We will record the time the caterpillar enters the chrysalises form. When the pupae firmly attaches itself to the paper towel, we will transport each pupae into a small clear compartment with holes. The pupae will be observed daily and we will also record the time the adult butterflies emerge.

**Appendix One:** Specialized Equipment and Supplies

***Live Organisms***

-30 butterfly eggs

-5 caterpillars

***Food***

-2 pounds of milk thistle leaves from Croatia (*Silybum marianum*)

***Equipment***

-35 plastic specimen jars

-3 petri dishes

-3 10 mL graduated cylinders

-1 top loading balance

-3 250 mL beakers

-1 roll of paper towels

**Husbandry Protocol**

*Taxonomy*

Name: Painted Lady Butterfly

Class: Insecta

Order: Lepidoptera

Family: Nymphalidae

Genus/Species: *Vanessa cardui*

*Natural History*

Painted Lady Butterflies start their life as eggs. Eggs are as small as pin heads and pale green in color. Eggs are usually laid on leaves so when caterpillars hatch, they have a source of food right away. They spend about 3-5 days for the incubation period. Afterwards is the larval stage where caterpillars emerge from the hatched eggs. Caterpillars are usually striped and can measure up to 1.25 inches long. They usually eat leaves for 5 to 10 days before it pupates. During pupate, the caterpillars form chrysalis (pupa). They hang upside-down from a leaf or branch where they form into adult butterflies. The internal structure changes while they are in the pupation period. The adult will emerge approximately 7 to 10 days after chrysalis has formed.

When the adult has, it hangs upside-down to pump blood into its wings before flying off. Adult Painted Lady butterflies are usually brown, black, or orange with white spots. The upper side is an orange-brown with darker wing bases and the underside has brown, gray and black pattern. They typically have a wingspan of 2 inch wingspan. They eat thistle leaves as well as clover nectar. These butterflies tend to migrate from colder climates to warmer climates. They hibernate in the South and in mild winters. Painted Lady Butterflies can be found almost everywhere. They are native to Africa, North America, Europe, and Asia. They mostly reside in open areas such as dunes, fields, and gardens. They usually live for about 2 weeks to a month.

*Housing Methods:*

* Eggs: plastic specimen jars
* Larvae: plastic specimen jars for larvae stage and then a cage or container during pupation
* Butterflies: Large container or cage with air holes to allow for air exchange but no openings big enough for them to escape.

*Handling and Transport Requirements*

If received as eggs:

* Prepare milk thistle leaves in plastic specimen jar. This way when they hatch they will have a source of food to feed and grow on in the beginning. Amount to be determined.
* Place eggs carefully into jars. Handling eggs very gently is important as to not drop or disturb them.

If received as caterpillars:

* Prepare milk thistle leaves inside the plastic specimen jar (about a spoonful) at the bottom. Amount to be determined.
* Using paintbrushes gently transfer the larvae into the cups.
* Taking a paper towel, poke few holds for caterpillars to breath and dampen it. Cover the jar with this damp towel. The purpose of this is to keep caterpillars moist so they do not dry out.
* Once caterpillars are fully-grown, they’ll transform into a chrysalis and need to be transferred to a larger house. They are extremely fragile at this point so wait 24 hours for the chrysalides to harden and then carefully remove the paper towel that they have attached to and tape it to the inside of the box or cage that we will use. Use strong tape such as masking or electrical tape. Put paper towels or butcher paper along the bottom of the container/cage to absorb any moisture.
* Pick a container/cage that will allow enough air exchange (mesh, air holes, etc.). The container/cage should not have openings big enough where a butterfly could escape.

*General Husbandry*

*Caring:*

* Caterpillars can survive in the plastic specimen jar until pupation. They will excrete round brown wastes on top of their food. However, it’s not necessary to remove the wastes.
* If the paper towel happens to dry out, replace it with another damp one to keep caterpillars from drying out.
* Replace food once it seems to deplete to keep from starving caterpillars.
* Be careful not damage chrysalides. If they have fallen, using clean fingers or insect forceps, brush of debris and allow to continue hanging by attaching its pointed tip to a cotton ball or household string.
* If it is necessary, mist the chrysalides a few times a day to keep them from drying out.
* Once they have emerged as adult butterflies, feed them by wetting a piece of cotton or paper towel in a solution of 5 tablespoons of sugar (brown or white) mixed with 1 cup of water. Place it in a shallow dish on the floor of the container/cage. Do not use honey because it may clog their mouth tubes. We could feed them bee pollen or slices of fresh fruit.
* Mist the adult butterflies as well so that their wings don’t dry out.
* Butterflies can be kept in container/cages for 2-4 weeks of their adult life span.

*Identification:*

* To keep each egg’s identity separate, we will label the plastic specimen jar from number 1-30. Caterpillars will remain in these same jars until transfer of chrysalides.

*Check up:*

* We will check on eggs, caterpillars and butterflies daily.
* We will record the day the eggs hatch, time of pupation, and emergence of adult butterfly.
* Checking on caterpillars every day will allow us to track the amount of food they eat and touch up on replacing their food, damp towel, or anything else.
* During pupation, we will check daily to make sure chrysalides are still hanging and haven’t fallen to the bottom. We’ll make sure they’ll be misted so they don’t dry out.
* As butterflies, it is important for us to make sure they do not escape, get enough food, and stay moist.

*Breeding*

* Painted Lady Butterflies undergo sexual reproduction. Eggs are laid a few hundred at a time on the chosen host plant.

*Disposition*

Butterflies taken care of in a laboratory setting are not advised to be released into the wild. It is recommended that Painted Lady Butterflies be kept in their container/cage for their entire 2-4 week life span. If we want to euthanize any larvae, chrysalids, or butterflies, then there are 2 methods. We could either place the larvae, chrysalids, or butterflies in a freezer for 48 hours or in 70% isopropyl alcohol for 24 hours. Then, using gloves, it should be wrapped in an opaque plastic bag that is sealed before placing it into a general garbage container.

**Appendix 2:** Timeline

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| **Week** | **Date** | **Activity** |
| **1** | 9/23-9/29 | Design experiment, create husbandry sheet for caterpillar/butterfly care, submit needed materials for approval |
| **2** | 9/30-10/5 | Once materials arrive, start preliminary experiment on caterpillars to determine the amount of food being fed to the control group. Check on eggs and caterpillars everyday. Once eggs hatch, begin testing. Record the time it took for eggs to hatch. |
| **3** | 10/6-10/12 | Observe the caterpillars and record the amount of time it takes for them to pupate. |
| **4** | 10/13-10/19 | Observe the caterpillars and record the amount of time it takes for them to pupate. |
| **5** | 10/20-10/26 | Observe the caterpillars and record the amount of time it takes for them to pupate. |
| **6** | 10/27-11/2 | Observe the caterpillars and record the amount of time it takes for them to pupate. |
| **7** | 11/3-11/9 | After the chrysalis is formed, observe and record the amount of time it takes for the butterfly to emerge from its cocoon. |
| **8** | 11/10-11/16 | After the chrysalis is formed, observe and record the amount of time it takes for the butterfly to emerge from its cocoon. |
| **9** | 11/17-11/23 | Start on research paper. |
| **10** | 11/24-11/30 | Finish research paper. |

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