The Effects of Temperature on *Vanessa cardui* Wing Size

Dionna Kennelly, Jarad Grigg, Asli Tabaru, Keaton Sentak

Department of Biology, Rutgers University, Camden, N.J. 08102

Edited by Daniel Pinolini

Abstract

Butterfly size can be affected by several external, environmental factors. Some of these factors are temperature, light exposure, and nutrient consumption. Temperature is known to have to have a great impact on the metabolism of invertebrates, speeding up growth and development stages. Metabolic rate can affect the size of butterflies and can result in a hastened metamorphosis phase. However, it is unknown how temperature affects mature *Vanessa cardui* butterflies, specifically their wingspan length. Here we show how increasing temperature decreases *V. cardui* wingspan and overall size. Previous research would suggest that our research agrees with the "temperature-size rule" which states that at lower temperatures ectotherms will grow more slowly, but will become larger as adults than individuals that live at warmer temperatures (Angilletta and Dunham, 2003). This study is meant to be a starting point to a much larger discussion involving how temperature could affect other aspects of the organism, such as color, flight patterns, or mating and pollination patterns.

Introduction

Butterfly wings are well known for their vibrant colors and unique, intricate patterns. In a previous experiment, *Vanessa urticae* larvae were exposed to different wavelengths of light, including red, blue, and green. From the conducted experiment, it was determined that light had a significant effect on the development of butterfly wings. When exposed to different wavelengths of light, butterfly wings developed with varying color intensities (Morgan, 1917). It is known that butterflies detect light through specific sensors present on their wings (Prum et al., 2006). Sensors present on the scales of the organism's wings contain holes, which filter and refract light rays that come in contact with the wing (Prum et al., 2006). Unlike their light sensors, butterflies detect temperature through their internal metabolism (Forster et al., 2011). As temperature increases, metabolism is quickened. Faster metabolism causes a faster metamorphosis phase, which can result in physical changes due to this accelerated growth stage (Forster et al., 2011).

Butterflies go through many stages throughout their life. First, female butterflies lay eggs which eventually grow into caterpillars, or larvae. Following this stage, when the larvae are large enough, they begin to enter the chrysalis stage of growth. During this stage, the larvae form cocoon structures out of silk-like fibers that are secreted from the organism (Walter,1997). Following metamorphosis within the chrysalis, butterflies emerge. After emergence, butterflies grow to full maturity and female butterflies can then begin to lay their own eggs, thus restarting a new life cycle (Walter, 1997)

Global warming has begun to alter the earth's environment, raising the core temperature of the earth slowly, but notably, within the past century (Maslin, 2004). The past two decades in the 20th century were the hottest recorded in 400 years, with temperatures in the Arctic increasing twice the global average (Maslin, 2004). *V. cardui* are known to be vastly adaptable and diverse creatures, with the ability to survive in many different climates (Braby, 2000). However, the development of many organisms, has begun changing to accommodate the steady rise in the earth's temperature (Klockmann et al., 2016). It has been reported that temperature has a significant effect on the survival rate of these butterflies and their ability to mate with similar species (Klockmann et al., 2016).

Temperature and light are environmental factors that are known to influence the development of organisms. An experiment was designed to test the lengths at which temperature affects the development of butterfly wings. It is anticipated that wing size will decrease as temperature increases in accordance to the temperature-size rule (Angilletta and Dunham, 2003). In the current study, *V. cardui* were exposed to varying temperature conditions. Following metamorphosis, various dimensions of the organism's wing will be measured and statistically analyzed to determine significance.

Materials & Methods

Organisms and culture condition

Painted-lady butterfly larvae (*V. cardui*) were purchased from Carolina Blue. Organisms were placed in individual environments to decrease competition among the larvae. Each larva was placed, with food provided from the cultures bought, inside small plastic containers. A small cheesecloth was placed under the lid of each container to provide the organism with a material to attach their cocoon to when entering the chrysalis stage of metamorphosis.

Experimental procedures

Throughout the experiment, all groups were kept under the same environmental conditions, with temperature as the only variable influencing development. Each organism was given approximately 3.57 grams of food throughout incubation. Three experimental groups of painted-lady butterfly larvae were subjected to environments of varying temperatures. The first group was kept at room temperature (22°C) throughout metamorphosis and contained 18 larvae. A second group contained 17 larvae and was kept in an incubation chamber held at a temperature of 28°C. The third and final experimental group contained 17 larvae and was kept in an incubation chamber held at a temperature of 33°C. Primarily, the wing size of each butterfly was examined to determine if these varying temperatures have an effect on the wingspan of these organisms. Caterpillars were monitored on a daily basis to track chrysalis formation and survival rate.

Statistical analysis

To determine if there were any statistically significant differences in wing size of the organisms, both the body size and forewing size were measured along with the wingspan. The forewing measurement is the diagonal length of the organism's wing from the center of the body to the top of its forewing. Measuring each of these three dimensions will substantiate the results, proving whether or not the wingspan is significantly varied across the three experimental groups. Following data collection, Shapiro-Wilk normality tests and T-Tests were run on each data category to determine whether the data displayed any significant differences.

Results

To test our hypothesis, we measured the lengths of wingspan, body, and forewing of kept at different temperatures (Fig.1). It was determined that *V. cardui* wingspan size was significantly different due to the difference in temperature between the two surviving groups, t-test, p=0.005. The ambient temperature significantly affected the body length (t-test, p=0.006), but not the forewing length (t-test, p=0.736).

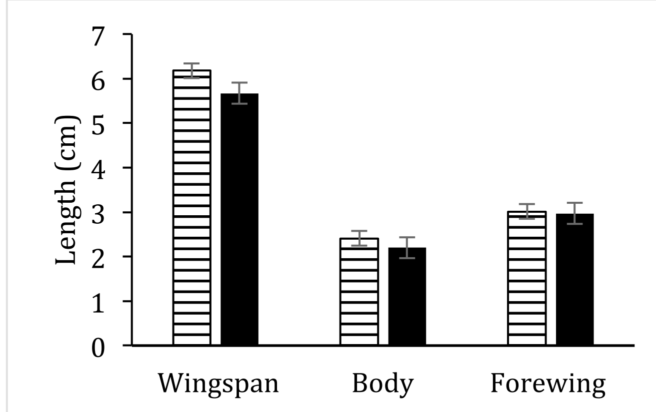

Figure 1: Average Wingspans, Body Lengths, and Forewing Lengths. This chart contains the average values for each of the three dimensions measured. Open horizontal line bars represent 22°C temperature environments. Solid filled bars represent 28°C temperature environments. Error bars represent standard deviation.

The first group of caterpillars out of the three temperature groups (22°C, 28°C, and 33°C) to form their cocoons were those housed in the 33°C environment. All the caterpillars in this group had entered into their cocoon by day five. The second group of caterpillars to enter the chrysalis stage of metamorphosis was the ones that were housed in the 28°C environment. These caterpillars had all entered into their cocoons by day seven. The room temperature group was the last group to enter the chrysalis stage of metamorphosis on day twelve of the experiment. Those kept at room temperature throughout metamorphosis all survived. This group had an average wingspan of 6.18 cm, body length of 2.40 cm, and an average forewing length of 3.01 cm. Four caterpillars died before transforming within the experimental group that was kept in the 28°C environment throughout metamorphosis. Of the 13 that survived, the average wingspan was 5.67 cm. The average body length was 2.20 cm, and the average forewing length was 2.97 cm. All caterpillars died in their cocoon before transformation within the 33°C environment.

Discussion

Based on the results, it was concluded that temperature does have a significant effect on the size of *V. cardui* wingspan and body length. Both the wingspan and body length resulted in p-values below the standard .05, indicating a significant difference in size when comparing the two surviving trials. Because of the significant difference in body length due to temperature, it could be speculated that the larger wing size was simply a result of a larger overall organism. The statistical analysis results do support the hypothesis that the larvae kept at higher temperatures will consequently possess smaller wingspans, and in turn result in smaller overall butterflies. The results, however, do not show a significant difference in the forewing size due to the different temperature environments. All 17 larvae kept in the 33°C control group did not complete metamorphosis. This result leads to the conclusion that this temperature was beyond the *V. cardui* environmental tolerance range.

Higher temperatures increase the metabolism of butterfly larvae. With a higher metabolic rate, it is possible that these butterfly larvae are using more energy than they are able to gain. When more energy is being used, the growth rate of the butterfly larvae could decrease. The amount of stress placed on the larvae could have influenced the development of the butterflies. When organisms are placed in environments with significant heat stress, they must expel more energy. As the organism expels more energy, they are unable to allot energy to simple growth and development (Kingsolver et al., 2013). Because of this uneven distribution of energy, organisms can end up being smaller than those that are not placed under stressful environmental conditions. *V. cardui* are known to migrate from Africa to Europe annually (Talavera and Vila, 2016). Countries in both Europe and Africa are some of the worst affected by the global warming crisis. Africa has seen significant loss of its forest's in the past two decades. Most European countries have also been affected in similar ways (Maslin, 2004). Without large enough wings, it is possible that the *V. cardui* would not be able to make this trip (Talavera and Vila, 2016). Possessing smaller wings could negatively affect other butterflies flight patterns as well.

We observed the difference between the wingspans of males and females (data not shown); however, the difference in sex was not the focus of this study and was not further analyzed. This might have been a contributing factor in the variation we observed (Fig. 1). On average, male *V. cardui* have smaller wingspans then female *V. cardui* (Braby, 2000). The varying size of these organisms based on sex could have affected our results, with more males or females possibly populating any one of our temperature environments. If this was an influencing factor, one could conclude that the overall smaller average wingspan resulting in the 28°C environment could have been due to this experimental group containing more males then females when compared to the experimental group kept at 22°C.

Acknowledgements

The authors would like to thank Dr. Kwangwon Lee for providing us with this research opportunity. We would also like to thank Dr. Amy Savage, and Daniel Pinolini for assisting us with our research.

References:

Angilletta, M.J., and Dunham, A.E. (2003). The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. Am. Nat. *162*, 332–342.

Braby, M.F. (2000). Butterflies of Australia:Their Identification, Biology and Distribution (Collingswood, Australia: CSIRO Publishing).

Forster, J., Hirst, A.G., and Atkinson, D. (2011). How do organisms change size with changing temperature? The importance of reproductive method and ontogenetic timing. Funct. Ecol. *25*, 1024–1031.

Kingsolver, J.G., Diamond, S.E., and Buckley, L.B. (2013). Heat stress and the fitness consequences of climate change for terrestrial ectotherms. Funct. Ecol. *27*, 1415–1423.

Klockmann, M., Schröder, U., Karajoli, F., and Fischer, K. (2016). Simulating effects of climate change under direct and diapause development in a butterfly. Entomol. Exp. Appl. *158*, 60–68.

Maslin, M. (2004). Global warming. [electronic resource] : a very short introduction. (Oxford ; New York : Oxford University Press, 2004.).

Morgan, T.H. (1917). Experimental Zoölogy (New York: Macmillan).

Prum, R.O., Quinn, T., and Torres, R.H. (2006). Anatomically diverse butterfly scales all produce structural colours by coherent scattering. J. Exp. Biol. *209*, 748–765.

Talavera, G., and Vila, R. (2016). Discovery of mass migration and breeding of the painted lady butterfly *Vanessa cardui* in the Sub-Sahara: the Europe–Africa migration revisited. Biol. J. Linn. Soc. n/a-n/a.

Walter, M. (1997). The Life Cycle of Moths and Butterflies. Rochester Institute of Technology.