Plasticity of pigmentation and thermoregulation of the harlequin bug, *Murgantia histrionica,* in response to developmental temperature

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Abstract

Sustaining homeostasis in a changing environment relies on an organism’s ability to maintain an internal temperature within a specific range through thermoregulation. When temperatures fall outside an optimal range, metabolic, reproductive and behavioral functions can be impaired. As poikilothermic ectotherms, insects must rely on mechanisms other than metabolic heat production to protect themselves from extreme temperatures.

Many insects, such as the harlequin bug *Murgantia histrionica*, demonstrate phenotypic plasticity in body color, possibly taking advantage of solar radiation to warm and cool their bodies. On its dorsal side, the harlequin bug displays geometric patterns resulting from a juxtaposition of black and color, and this ratio may show variation among individuals. Previous studies demonstrated that both thermoperiod and photoperiod play an influential role in determining this pigmentation. In order to investigate the impact of temperature on pigmentation patterns, we reared 4th instar harlequin nymphs in two thermal environments, mimicking the seasonal temperatures observed in their natural geographic range, and quantified the black to color ratio using digital imagery. We were unable to induce a darker phenotype from 4th instar thermoperiod exposure alone, indicating that photoperiod may play an important role in melanization, and that this melanization may be determined before the 4th instar.

In order to assess differential thermoregulation capabilities in response to melanization, we monitored the temperature of adult wild population harlequin bugs in dark and basking conditions. Our data showed that bugs with a greater proportion of black in their dorsal patterning reached higher body temperatures when exposed to light than less melanized bugs, indicating that melanization may play an important role in thermoregulation.

Introduction

- In temperate environments, where temperature and sunlight differ greatly over the course of the year, maintaining homeostasis through thermoregulation may be one of the key factors determining species survival. Poikilothermic ectotherms such as insects, who are unable to sustain body temperature within an optimal range exclusively through metabolic heat production, must rely on other mechanisms to protect themselves.
- The theory of thermal melanism suggests that insects can alter their coloration by increasing production of the pigment melanin in colder environments, thereby enabling the body temperature to reach higher temperatures (Trullas *et al.* 2007).
- Evidence of melanin plasticity has been observed in several different insect species. For example, *Lepidoptera* larvae produce more melanin when exposed to colder temperatures during a specific critical period during development (Hazel 2002), and adult *Pieris rapae* and *Papilio polyxenes* exhibit darker wings in wintrier environments (Stoehr & Gouox 2008, Hazel 2002). In contrast, melanin patterns in Ladybirds (scientific name) have been shown not to be determined during a critical stage in development, but rather by the amount of total time spent in cold thermoperiods (Michie *et al.* 2010).
- To explore inducers of thermal melanism we chose to focus on the Harlequin bug, *Murgantia histrionica*, a pest of the *Brassicaceae* family that has colonized much of the southeastern United States. Adult bugs display a geometric pattern on their dorsal side resulting from a juxtaposition of black and yellow. Individuals show variation in this ratio, and its plasticity has been previously observed (Figure 1, Whitman & Agrawal 2009). However, it has not yet been determined whether the variation is induced by seasonal differences in thermoperiod, photoperiod, or both.
- We sought to determine if the Harlequin bug’s melanism plasticity could be induced by thermoperiod alone by rearing nymphs to adulthood in thermal environments mimicking the seasonal temperatures observed in their natural geographic range. We also monitored the temperature of adult wild harlequin bugs in dark and basking conditions in order to assess whether there is a relationship between degree of melanization and body temperature.

Methods

- Twenty-two adult male bugs (n = 22) from Eastern VA were placed in a 26°C environmental chamber under an unlit lamp. After 3 minutes the light was activated for 8 minutes. We recorded bug temperature and air temperature every 20 seconds (Figure 2).
- Bugs used in our rearing experiment were the first filial (F1) offspring of adults collected from Athens, OH. When bugs molted to the 4th instar we randomly distributed nymphs between two thermoperiod conditions, a “fall” chamber (n = 25) set at 12°C and a “summer” chamber (n = 25) kept at 26°C, both on a 12hr:12hr light:dark cycle. Both summer and fall chambers were equipped with basking lights. Upon molting to adulthood, we photographed bugs for pigment analysis. Melanization was quantified as percent black patterning of the total dorsal surface area.
- For our temperature trials, we performed a linear regression on percent black surface area against body temperature increase per gram of bug weight. For our rearing experiments, we performed a 2-sample t-test on the average summer and fall percent black surface area.

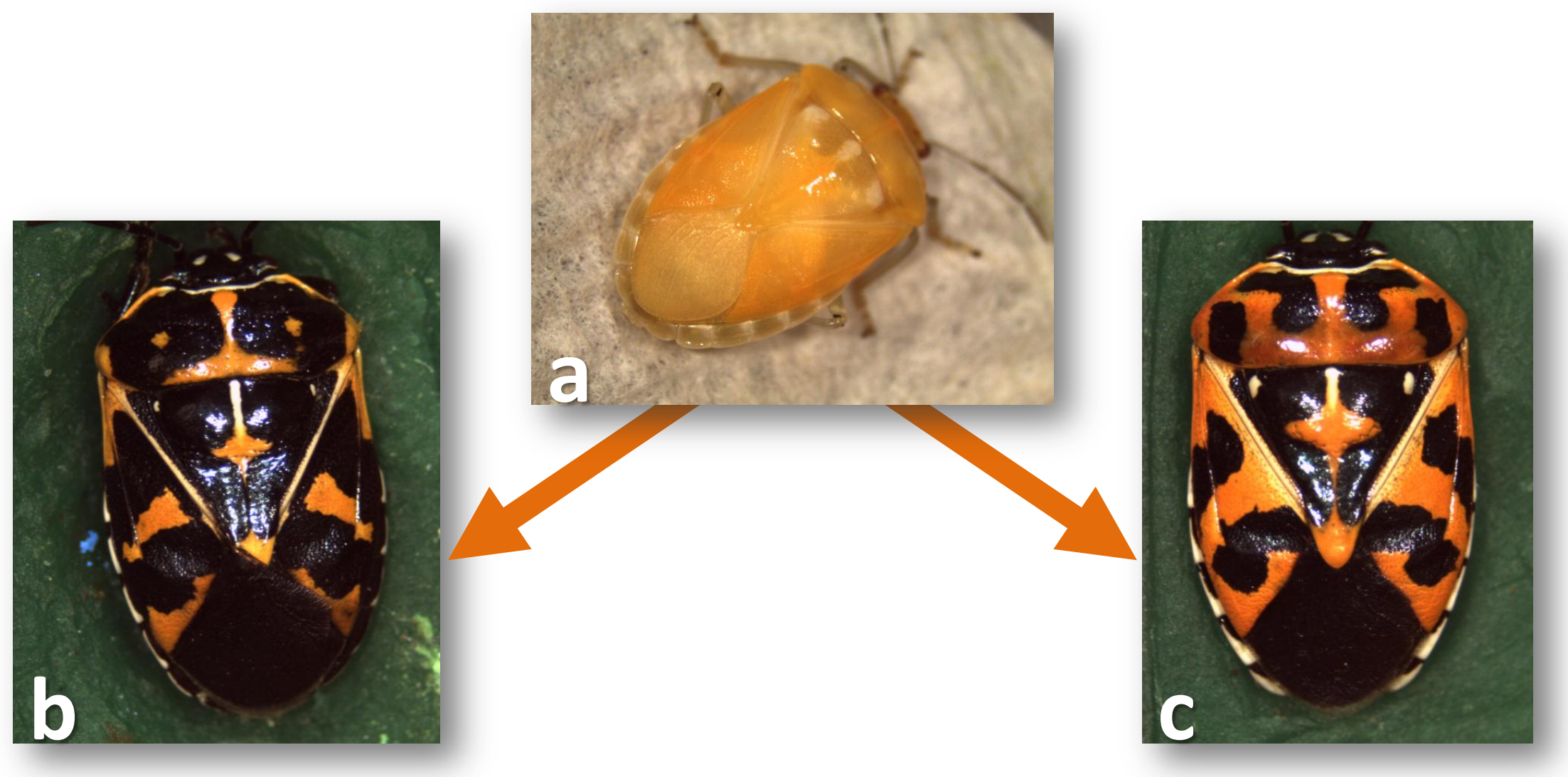

Figure 1. Different degrees of melanization observed in adult *M. histrionica.* (a) Newly molted unmelanized individual. (b) Individual with a high degree of melanization. (c) Individual with a low degree of melanization.

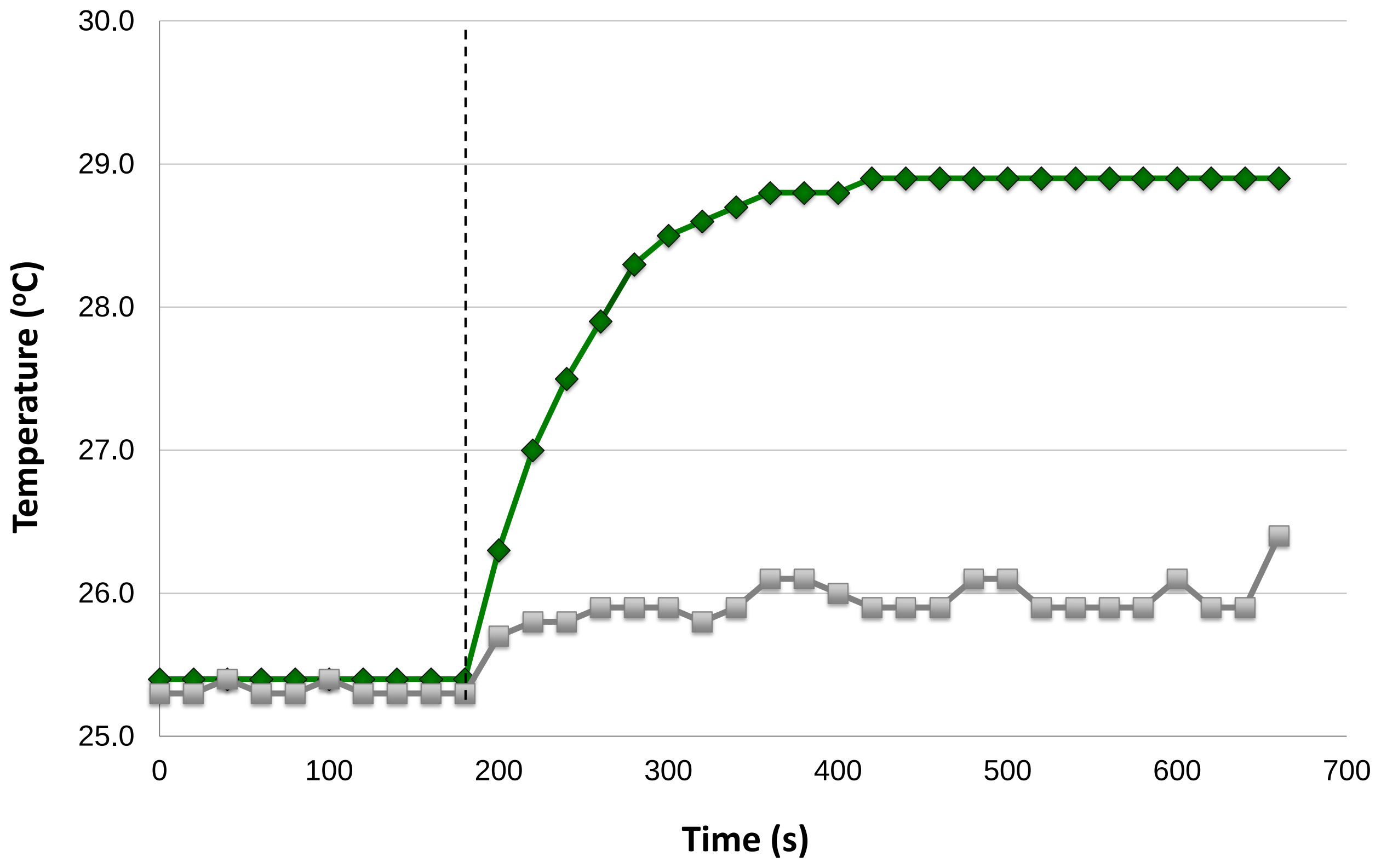

Figure 2. Sample graph of 0.0575 g adult male *M. histrionica* body temperature (green line) v. air temperature (grey line) in 26°C environmental chamber in dark and underneath light. Vertical dashed line represents when lamp was turned on (180 seconds). Temperature increase per gram = .880(proportion of black pigment) + 9.419

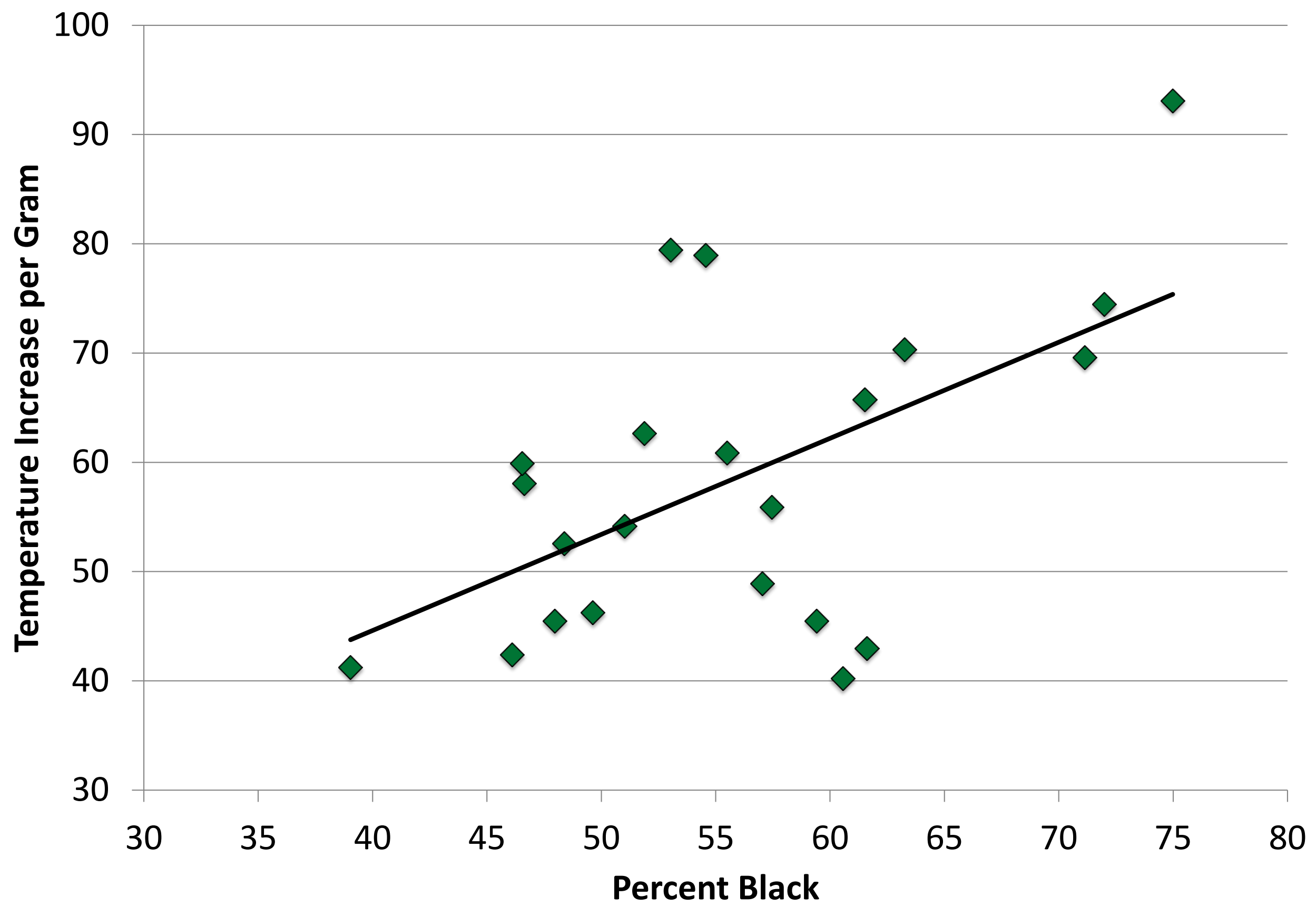

Figure 3. Linear regression of percent black dorsal surface area v. *M. histrionica* body temperature increase per gram (R^2 = 0.310, p = 0.007 n = 22).

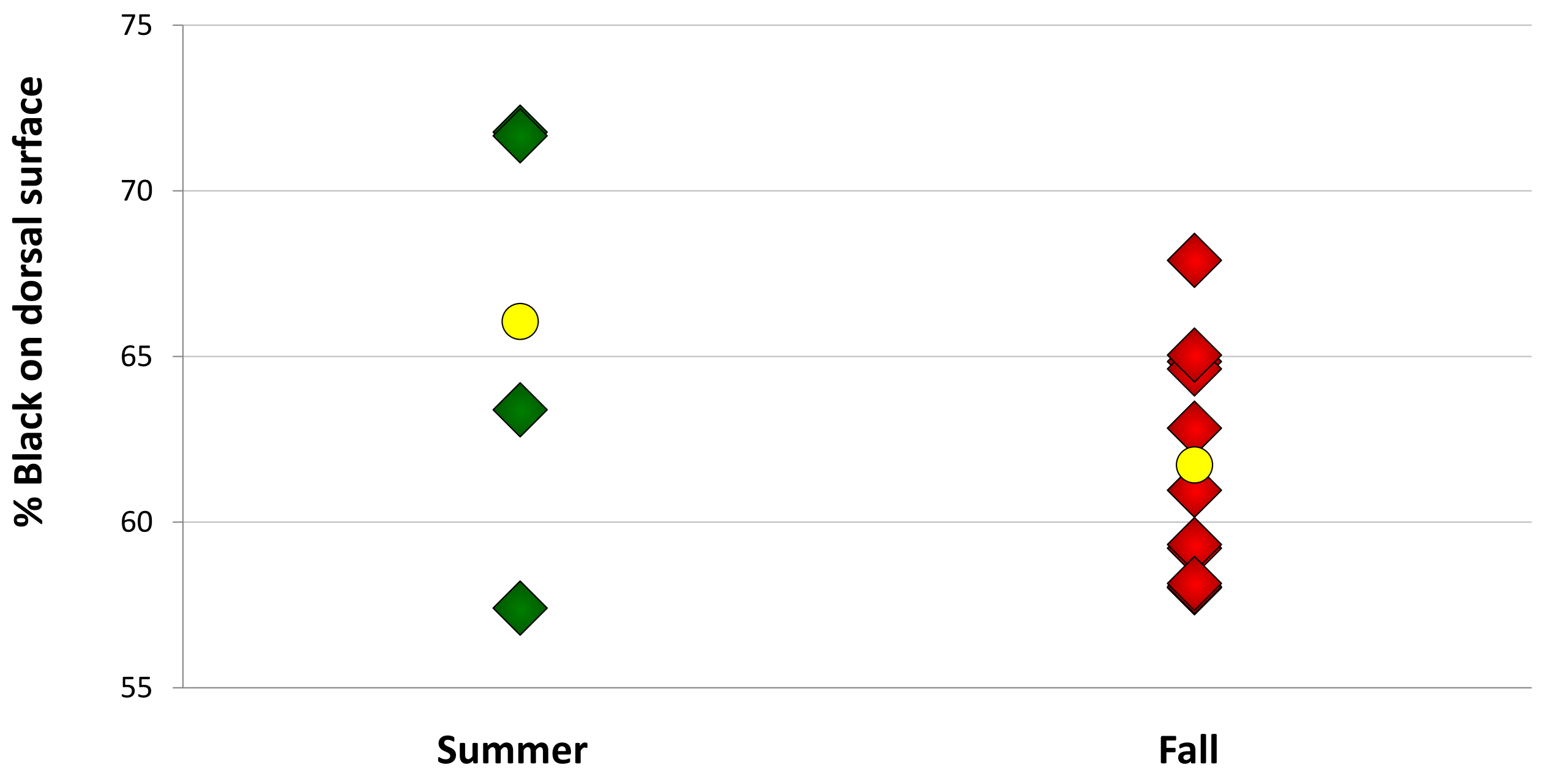

Figure 4. Average percent melanization of total surface area of *M. histrionica* moved to summer and fall environments as 4th instars. 2-sample t-test, p = 0.320, n_{SU} = 4, n_{FA} = 11. Error bars represent SEM. Yellow points represent mean.

Results

Temperature Trials:

- Our results indicated that there is a relationship between melanization and body temperature. Bugs with a greater proportion of black in their dorsal pattern were able to increase their body to higher temperatures than bugs with less black (Figure 3, linear regression, R^2 = 0.310, p = 0.007 n = 22).

Environmental Treatments:

- Contrary to our hypothesis, we found that bug melanization was not influenced by the thermoperiods experienced during the 4th and 5th instars. We observed no difference in percent black between the two treatment groups (Figure 4, 2-sample t-test, p = 0.320, n_{SU} = 4, n_{FA} = 11). The average melanization for the fall group was 61.72% black (SEM ± 1.0), while the average melanization for the summer group was 66.06% (SEM ± 3.5). However, due to high mortality rates only a total of 4 summer and 11 fall bugs could be examined.

Discussion

- We found that melanization did influence body temperature, as more melanized individuals reached higher body temperatures than less melanized individuals. If this trait is indeed under selection, it must confer a fitness advantage. Darker individuals that are able to withstand colder conditions may have larger periods of activity, and consequently more success in acquiring mates, food, and escaping predators (Trullas *et al.* 2007).
- Cold can retard growth --> Active later into season and hatch earlier into spring
- We were unable to induce greater melanization by exposing 4th instars to different thermoperiods. These experiments suggest that melanization may either be determined before the 4th instar, may be a result of photoperiod rather than thermoperiod, or may be a result of a combination of both thermo- and photoperiod. Unfortunately, the high mortality of the summer treatment reduces our certainty in the reliability of our results.
- In the future we hope to determine the role of photoperiod in the patterning of *M. histrionica*. While selection favors increased plasticity, unpredictability in environmental conditions may prevent plasticity from producing optimal phenotypes. Thus, photoperiod, which exhibits less fluctuation, may be a more accurate indication of season than temperature (Kingsolver & Huey 1998).
- Eventually, we hope to ask to whether or not this plasticity is genetically determined. While previous studies have suggested familial influence in melanization, this is an exciting and unexplored territory in *M. histrionica* studies (Hazel 2002, Michie *et al.* 2011, Davis *et al.* 2005).

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