

Thermoregulation in Molting and Feeding *Danaus plexippus* Caterpillars

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PRÉCIS

Many insects rely on thermoregulation to maintain body temperatures independent of the surrounding environment. Basking is one of the most efficient ectothermic tactics. Several studies have examined the effects of temperature on larval growth and development, melanic coloration, and behavioral characteristics. However, thermoregulation during molting has been less well studied. Based on evidence from previous studies done on thermal behavior, I hypothesized that monarch caterpillars would thermoregulate during the molting process in a manner different to thermoregulatory patterns observed during feeding. This study also assessed whether or not caterpillars are behaviorally restrained from thermoregulating while molting.

To address the issue, an observational study of monarch butterflies was performed at Pinjarra Hills in Brisbane, Australia. Under natural conditions, monarch larvae were monitored on milkweed plants over several days both during a hot time (November 2006) and a cooler time (May 2007) of year. For each located instar, body temperature was recorded along with ambient temperatures from the surrounding environment, such as leaf surfaces in the sun and shade, the stem, bud, and ground. The stage of each instar, location within the sun or shade, and other behavioral activities were recorded as well. Full statistical analysis was not performed, although t-tests were used to test basic patterns and make inferences.

Regression slopes showed that monarch larvae thermoregulate to a significant degree while feeding in November. Slopes also showed that larvae do not thermoregulate when molting regardless of month. During the molting process larvae must conform to the ambient temperatures available. Molting body temperatures tended to fluctuate throughout the day, rising and falling in relation to sun exposure. Feeding body temperatures appeared to remain more stable (i.e. thermoregulable) regardless of the ambient temperatures. A seasonal difference was

evident; more variation in leaf temperatures and body temperatures was seen in November (and the only statistically significant difference) when temperatures were higher, while the cooler period of May made thermoregulation difficult for even the feeding larvae. The results also emphasized the problem of overheating, as larvae preferred the partial sun and shade offered underneath milkweed leaves.

This paper provides basic information on thermoregulation during molting, but more data are needed. Additional studies should focus more on available ambient temperatures and physiological aspects of molting. It is also possible that pseudoreplication and a lack of randomization had an effect on the outcome of the results, which future studies could avoid.

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INTRODUCTION

The study of ecology encompasses a broad scientific field that explores the consequences of interactions between biotic and abiotic environments. For smaller organisms, microhabitats and microclimates (including temperature, humidity, radiation, wind, etc.) are a central part of the environment. The thermal environment is one variable that affects all organisms. Environmental temperature influences the rates of physiological processes, such as growth and development rates, in ectothermic organisms specifically (Nice and Fordyce, 2006). Many ectotherms have developed adaptive mechanisms to cope with their thermal environment and can respond behaviorally and/or physiologically to diurnal and seasonal temperature changes.

Organisms that cannot thermoregulate must allow their bodies to conform, more or less, to the surrounding temperature. Those who can use mechanisms of thermoregulation possess a variety of ways in which to control their body temperature in relation to the ambient temperature for maximum growth and development. To a certain degree, some insects can utilize endothermic processes to generate their own internal heat (James, 1986). However, this alone is not sufficient for survival and is only used continuously by terrestrial birds and mammals (Heinrich, 1981). Insects instead display a number of tactics for thermoregulation (Nice and Fordyce, 2006). Many insects can raise their body temperature several degrees Celsius above ambient temperature. With their small body size and weak endothermic capacity they rely on heat acquired from the environment through radiation, convection, or conduction to determine and maintain their body temperature (Heinrich, 1981). The use of sun-radiated heat as an ectothermic tactic is often the most efficient method and readily available source (Heinrich, 1981). In particular, many caterpillars use thermoregulation tactics such as basking, aggregation, tent making, coloration, setae, and behavioral postures and movements to regulate their body

temperatures in variable thermal conditions (Stamp and Casey, 1993). Larval phenotypic plasticity, often seen in seasonal polyphenism or between instar stages, and microhabitat shifts are key adaptations used by some caterpillars. The efficiency of these adaptations facilitates more rapid growth during the larval stage, reducing the amount of time larvae remain in their most vulnerable state as prey and increasing the probability of reaching the minimum body-mass limit for proper pupation (Nice and Fordyce, 2006). A trade-off may occur here as ectothermic regulation does not come without risks, such as overheating and predation (Rawlins and Lederhouse, 1981). Daily temperature fluctuations can cause heat avoidance mechanisms to be employed during certain times of the day, and basking is a behavior that leaves the caterpillar visually conspicuous to surrounding predators (Rawlins and Lederhouse, 1981).

The common monarch butterfly, *Danaus plexippus* L. (Lepidoptera: Nymphalidae), is well known for its extensive migratory patterns from breeding to over-wintering sites throughout North America (Zalucki and Clarke, 2004; Davis *et al.*, 2005) and Australia (Clarke and Zalucki, 2004). As a result of their expansion into more temperate climates following the spread of their food source, the common milkweed (*Asclepias* spp.), they have evolved extensive thermoregulation capabilities and are known to bask in direct sunlight throughout both the larval and adult life stages (James, 1986). Monarch larvae are characterized by white, yellow, and black stripes and both larvae and adults use increased melanization to absorb the maximum amount of thermal energy during certain times of the year (Davis *et al.*, 2005). Their plasticity in coloration is one method used to allow them to locally adapt to their regional environments, as some larvae may be nearly all black in a colder environment in order to absorb more radiation, and mostly white and yellow in a warmer environment. Like any basking insect, monarch larvae must deal with the risk of overheating as well, and do so by altering their behavioral postures and

location on the milkweed, showing a behavioral tendency to get out of the heat during the hottest part of the day (Davis *et al.*, 2005).

A considerable amount of research has been done concerning the effects of temperature on larval growth and development (Hazel, 2002; Kingsolver, 2000; Rawlins and Lederhouse, 1981; Zalucki, 1982). However, the relationship of temperature and molting between instars is poorly understood. Each monarch caterpillar undergoes five growth stages (instars) in which they shed their exoskeleton as they grow, choosing one location on the milkweed plant as they perform the molting process. Evidence from previous studies done on thermal behavior (Malcolm and Zalucki, 1993; Solensky and Larkin, 2003) suggests that temperature is important during these molting periods and that at any instar stage, larvae would try to maintain a high body temperature for maximum absorption of thermal energy. However, there are several risks associated with molting, such as vulnerability to predation and moisture loss due to overheating, that may play a role in the location chosen by larvae.

In this study, the microhabitat temperatures chosen by monarch larvae for molting are compared to the temperatures chosen for feeding and the ambient temperatures available from various parts of the surrounding plant. It was hypothesized that monarch caterpillars would thermoregulate during the molting process in a manner different from thermoregulatory patterns observed during feeding. When choosing molting sites, larvae must indeed take into consideration predators and overheating, but the primary concern could well be finding a warm environment so they can maximize rapid transit through this vulnerable state as they shed their exoskeleton. Molting may be a time for basking as well. Several different results could be obtained from this study. First, it could be found that monarch larvae do not thermoregulate at all during the molting process. This result would lead to the question of whether or not

caterpillars are behaviorally restrained from thermoregulating while molting and what impacts this may have on developmental rates. Second, monarch larvae could be found to thermoregulate to a certain extent during the molting process in a manner reflecting thermoregulation during feeding. With this result, it would appear that the molting process has no effect on the normal thermoregulatory behavior of instars. Third, monarch larvae could be found to thermoregulate to a certain extent during the molting process but in a manner different to what was observed during feeding. This result could be found as an increase or decrease in thermoregulatory behavior during molting, perhaps due to a preference for different thermal environments. Any of these potential results will further emphasize the need for ectotherms, particularly insects and more specifically caterpillars, to constantly thermoregulate throughout the day and during each stage of growth.

METHODOLOGY

November – Hot Period. Under natural conditions of temperature and radiation, monarch larvae (*D. plexippus*) were monitored for a one week period (five days) during the month of November 2006, at a specific open range field site at Pinjarra Hills, (-27° 32' S; 152° 54' E) Brisbane. Larvae, ranging from first to fifth instars, were either found and observed *in-situ* or taken from another site as eggs and positioned on randomly selected milkweed after hatching. The addition of first instars allowed for the observation of a greater number of earlier-stage larvae, as they are harder to locate at random. At the beginning of the observatory period, the location and instar stage of each previously positioned or haphazardly found larvae was marked on the milkweed plant. This allowed for easier findings later in the week by giving indication as to what stage of larvae should be found nearby the original plant. New larval positions found later in the week were marked accordingly.

For each instar, body temperature was measured mid-length using an infra-red temperature gun (Raytek® *PM Plus*), as well as the ambient temperature of the surrounding leaf surface at the time (whether in the sun or shade). The stage of the instar, location within the sun or shade, and other behavioral activity were recorded for each temperature measurement as well. During each observatory period, rotations between all the located larvae were continuously made. Throughout the day, additional ambient surface temperatures were taken at various locations including leaves at the top of the milkweed plants, leaves located lower to the ground, the bud, stem, stalk, and the grass/ground. These measurements were taken in order to get a sense of the ambient environmental temperatures available to the larvae at different periods of the day. The behavioral activities of the larvae were observed in terms of location within the sun or shade and level of active movement or quiescence. From this data, an estimate of the proportion of larvae performing each activity during the morning, mid-day, and afternoon periods could be tallied in order to assess the affects of daily temperature and radiation fluctuations. Estimates of the number of larvae found at different locations throughout the day could also be found and compared to the average body temperatures. Molting larvae were especially observed at the end of each instar stage to determine if thermoregulation occurred.

May – Cooler Period. During the month of May 2007, monarch larvae (*D. plexippus*) were again monitored under natural conditions of temperature and radiation. Data was taken from the same open range field site of milkweed at Pinjarra Hills, but also from a new site across the road in the same area. This second site was used because it provided a more ample supply of healthy milkweed plants that had not been destroyed by bovines or a lack of water. Parasites were evident at both locations. Time restrictions and limited access to the field site prevented data from being collected every consecutive day for a week like it was in November. As a result,

data was collected one or two days a week for four weeks total. The same approximate amount of data was still collected during both months, and for this particular study, the specific days should not matter. Observations were made in both November and May to allow for the inclusion of data taken from both a hot environment and a cool environment.

In the same manner as before, larvae of all instar stages were found and observed *in situ*. It was not necessary this time to collect eggs from other sites and position them on randomly selected milkweed plants. An abundance of eggs and larvae were already present. The observations and recorded measurements taken in the field were consistent with the pattern set up in November with the same level of focus on feeding and molting larvae from each instar stage. Data from each month was then taken and displayed in graph format in order to visualize trends. Molting and feeding data was additionally separated and compared to address the research question of whether or not thermoregulation is occurring during the molting period.

Analysis. Apart from categorizing the data based on location, activity, and number of instars, p-values were calculated for each of the average differences (t-test using $\alpha = 0.05$) to give some indication of the level of statistical significance present. Regression lines were established to compare the relationship between leaf temperatures and individual molting and feeding body temperatures. In some cases, line graphs were used with polynomial trendlines to display diurnal fluctuations in temperatures over time. Histograms were utilized to show the major average differences between temperatures over time and visually compare them to determine any patterns. Full statistical analysis was not performed in this research study. Descriptive statistics were simply applied to make statistical inferences about the relationship between feeding and molting monarch larvae.

RESULTS

The initial data recorded for November was taken over five days between the times of 8:05am and 5:14pm. Around 15 individual larvae were found per day and measured more than once, giving a total of 155 larvae for the week (Table 1). Of this total, 36 larvae were found to be feeding, 18 larvae were molting, and 101 larvae were engaged in other activities. The initial data recorded in the month of May was taken over six days, spread out within a four week period (larvae monitored one or two days a week), between the times of 9:33am and 3:17pm. A total of 167 larvae were measured, including 22 feeding larvae, 32 molting larvae, and 113 larvae engaged in other activities (Table 1). Each instar's level of activity, location, and body temperature were measured. The ambient temperature of parts of the surrounding plant and environment were randomly taken throughout the day as well, coinciding with the location of the instars. These included temperatures taken from the surface of a leaf in the sun, the surface of a leaf in the shade, the bud, a leaf higher or lower than the location of the instar, the stem, stalk, and the ground or grass (Table 2).

A categorization of how many larvae were found at each location within the available environment, along with the average body temperature at each location during the morning, midday, and afternoon periods of the testing, was established for both November and May (Tables 3 and 4 respectively). Numbers show the majority of larvae were found on the leaf surface and within the shade. During the hot November month, larvae tended to seek more protection from the sun's radiation as the day wore on. However, location within the shade may not have been intentional during May, as the sun was not always prevalent. To find further general patterns within the data, larvae were categorized by level of activity and resulting average body temperatures in order to show the number of larvae feeding, crawling, molting, and

basking either in the sun, partial sun, or shade (Tables 5 and 6). Data from both months exhibit a decrease in average body temperatures going from the sun, to partial sun, to shade. In November, most feeding and molting was performed in the partial sun, while in May, most was done in the shade. However, all body temperatures were 4-5°C lower in May due to the reduced intensity (or absence) of the sun during the winter period.

Comparisons were made between temperature measurements for the larvae and leaf surfaces. Leaf surfaces reached a maximum of 36.4°C in the hot November sun and a minimum of 20°C in May. In both months, a parabolic curve is seen as the temperatures of the leaves rise and fall in a pattern mirroring the rise and fall of the sun throughout the day (*Fig. 1 and Fig. 2*). An overall significant difference is evident in November between the temperatures taken in the sun and those in the shade ($p < 0.0001$; *Fig. 3*). However, splitting the temperature measurements up over three time periods of 8:00 – 10:59, 11:00 – 13:59, and 14:00 – 18:00 showed significant differences throughout the morning and midday time zones but not throughout the evening (*morning* $p = 0.0372$; *midday* $p < 0.0001$; *evening* $p = 0.149$; *Fig. 3*). The same overall significant difference seen in November is not evident in May, and a significant difference was not found within any of the three time zones (*morning* $p = 0.855$; *midday* $p = 0.738$; *evening* $p = 0.986$; *overall* $p = 0.922$; *Fig. 3*). The heavy radiation from the sun is probably what creates the significant variability in November.

With regard to the body temperature of all the monarch larvae, regardless of activity, different patterns were measured throughout November and May. The November data displayed a curve matching the pattern of the sun with a maximum midday temperature of 38.1°C and a minimum of 24.8°C in the afternoon (*Fig. 4*). With the May data, a pattern was seen to show a

general increase in temperature throughout the entire day with a minimum temperature of 21.4°C in the early morning and a maximum of 34.3°C in the afternoon (*Fig. 5*).

To specifically address the hypothesis that monarch caterpillars will thermoregulate during the molting process in a manner different from thermoregulatory patterns observed during feeding, measurements taken from feeding and molting monarch larvae were compared. The molting body temperatures of both months display larger parabolic curves that rise to a maximum at midday before decreasing again in the same manner as the sun (*Figs. 6 and 7*). This evidence, graphically viewed as scatter plots, supports the notion that the body temperatures of feeding larvae stay at a more constant level throughout the day instead of rising and falling like the body temperatures of molting larvae. This provides a crucial result, suggesting that feeding larvae thermoregulate while molting larvae conform to ambient temperatures. As expected when comparing results between the different months, the body temperatures in May are lower than the body temperatures in November. The chief difference between the two months is in the fact that the November curves seem to decrease more at the end of the afternoon measurement period, while the lowest part of the May curves are in the morning period before the temperature has had a chance to rise throughout the day (*Figs. 6 and 7*).

A histogram representing the differences between the average body temperatures of feeding and molting monarch larvae is provided in Figure 8. From the November data, it appears that the feeding body temperatures are quite a bit higher in the morning and afternoon time periods and lower than the molting body temperatures during midday, although these results are not statistically significant (*morning* $p = 0.132$; *midday* $p = 0.569$; *evening* $p = 0.140$; *overall* $p = 0.0679$). The May data illustrates an even smaller difference between the molting and feeding body temperatures and again is not statistically significant (*morning* $p = 0.533$; *midday* $p =$

0.328; evening $p = 0.952$; overall $p = 0.534$). Molting body temperatures appear slightly higher than feeding body temperatures throughout the day, with almost no difference evident in the afternoon. This follows the general pattern seen so far in regard to less variability in the cooler months.

To look further into the relationship between the feeding and molting body temperatures, it is necessary to compare them to the surrounding leaf temperatures because thermoregulation is defined as maintenance of a constant internal body temperature independent from the environmental temperature. Line plots comparing the body temperatures and the temperatures of the leaf surfaces in the sun and shade can be plotted together to illustrate the individual rise and fall patterns of each (Figs. 9-12). However, a better graphical representation again comes from looking specifically at the average differences in a histogram (Figs. 13 and 14). When comparing average body temperatures of feeding larvae with average leaf temperatures, a consistent trend shows all body temperatures of feeding larvae to be above leaf temperatures during the evening time period regardless of month and location of the leaf (Figs. 13). Of all the individual p-values calculated for average differences based on time period, month, and location of leaf in sun or shade, the only significant difference was found during the morning time period of November between the feeding body temperatures and the leaf temperatures of leaves located in the shade ($p = 0.0253$; Fig. 13). When ignoring time periods and looking at overall differences throughout the day based on location of leaf and month, the only significant difference was again found during November between the feeding body temperatures and the leaf temperatures of leaves located in the shade ($p = 0.0144$; Fig. 13).

With regard to the molting body temperatures, a clear trend is illustrated in Figure 14 where the body temperatures of the midday measurement period tend to be higher than the leaf

temperatures regardless of month and leaf location. Compared to the feeding larvae, this result suggests that molting larvae conform to surrounding ambient temperatures instead of thermoregulating. The largest individual difference between the body temperatures of molting larvae and leaf temperatures, again based on time period, month, and location of leaf in sun or shade, is also seen during November with leaves in the shade. However, this larger difference occurs during the midday time period instead of the morning, and no statistical significance was found between the two ($p = 0.113$; *Fig. 14*). Contrary to the feeding body temperatures, an overall significant difference was not found throughout the day between the molting body temperatures and the leaf temperatures of leaves located in the shade during November ($p = 0.367$; *Fig. 14*). No significant difference was found between any of the molting body temperatures and leaf temperatures regardless of time period, month, or location of the leaf.

To visualize this data in a different way, scatter plots were created with the leaf temperatures on the x-axis and either the feeding or molting body temperatures on the y-axis (*Figs. 15 and 16*). The positive regression between all leaf temperatures and body temperatures is quite evident. In both graphs, the May data portray similar linear regression lines with slopes close to one. The minor difference is that the feeding body temperature data for leaves in the sun increases with a slightly higher rate. For the data collected in November, the body temperatures are consistently higher when compared to leaves in the shade versus leaves in the sun. This is consistent with the results illustrated in the histograms. The major difference between the feeding results and molting results is that the linear lines for the November feeding data exhibit a lower rate, or slope. At low leaf temperatures the feeding body temperatures start higher than the molting body temperatures do, but then progress slower. This crucial result is evidence for thermoregulation in feeding monarch larvae throughout the month of November. A similar

change in slope throughout November is not as evident in molting monarch larvae, suggesting a lack of thermoregulation. One other minor discrepancy shown by the scatter plots is that the differences between molting body temperatures in the shade and sun decrease at lower leaf temperatures.

DISCUSSION

Monarch caterpillars are small organisms and any change in body temperature will be minimal. Milkweed plants, and the surrounding environment, do not display enormous temperature changes either. However, a change of 1-2°C per degree day of a caterpillar's life cycle can add up to make a significant difference overall in growth and developmental rates (Rawlins, 1981). Variation within the environment in the temperature of plant surfaces is quite evident when comparing temperatures taken during the hotter month of November and cooler month of May, and also when comparing surfaces located in the sun versus in the shade.

In May, the differences between the surface temperatures of leaves in the sun and shade are minimized due to the lower intensity (or absence) of the sun. In the hotter month of November, a significant difference is evident between the surface temperatures of leaves with those located in the sun being considerably higher. Although basking is their main method of thermoregulation, monarch larvae tended not to reside in direct sunlight, probably because the sun was too intense. Monarchs often used milkweed leaves as protection from the radiation. Many could be found resting in either partial sun regions of the plant (whether on a leaf, stem, or for smaller instars inside the top bud) or in complete shade on the underside of leaves.

One interesting result found was that the larvae consistently had higher body temperatures when resting on the underside of a leaf in the shade versus on the top surface in the sun. This is surprising as the leaf temperatures are warmer in the sun. Perhaps the radiation is

reflected away too much by the glossier surface of the tops of the leaves, or the wind creates a chill factor that hinders thermoregulation out in the open, or larvae that had recently moved out of the hot sun were instead being measured in the shade. On the other hand, perhaps the sheltered area on the underside of leaves, where most molting larvae were found, may simply provide a more stable environment with less fluctuation of daily temperatures.

As the sun fluctuates throughout the day, the temperatures of the plant surfaces rise and fall with a similar pattern as they absorb the sun's radiation. Because thermoregulation is defined as maintenance of a constant internal body temperature independent from the environmental temperature, this daily fluctuation of ambient temperatures is crucial for comparisons with body temperatures in determining whether or not thermoregulation has occurred. It is for this reason that it is harder to determine the level of thermoregulation in May when temperature differences are minimized.

With regard to the body temperatures of feeding monarch larvae, results show that feeding larvae do not thermoregulate during the cooler month of May. This is evident in the regression lines comparing leaf temperatures and feeding body temperatures for the month of May (*Fig. 15; May regression slope for sun = 1.09; May regression slope for shade = 0.985*). With the regression lines close to one, feeding body temperatures are simply conforming to the surrounding ambient temperatures. This lack of thermoregulation is most likely due to the limited amount of radiation available at this time of year.

However, in November, feeding monarch larvae do utilize thermoregulation to a significant degree. During the cooler parts of the day when leaf temperatures are at their lowest, larvae are able to raise their body temperature above ambient. This is seen in regression lines for both location in the sun and shade (*Fig. 15; Nov. regression slope for sun = 0.592; Nov.*

regression slope for shade = 0.597). Feeding larvae demonstrate the ability to lower their body temperatures when overheating is an issue by locating cooler, often shadier, regions of the milkweed plant in which to reside. When leaf temperatures are at their highest (most likely during the middle of the day) regression lines show a decrease in the body temperatures of larvae located in either the sun or shade (*Fig. 15*). In this way, larvae can adjust for the diurnal fluctuations of the sun as they attempt to maintain a steady internal body temperature, as close to the optimum (ca 30°C) as possible, throughout the entire day.

Previous studies support the idea that monarch larvae undergo thermoregulation quite often in an attempt to increase their rate of growth and development (Rawlins and Lederhouse, 1981; Zalucki, 1982). It has been well-documented that temperature is highly important to physiological processes and can be regulated by color plasticity and basking (Davis *et al.*, 2005; James, 1986). The observational data presented herein support the idea that monarch larvae thermoregulate during normal day to day activities. However, the fact that thermoregulation only occurs to a significant degree in feeding monarch larvae during November suggests that the adaptive thermoregulatory mechanism is limited when radiation levels are limited.

Data from this study also suggests that thermoregulation is significantly limited while larvae are going through the molting process. Thermoregulation was not found to occur to any significant degree for molting monarch larvae regardless of month or location within the sun or shade (*Fig. 16; Nov. regression slope for sun = 0.775; Nov. regression slope for shade = 0.957; May regression slope for sun = 0.947; May regression slope for shade = 0.981*). Body temperatures of the molting larvae fluctuated more than those of the feeding larvae in a pattern matching the rise and fall of the sun (*Figs. 6 and 7*). The fact that the molting body temperatures are lower in the morning and afternoon, when ambient temperatures are cooler, indicates that the

larvae are not maintaining a stable internal body temperature, but rather appear to be conforming to the ambient temperatures. This can lead to a problem during midday when the sun is the hottest. Results show that the molting body temperatures are higher than those for feeding larvae during midday regardless of the month (*Figs. 6, 7, 14*). Once molting larvae have chosen a spot and began the molting process, they cannot adjust for changes in the surrounding ambient temperatures. Naturally, at midday when the sun heats up molting larvae do as well, as they cannot hide within the shade to help maintain a steady optimum temperature during the course of a day.

The primary test of the hypothesis that molting larvae thermoregulate was measured by the slope between body temperatures and ambient temperatures. The regressions for molting body temperatures and leaf surface temperatures show regression lines close to one during the month of May similar to those seen in feeding monarch larvae (*Fig. 16*). In contrast to the feeding larvae, in November regression lines were still close to one (*Fig. 16*). Although molting body temperatures in the shade show slight thermoregulation, it is not significant enough to make the inference that larvae actively undergo thermoregulation during the molting process.

Based on current knowledge, it was originally hypothesized that monarch caterpillars would thermoregulate during the molting process in a manner different from thermoregulatory patterns observed during feeding. It was assumed that location within a warm environment would still be important while molting and necessary for rapid transit through the vulnerable molting process. The results of this study suggest that although monarch larvae thermoregulate while feeding in November, they do not thermoregulate while molting. This could be due to several factors, such as reducing exposure to predators by molting sheltered underneath leaves or reducing the risk of overheating in direct sunlight by molting in the shade. The important

question that is raised by these results is whether or not caterpillars are actually behaviorally/physiologically restrained from thermoregulating during the molting process. If this is the case, then choice of location must be due to another factor altogether.

As a rare study of thermoregulation and molting, this paper opens the door for further research. More extensive experimental studies should be done to test the idea of thermoregulating constraints during molting. Additional work should increase replication and randomization of data measurements as well. The new knowledge gained through this study about the lack of thermoregulation during the molting process raises questions about explanatory mechanisms and emphasizes its importance during the growth of each instar.

CONCLUSION

Feeding monarch larvae thermoregulate to a significant degree in November, while conforming to ambient temperatures in May. Molting monarch larvae do not thermoregulate at all regardless of month. During the molting process, larvae must conform to the fluctuations in ambient temperature provided by the diurnal rise and fall of the sun. The data presented in this study regarding feeding monarch larvae support earlier thermoregulation studies (Hazel, 2002; Kingsolver, 2000; Rawlins and Lederhouse, 1981; Zalucki, 1982). However, the relationship of temperature and molting between instars is poorly understood. The molting data collected raise questions about mechanisms involved in the molting process. It is possible that monarch larvae are physiologically constrained from thermoregulating while molting. Further experimental studies should be done to test the idea of thermoregulatory constraints, while working to increase replication and randomization of data measurements as well.

APPENDICES

Appendix A: Tables for November and May

Table 1. Instar counts for all the larvae found and measured. A few *Danaus chrysippus* (D.C.) larvae were also found, although their data was not used in the feeding and molting graphs.

COMPLETE INSTAR COUNT			
NOV.		MAY	
I	27	I	52
II	26	II	32
III	24	III	17
IV	10	IV	25
V	57	V	36
V (D.C.)	11	V (D.C.)	5
Total	155	Total	167

MOLTING COUNT ONLY			
NOV.		MAY	
I – II	6	I – II	10
II – III	3	II – III	5
III – IV	6	III – IV	11
IV – V	3	IV – V	6
Total	18	Total	32

Table 2. Average temperature recordings for different parts of the surrounding environment measured in November and May. Numbers show that each part of a plant absorbs radiation to a different degree.

Nov.	Leaf sun	Leaf shade	Bud	High Leaf	Low Leaf	Stem	Stalk	Ground
AVG:	31.1	29.7	28.5	30.1	30.7	31.9	30.3	32.1
May	Leaf sun	Leaf shade	Bud	High Leaf	Low Leaf	Stem	Stalk	Ground
AVG:	26.3	26.3	27.5	25.8	28	27.5	26.7	28.6

Table 3. November breakdown of the number of larvae found at each location within the available environment. Total number of larvae found and the average body temperature at each location are given for the morning, midday, and afternoon periods of the testing.

Time	Light	Plant Region	# Found	Total #	AVG Body Temp	
8-10:59	sun	leaf	5	9	31.7	
		stalk	3		30.9	
		grass	1		36.4	
	partial sun	leaf	8	11	29.8	
		bud	2		30.7	
		grass	1		27.7	
	shade	leaf	8	17	30.8	
		stem	1		29.1	
		stalk	2		33.1	
grass		6	32.8			
leaf		5	32.2			
bud		1	35.3			
11-13:59	sun	grass	1	7	33.2	
		leaf	7		33.2	
		bud	2		31.6	
	partial sun	flower	1	13	34.5	
		stem	2		33.4	
		grass	1		38.1	
		leaf	24		41	32
		bud	5			30
		stem	1			33.1
	14-18:00	sun	stalk	3	11	34.6
			grass	8		33.5
			leaf	6		31
bud			2	26.9		
partial sun		stem	2	17	30.8	
		stalk	1		33.1	
		leaf	11		30.5	
		flower	3		31.7	
		stalk	2		29.8	
shade	grass	1	29	33.7		
	leaf	19		28.6		
	bud	7		29.4		
	stem	1		33.2		
	stalk	1		32		
	grass	1		37.7		

Table 4. May breakdown of the number of larvae found at each location within the available environment. Total number of larvae found and the average body temperature at each location are given for the morning, midday, and afternoon periods of the testing.

Time	Light	Plant Region	# Found	Total #	AVG Body Temp
8-10:59	sun	none	0	0	0
		leaf	3	4	27.6
	shade	stem	1		29.2
		leaf	41	65	24.8
		bud	7		25.6
		flower	1		25.6
		stem	2		27.3
		stalk	2		25.9
		grass	11		25.3
		web	1		22.4
		11-13:59	sun	leaf	6
leaf	9			15	28.2
shade	bud		3		30.3
	stem		1		29.7
	seed pod		2		27
	leaf		27	35	27.4
	bud		2		28.1
	stem		2		26.1
	stalk		2		31.4
	grass		2		27.9
14-18:00	sun	leaf	9	11	27.9
		stem	1		28.9
		grass	1		31.4
	partial sun	leaf	6	7	26.1
		stem	1		32
		grass	1		28.7
	shade	leaf	17	24	27.5
		bud	3		25.7
		stem	3		26.1
		grass	2		28.3

Table 5. November breakdown of the level of activity of each larva found and their location within the sun, partial sun, or shade. Resulting average body temperatures and the number of larvae feeding, crawling, molting, and basking are given.

Exposure	Movement	Activity	# Found	Avg Body Temp
sun	active	feeding	6	31.9
sun	active	crawling	1	33.2
sun	inactive	molting	1	33.2
sun	inactive	basking	19	31.3
partial sun	active	feeding	16	31.3
partial sun	active	crawling	1	33.7
partial sun	inactive	molting	11	30.6
partial sun	inactive	basking	13	32.0
shade	active	feeding	14	32.0
shade	active	crawling	3	31.5
shade	inactive	molting	6	29.0
shade	inactive	resting	64	31.2

Table 6. May breakdown of the level of activity of each larva found and their location within the sun, partial sun, or shade. Resulting average body temperatures and the number of larvae feeding, crawling, molting, and basking are given.

Exposure	Movement	Activity	# Found	Avg Body Temp
sun	active	feeding	5	28.7
sun	active	crawling	0	0
sun	inactive	molting	1	28.9
sun	inactive	basking	11	28.0
partial sun	active	feeding	6	28.8
partial sun	active	crawling	1	25.6
partial sun	inactive	molting	5	28.5
partial sun	inactive	basking	14	27.9
shade	active	feeding	11	24.7
shade	active	crawling	5	24.6
shade	inactive	molting	26	26.9
shade	inactive	resting	82	24.9

Appendix B: Figures

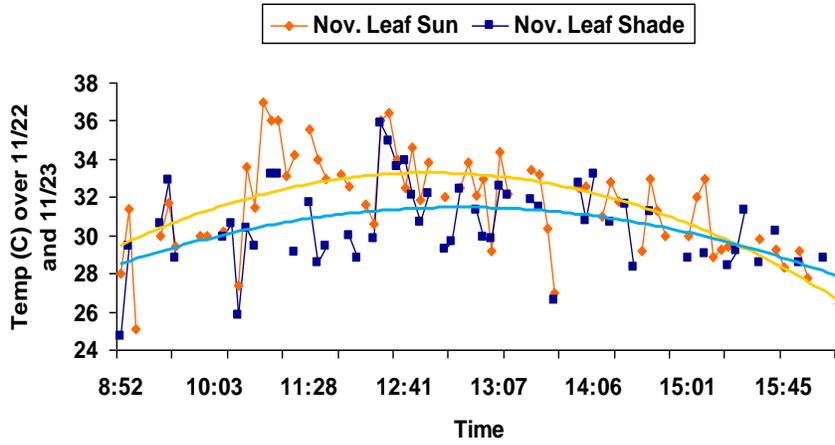


Figure 1. Representation of the diurnal fluctuations in leaf temperatures in the sun and shade measured over two field days in Nov. with polynomial trend lines. Recorded at Pinjarra Hills.

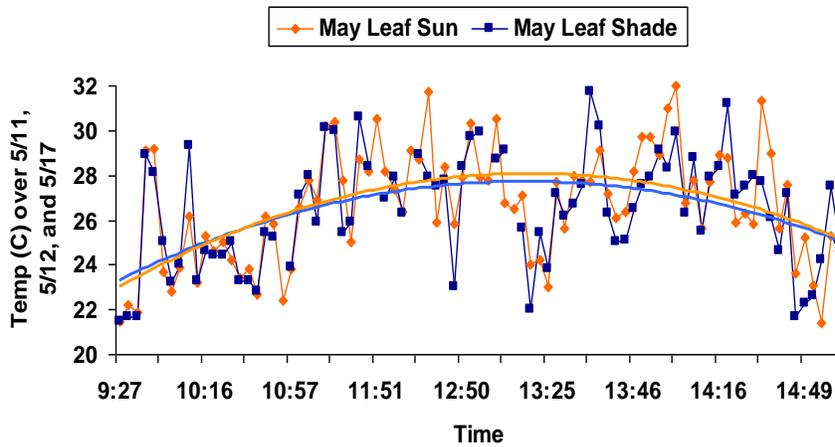


Figure 2. Representations of the diurnal fluctuations in leaf temperatures in the sun and shade measured over three field days in May with polynomial trend lines. Recorded at Pinjarra Hills.

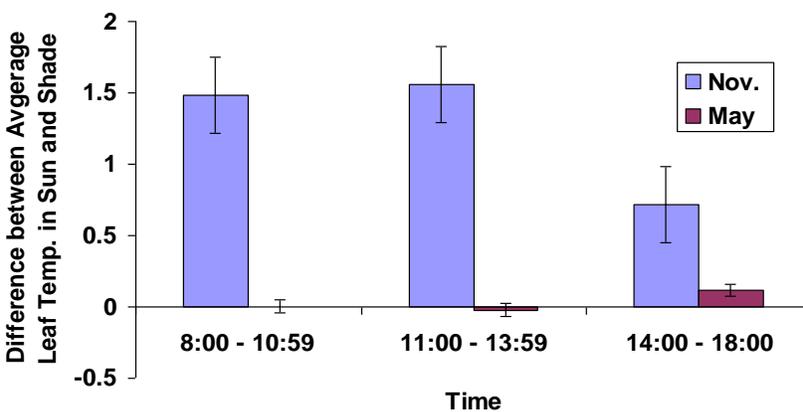


Figure 3. Differences between the average leaf temperatures in the sun and shade ($T_{Sun} - T_{Shade}$) over different time periods in the different seasons of Nov. and May. Standard error bars are shown. Recorded at Pinjarra Hills.

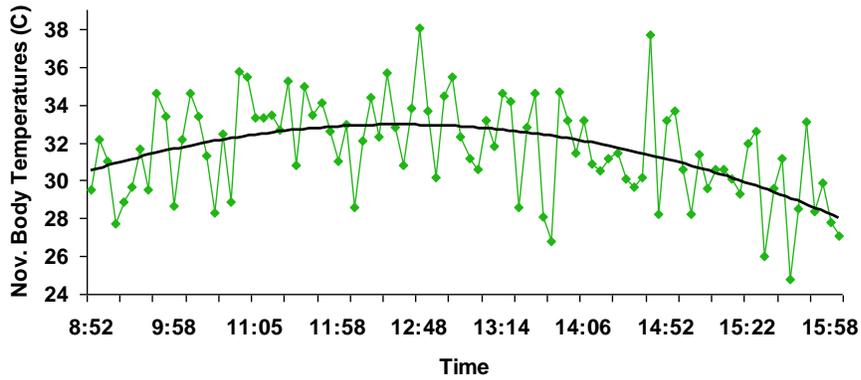


Figure 4. Representation of the diurnal fluctuations of all monarch body temperatures, of any instar stage at various locations on milkweed plants, measured in Nov. with a polynomial trend line. Recorded at Pinjarra Hills.

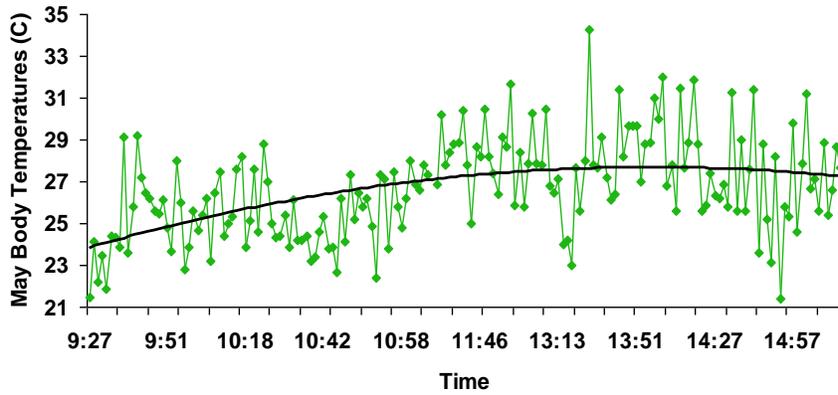


Figure 5. Representation of the diurnal fluctuations of all monarch body temperatures, of any instar stage at various locations on milkweed plants, measured in May with a polynomial trend line. Recorded at Pinjarra Hills.

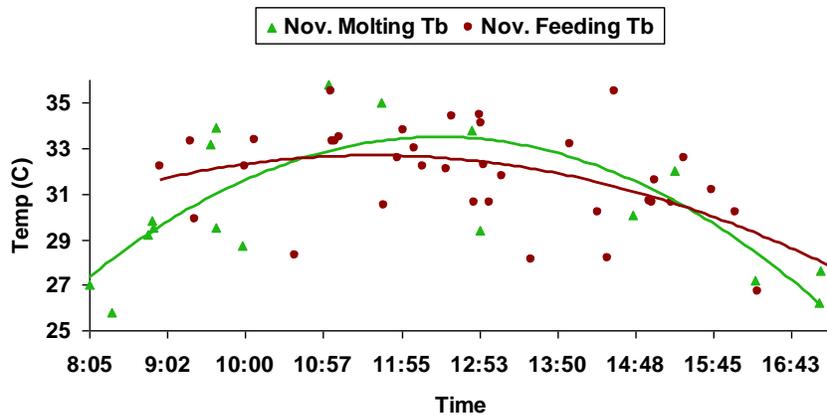


Figure 6. Scatter plot representation and polynomial best fit lines for all feeding and molting body temperatures of monarch larvae (all instar stages) at various locations on milkweed plants in November. Recorded at Pinjarra Hills.

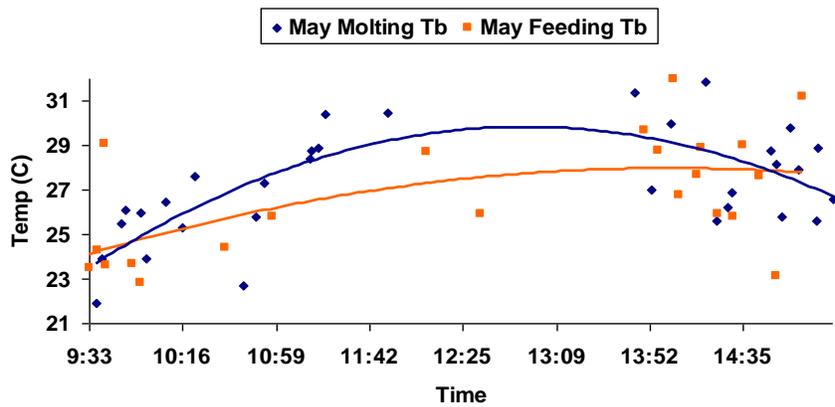


Figure 7. Scatter plot representation and polynomial best fit lines for all feeding and molting body temperatures of monarch larvae (all instar stages) at various locations on milkweed plants in May. Recorded at Pinjarra Hills.

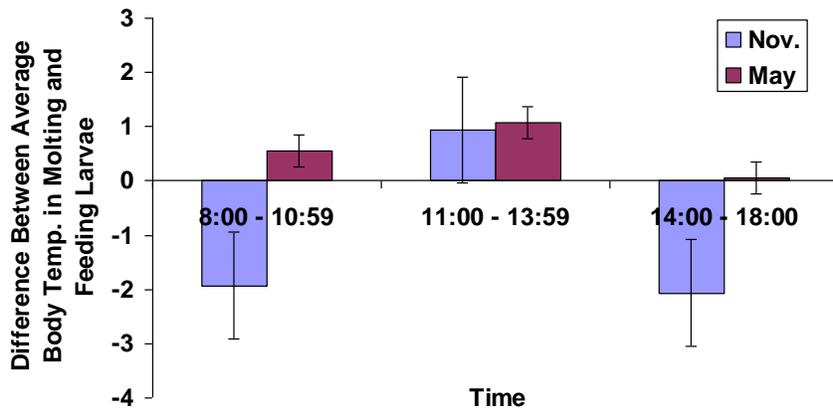


Figure 8. Comparison of the overall differences between the average body temperatures of all molting and feeding monarch larvae ($T_{\text{Molting}} - T_{\text{Feeding}}$), of any instar stage, at various locations on milkweed plants over different time periods in the different seasons of Nov. and May. Standard error bars are shown. Recorded at Pinjarra Hills.

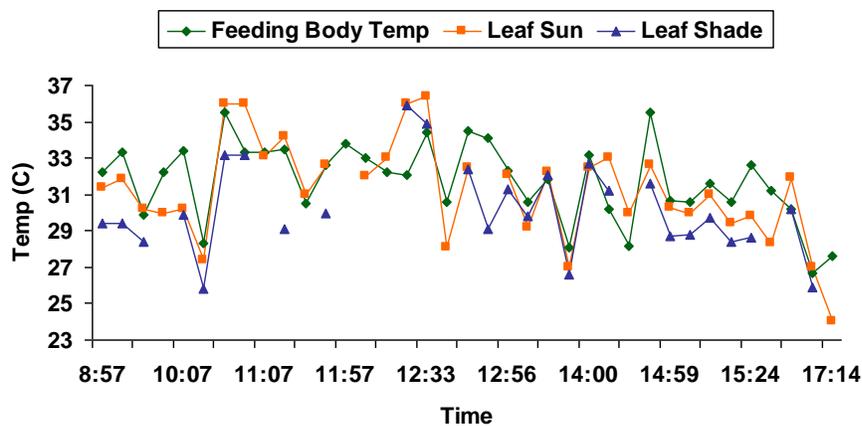


Figure 9. Comparison of the feeding body temperatures, of any instar stage at various locations on milkweed plants, and the corresponding leaf temperatures in the sun and shade during Nov. Recorded at Pinjarra Hills.

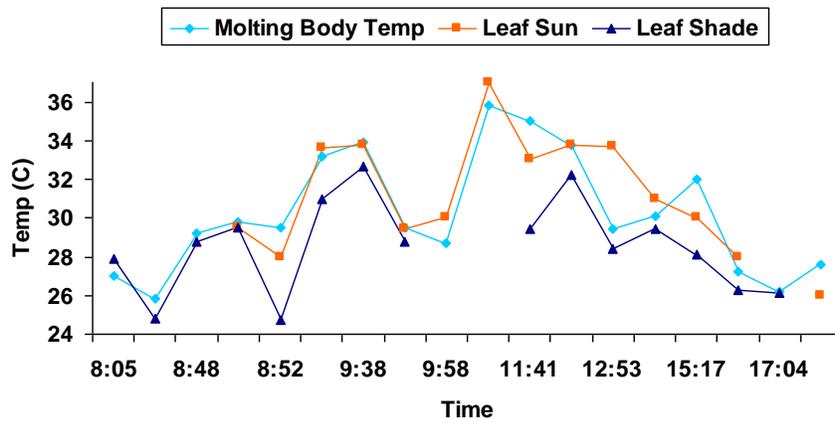


Figure 10. Comparison of the molting body temperatures, of any instar stage at various locations on milkweed plants, and the corresponding leaf temperatures in the sun and shade during Nov. Recorded at Pinjarra Hills.

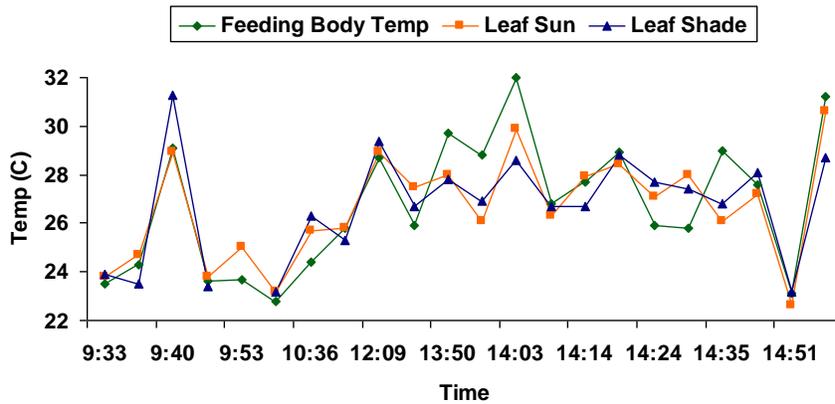


Figure 11. Comparison of the feeding body temperatures, of any instar stage at various locations on milkweed plants, and the corresponding leaf temperatures in the sun and shade during May. Recorded at Pinjarra Hills.

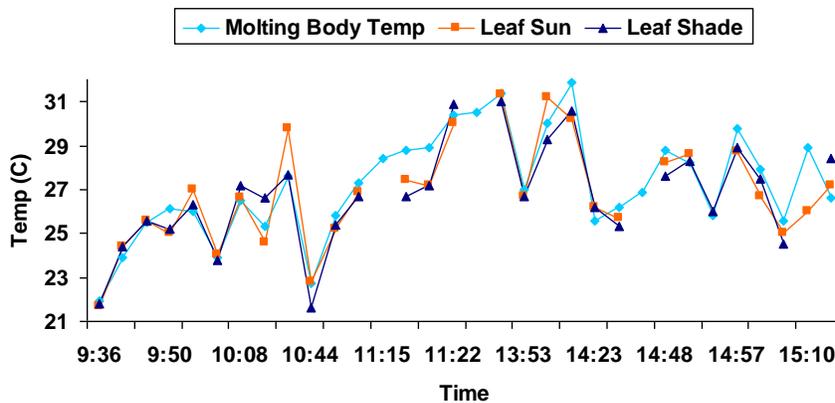


Figure 12. Comparison of the molting body temperatures, of any instar stage at various locations on milkweed plants, and the corresponding leaf temperatures in the sun and shade during May. Recorded at Pinjarra Hills.

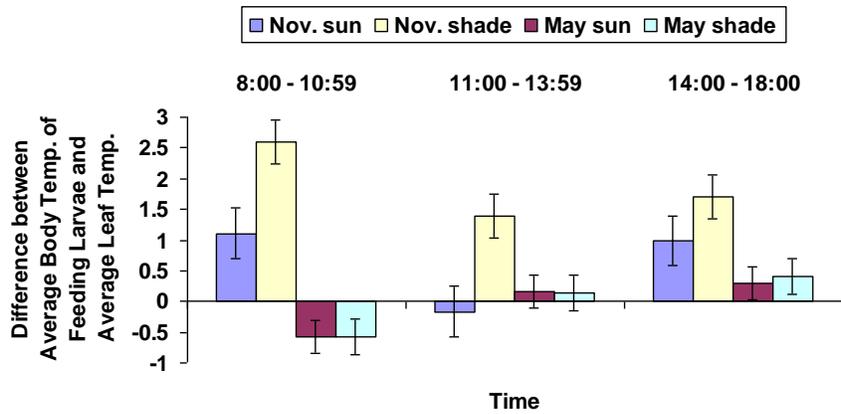


Figure 13.

Differences between the average body temperatures of feeding monarch larvae, taken from any instar stage at various locations on milkweed plants, and the average leaf temperatures in the sun and shade ($T_{\text{Feeding}} - T_{\text{Leaf}}$) over different time periods during the different seasons of Nov. and May. Standard error bars are shown. Recorded at Pinjarra Hills.

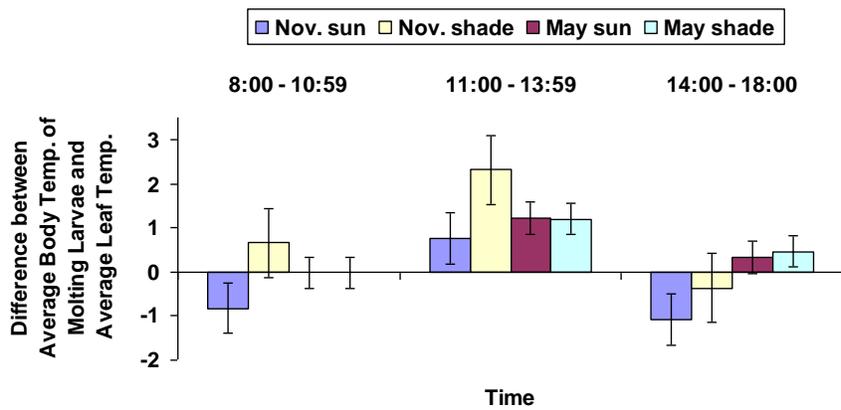


Figure 14.

Differences between the average body temperatures of molting monarch larvae, taken from any instar stage at various locations on milkweed plants, and the average leaf temperatures in the sun and shade ($T_{\text{Feeding}} - T_{\text{Leaf}}$) over different time periods during the different seasons of Nov. and May. Standard error bars are shown. Recorded at Pinjarra Hills.

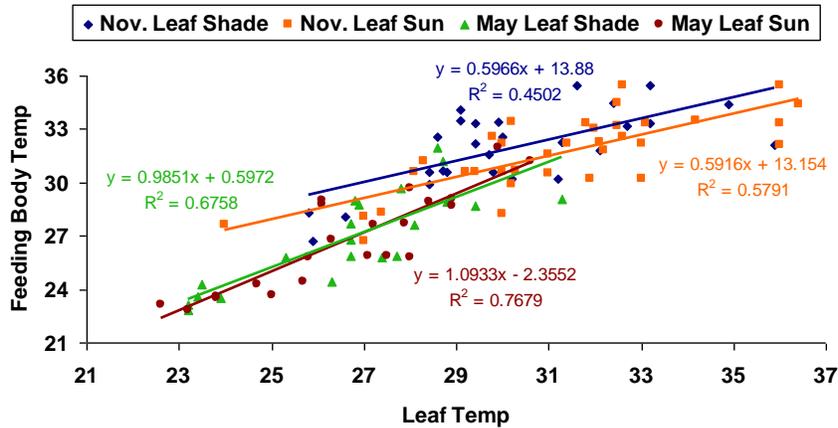


Figure 15. Regression between all feeding body temperatures, taken from any instar stage at various locations on milkweed plants, and leaf temperatures. Broken down according to the different seasons of Nov. and May and location within the sun or shade. Recorded at Pinjarra Hills.

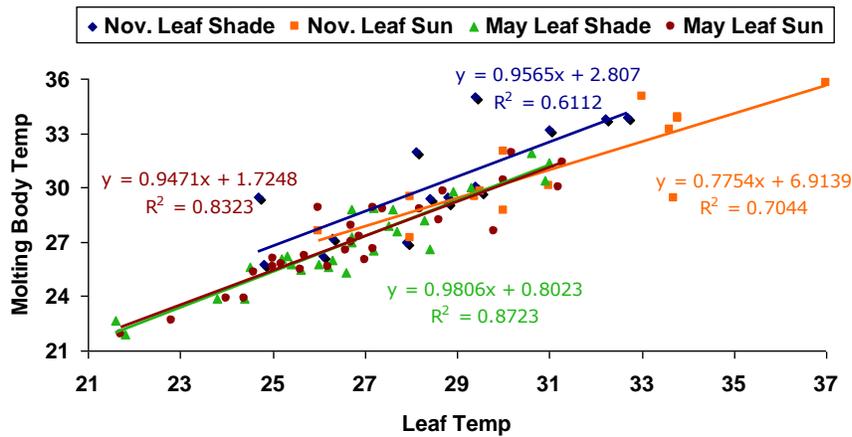


Figure 16. Regression between all molting body temperatures, taken from any instar stage at various locations on milkweed plants, and leaf temperatures. Broken down according to the different seasons of Nov. and May and location within the sun or shade. Recorded at Pinjarra Hills.

REFERENCES:

Clarke, Anthony R., Zalucki, Myron P. (2004) Monarchs in Australia: on the winds of a storm?

Biological Invasions, **6**: 123-127.

This paper uses historical records to hypothesize the source of the founder population of monarch butterflies in Australia. It discusses the date of arrival, source of the populations, and their spread across the continent. A decent background is gained on the origins of *D. plexippus* L. to supplement further research on the species.

Davis, Andrew K., Farrey, Bethany D., Altizer, Sonia. (2005) Variation in thermally induced melanism in monarch butterflies (Lepidoptera: Nymphalidae) from three North American populations. *Journal of Thermal Biology*, **30**: 410-421.

This source directly deals with the monarch butterfly, providing valuable background information on the species and discussing the melanistic adaptation of the larvae during development. Coloration is extensively discussed and analyzed in correspondence to temperature. This study also looks into coloration of adult butterflies and clearly shows the types of statistical tests used to analyze the data.

Dyck, Hans Van, Matthysen, Erik, Wiklund, Christer. (1998) Phenotypic variation in adult morphology and pupal colour within and among families of the speckled wood butterfly *Pararge aegeria*. *Ecological Entomology*, **23**: 465-472.

Unlike the other sources, this experimental study focuses on butterfly wing color and the functions of the wings in speckled wood butterfly. It analyzes differences in adult morphological traits among families, sexes, and pupal color types at the intraspecific level. It provides further information as to the importance of thermal biology to not only the larvae, but the adult stage of butterflies.

Hazel, Wade N. (2002) The environment and genetic control of seasonal polyphenism in larval color and its adaptive significance in a swallowtail butterfly. *Evolution*, **56** (2): 342-348.

Hazel examines seasonal polyphenism in swallowtail butterflies and the seemingly darker coloration of larvae brought up in autumnal conditions compared to those raised in midsummer conditions. He suggests a genetic basis for the polyphenism. This source provides general information as to how caterpillar coloration influences growth rate and body temperature and is a good comparison to monarchs as the swallowtail larvae can have varying degrees of black.

Heinrich, Bernd, ed. *Insect Thermoregulation*. New York: John Wiley & Sons, 1981.

This book offers a substantial amount of general information concerning insect thermoregulation. It addresses temperature regulation, behavioral and physiological mechanisms of thermoregulation, performance of insect muscle, and ecological and evolutionary perspectives. Most useful to this research paper are its definitions and thermoregulatory taxonomy.

James, David G. (1986) Thermoregulation in *Danaus Plexippus* (L.) (Lepidoptera:

Nymphalidae): Two Cool Climate Adaptations. *General and Applied Entomology*, **18**.

Two cool climate adaptations for thermoregulation in *Danaus Plexippus* (L.) are examined in this research paper. It provides evidence supporting the use of delta wing basking and melanic coloration in larvae to enhance body temperatures. Dealing directly with monarchs, this paper is to the point and clearly outlines the methodology used to test such adaptations.

Kingsolver, Joel G. (2000) Feeding, growth, and the thermal environment of cabbage white

caterpillars, *Pieris rapae* L. *Physiological and Biochemical Zoology*, **73** (5): 621-628.

Kingsolver demonstrates a new method of testing the relationship between caterpillar growth and the thermal environment. He uses wild-caught cabbage white caterpillars in his lab studies and both wild-caught and pre-made caterpillar models in his field studies to test how food consumption and the thermal environment affects growth rate. His results provide a decent indication as to the daily fluctuations in body temperature of the caterpillars and compare larval growth at different temperatures to overall growth.

Kuhrt, Ute, Samietz, Jorg, Dorn, Silvia. (2005) Thermoregulation behaviour in codling moth

larvae. *Physiological Entomology*, **30** (54-61).

Temperature gradient experiments are used in this research paper to examine the thermoregulation behaviour of codling moth larvae feeding within apples and mature larvae searching for cocoon sites. Results are used to help explain the exploitation of temperature differences, especially within feeding larvae. This study also brings forth the idea that during the insect life cycle thermoregulation behaviour can change or disappear.

Malcolm, Stephen B. and Myron P. Zalucki, eds. *Biology and Conservation of the Monarch*

Butterfly. Los Angeles: Natural History Museum, 1993.

Malcolm and Zalucki have successfully put together multiple papers on the biology and conservation of monarchs that encompass a wide variety of topics. A detailed chapter outlines temperature and thermoregulation in monarch butterflies. The monarch's

complex life history is analyzed along with the ecological influence of temperature on all life stages.

Nice, Chris C., Fordyce, James A. (2006) How caterpillars avoid overheating: behavioral and phenotypic plasticity of pipevine swallowtail larvae. *Oecologia*, **146**: 541-548.

This source addresses how caterpillars avoid thermal maximum temperatures by climbing higher up on a non-host plant. It looks at the maladaptive results of thermoregulation and provides some background information on color polyphenism in pipevine swallowtail larvae. It addresses microhabitat shifts and optimal growth temperatures.

Rawlins, John E., Lederhouse, Robert C. (1981) Developmental Influences of Thermal Behavior on Monarch Caterpillars (*Danaus plexippus*): An Adaptation for Migration (Lepidoptera: Nymphalidae: Danainae). *Journal of the Kansas Entomological Society*, **54** (2): 387-408.

An abundance of data is collected on the relationships between temperature, growth and development rates, and behavioral responses to solar radiation in monarch caterpillars. A decent summary concerning the different percentages of time spent doing different activities and in what locations is outlined. This paper brings forth significant knowledge on the field behavior of monarchs necessary for further research.

Solensky, Michelle J., Larkin, Elizabeth. (2003) Temperature-induced variation in larval coloration in *Danaus plexippus* (Lepidoptera: Nymphalidae). *Entomological Society of America*, **96** (3): 211-216.

By placing monarch larvae in temperature-specific rearing facilities, Solensky and Larkin measure temperature-induced variation in larval coloration after rearing. Mortality and development rates are also analyzed with clear graphs to back up the results. This source specifically mentions monarch larvae in Australia and the benefits of plasticity in body coloration.

Stamp, Nancy E., and Timothy M. Casey, eds. *Caterpillars: Ecological and Evolutionary Constraints on Foraging*. New York: Chapman & Hall, 1993.

This book deals with all aspects affecting the foraging patterns of caterpillars. It deals with ecological and evolutionary constraints, consequences, and environmental variations. It provides general background information concerning thermoregulation and provides graphs and reasons for such strategies.

Talloe, W., Dyck, H. Van, Lens, L. (2004) The cost of melanization: butterfly wing coloration under environmental stress. *Evolution*, **58** (2): 360-366.

The cost of melanization and the constraints on thermoregulation were explored in this environmental study together with the effects of host-plant stress on life-history traits. Melanin was discussed in more detail along with the factors influencing the degree of melanization. This source shows a correlation between environmental conditions and wing coloration, demonstrating that color is not only based upon temperature.

Zalucki, Myron P., Clarke, Anthony R. (2004) Monarchs across the Pacific: the Columbus hypothesis revisited. *Biological Journal of the Linnean Society*, **82**: 111-121.

The assumptions that monarchs have always been common in North America and that their migratory behavior has developed over evolutionary time are challenged in this paper. The movement of monarchs suggested in the 'Columbus hypothesis' is revisited and a new analysis is presented. Additional light is shed on the origins and spread of monarchs across the Pacific.

Zalucki, Myron P. (1982) Temperature and Rate of Development in *Danaus Plexippus* L. and *D. Chrysippus* L. (Lepidoptera: Nymphalidae). *J. Aust. Ent. Soc.*, **21**: 241-246.

Zalucki focuses on describing the effects of temperature on development and mortality in *D. plexippus* L. and *D. chrysippus* L. while comparing the results for both species. He develops a time scale based on the developmental zero and day-degrees spent in any one stage and uses linear models to clearly depict the relationships found. Competition between the two species can therefore be examined based on the effects of the thermal time scale.