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Chris C. Nice · James A. Fordyce

How caterpillars avoid overheating: behavioral and phenotypic plasticity of pipevine swallowtail larvae

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Abstract We tested the hypothesis that larvae of the pipevine swallowtail butterfly, *Battus philenor*, employ behavioral and phenotypic plasticity as thermoregulatory strategies. These larvae are phenotypically varied across their range with predominantly black larvae (southeastern USA and California) and red larvae (western Texas, Arizona) occurring in different regions. Two years of field observations in south Texas indicate that the proportion of red larvae increases with increasing daily temperatures as the growing season progresses. Larvae were also observed to shift their microhabitats by climbing on non-host vegetation and avoided excessive heat in their feeding microhabitat. Larvae of ten half-sib families from populations in south Texas and California, reared under different temperature regimes in common garden experiments, exhibited plasticity in larval phenotype, with larvae from both populations producing the red phenotype at temperatures greater than 30°C and maintaining the black phenotype at cooler temperatures. However, larvae from Texas were more tolerant of higher temperatures, showing no decrease in growth rate in the highest temperature (maximum seasonal temperature) treatment, compared to the California population. In a field experiment, black larvae were found to have higher body temperatures when exposed to sunlight compared to red larvae. These results suggest that microhabitat shifts and the color polyphenism observed in pipevine swallowtail larvae may be the adaptive strategies that enable larvae to avoid critical thermal maximum temperatures.

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C. C. Nice (✉)
Department of Biology, Population and Conservation Biology Program, Texas State University, San Marcos, TX 78666, USA
E-mail: ccnice@txstate.edu

J. A. Fordyce
Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996, USA

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Introduction

The rates of physiological processes in ectothermic organisms, including growth and development, are determined in part by environmental temperature (Lyons 1994; Smith and Ward 1995). Many ectotherms possess adaptive behaviors and morphologies that allow regulation of body temperature above or below ambient temperatures. Insects, for example, display a remarkable diversity of strategies for thermoregulation in almost all life stages in response to a variety of environmental factors (Heinrich 1996). These strategies commonly include behaviors and/or phenotypic plasticity that maximize temperature-dependent growth rates and which, therefore, often affect their fitness (Huey and Kingsolver 1989; Nylin and Gotthard 1998). Plastic and behavioral responses, as opposed to fixed or invariant traits, enable insects to respond to seasonal or daily variation in their thermal environments (e.g. Shapiro 1976; Kukal and Dawson 1989; Bryant et al. 2000; Fitzgerald and Underwood 2000; Hazel 2002). Many of the behavioral responses of insects to varying thermal environments involve microhabitat shifts or modifications. For example, in the Lepidoptera, behaviors such as basking (Forsman 2000), gregariousness (Stamp 1980; Bryant et al. 2000) or the construction of shelters (i.e., tents) (Casey et al. 1988; Joos et al. 1988) have been shown to effectively regulate body temperatures.

Many of the plastic responses observed in insects involve pigment changes in seasonally changing thermal environments. Some of the most well-studied examples are found in the Lepidoptera. For example, many butterflies exhibit seasonal polyphenisms in wing-pigmentation patterns (Shapiro 1976). Increased melaninization of the wings of early spring forms of certain butterflies facilitates increased absorption of solar radiation (Watt 1968; Shapiro 1976; Douglas and Grula 1978; Van Dyck

and Matthysen 1998) enhancing metabolic processes and increasing activity at lower temperatures compared to summer forms which have less melanin deposited on the wings. Caterpillars of many groups of butterflies and moths exhibit plastic variation in coloration (Sherman and Watt 1973; Shapiro 1976) that often has thermoregulatory consequences. For example, Hazel (2002) demonstrated that larvae of the black swallowtail butterfly, *Papillio polyxenes*, exhibit a seasonal polyphenism producing darker caterpillars late in the growing season. When exposed to sunlight, the darker autumnal form maintained higher body temperatures and increased growth rates compared to the lighter midsummer form. The increase in melanin in late-season larvae of *P. polyxenes* appears to increase activity levels and growth rates, thus increasing the probability that larvae will reach the minimum body-mass threshold required for pupation and concomitant winter diapause (Hazel 2002).

While low temperatures present demonstrable problems for larvae, high temperature situations also present challenges. Larvae facing critical maximum temperatures may experience heat torpor or death (Heinrich 1993). Here, we investigate the possibility that a reduction or loss of dark coloration could have adaptive, thermoregulatory consequences in warm climates or seasons. Our focus is on larvae of the pipevine swallowtail, *Battus philenor*, which employ both behavioral and phenotypic plasticity as strategies for the avoidance of high microhabitat temperatures.

Battus philenor is distributed throughout the southern USA and Central America with a disjunct population occurring in California (Racheli and Pariset 1992; Fordyce and Nice 2003). Larval host-plants for members of the genus *Battus* are species of *Aristolochia* (Aristolochiaceae). The large caterpillars have characteristic aposematic coloration (Nishida 2002). Larvae are black with small red or orange prominences in California and the southeastern USA (Garth and Tilden 1986; Scott 1986; Opler 1992), but nearly entirely red in Arizona to western Texas (Neck 1996, JAF and CCN, personal observation). However, in south Texas, both black and red caterpillars are observed (Neck 1996; Tveten and Tveten 1996, CCN personal observation) (Fig. 1). First instar larvae appear to lack dark pigment and larval color becomes evident by the second instar. The caterpillars in south Texas feed on the procumbent vines of *A. erecta*, but are often seen climbing to greater heights on non-host vegetation. This behavior coincides with increasing daily temperatures. A possible explanation for the observed climbing behavior is that larvae move away from the soil surface where temperatures may be significantly higher than air temperatures above the ground (Gass and Barnes 1998). In addition to this behavior, it is possible that the larval color polymorphism may be an adaptive, plastic response to the thermal changes occurring in the microhabitats of these larvae on a seasonal basis and may serve a thermoregulatory function.

In this study, we investigated whether larval color in *B. philenor* is a phenotypically plastic response to temperature and, if so, whether this plasticity and the observed climbing behavior serve to reduce larval body temperatures in the face of high microhabitat temperatures. We asked the following questions.

1. Can the observed climbing behavior of larvae be attributed to avoidance of high temperatures in the microhabitat where the larvae feed?
2. What are the frequencies of the red and black phenotypes in south Texas in relation to seasonal changes in temperature?
3. Do ambient temperatures contribute to the development of the red and black phenotypes; and are there heritable differences in larval color responses that could explain the observed geographic patterns in larval color?
4. Does larval color variation have thermoregulatory consequences?

Materials and methods

Field observations

To test the hypothesis that the behavior of larvae climbing to heights on non-host vegetation may be an effective strategy to avoid high temperatures in the microhabitats where larvae feed, we quantified larval height and host-plant height and measured the temperatures in each of these microhabitats in the field. All field measurements were made in South Central Texas at Freeman Ranch (Hays Co.), a field station operated by Texas State University. At the approximate midpoint of

Fig. 1 The red and black larval phenotypes of *B. philenor* observed in two half-siblings from Texas. The black larva was reared at 30°C and the red larva was reared at 36°C. Photograph by Chris C. Nice

the larval developmental period for *B. philenor*, 28 larvae were haphazardly chosen and their height above the ground was measured. These measurements were recorded for 19 larvae in 2003 over the period May 5–24, 2003, and for 9 larvae on May 31, 2004. Mean height observed for larvae was compared to mean height of the host-plant, *A. erecta*, ($n = 94$) using a *t* test. Temperatures were recorded at the respective heights for 26 larvae ($n = 22$ in 2003, $n = 4$ in 2004) and at the nearest *A. erecta* host-plant using a Traceable Dual-Channel Thermometer (Fisher Scientific) and compared using a paired *t* test. All measurements were made within one hour of solar noon in order to minimize variation in insolation.

Observations of seasonal changes in the color of *B. philenor* larvae were made at Freeman Ranch. Individual black and red larvae were counted in 2003 on 9 days beginning soon after the first appearance of larvae, April 24, and ending when the number of larvae began to decline sharply, June 12. This census was repeated in 2004 on 8 days from April 17 to June 14. A total of 179 larvae were recorded as either red or black (95 in 2003, 84 in 2004). Occasionally, some larvae were encountered that were somewhat purple and apparently intermediate in color. These few individuals were conservatively scored as ''black''.

To establish if a relationship exists between larval color and temperature, we used logistic regression to test whether maximum daily temperatures predict the frequency of the red and black larval phenotypes. Maximum daily temperatures were averaged across a 5-day period including the actual date of observation and the preceding 4 days using data from the nearest monitoring station (San Marcos, TX, USA) obtained from the National Oceanic and Atmospheric Association (NOAA) via their website at http://www.srh.noaa.gov. These 5-day averages represent an ''average'' of the recent thermal environment experienced by larvae. The year of the observations (2003 or 2004) was included in the model to account for between-year variation.

The effect of temperature on larval color and performance

To test the hypothesis that ambient temperatures are the cues underlying the larval color polymorphism observed in *B. philenor* larvae, common garden experiments were conducted in temperature-controlled environments in the laboratory at Texas State University. These experiments were designed to assess the relative contributions of heritable variation and phenotypic plasticity and therefore included larvae from several families from both Texas (Freeman Ranch, Hays Co.), where the larval color polymorphism is observed, and California (Stebbins Cold Canyon Reserve, Yolo Co.), where larvae are black. Eggs were obtained from five wild-caught female *B. philenor* from each of these populations. These eggs constituted ten half-sib families, and possibly full-sib families given sperm precedence observed in swallowtail butterflies (Clarke and Sheppard 1962). Eggs were allowed to hatch and the larvae from each family were divided among three treatments with constant temperatures of 24, 30 and 36°C. A minimum of three larvae from each female were reared at each of the temperature treatments. In some cases as many as 11 larvae/female/treatment were reared, depending on the number of eggs available per female. In total, 51 larvae were reared at 24°C, 49 at 30°C and 47 at 36°C. Additionally, 17 larvae from five Texas females (2–6 larvae/female) were reared at 40°C, close to the maximum temperature (39°C) recorded at the nearest NOAA weather station. All larvae were reared in the dark (i.e. 0L:24D cycle except for a brief period each day when food was added and waste removed) to control for the effects of day length. All larvae were fed with leaves of *A. erecta ad libitum*. These leaves were collected from Freeman Ranch as needed. Larvae were inspected on a daily basis and scored as black or red upon molting to the second instar.

Larval performance under the temperature treatments was assessed by measuring time to pupation (days) and weight at pupation (mg). Two mixed model analyses of variance (ANOVA) were used to test for effects of family, population (CA vs. TX) and temperature on time to pupation and pupal weight. Family was considered a random effect nested within population for each analysis. *F* ratios were calculated following Sokal and Rohlf (1995).

The effect of larval color on body temperature

To determine if color had an effect on larval body-temperature accumulation we conducted an experiment where temperature was measured in the bodies of red and black larvae exposed to sunlight. Ten pairs of lab-reared red and black larvae matched for body mass were killed by freezing. Each larva received a small incision between the first pair of prolegs on the ventral surface of the third abdominal segment. A small, flexible temperature probe was inserted into each larva. These probes were connected to a Traceable Dual-Channel Thermometer (Fisher Scientific) so that the internal body temperatures in the red and black larvae could be recorded simultaneously. Each pair of larvae were placed on a clear plastic petri dish and chilled in a cooler of ice until their body temperature had stabilized for 5 min. The petri dishes with larvae were then exposed to direct sunlight in the field, 30 cm above the soil surface at the approximate mean height of climbing larvae (see Results). All replicates were carried out in full sunlight between 11:00 and 14:00 local time. Internal body temperatures were recorded every 30 s for 14 min. The effect of color on larval temperature was determined using repeated measures ANOVA, blocked by pair, on the difference between ambient temperature and internal larval body temperature measurements at 150, 300, 450, 600, and 750 s.

Results

Field observations

All values are expressed as mean ± 1 SE. Larvae of *B. philenor* that climbed non-host vegetation experienced cooler microhabitat temperatures compared to temperatures recorded at the nearest host-plants. Larvae climbed to heights on non-host vegetation ($n = 29$, 28.8 ± 2.9 cm) that significantly exceeded the mean height of the host, *A. erecta* ($n = 94$, 16.2 ± 0.6 cm; unpaired t test, df = 26, $t = 5.308$, $P < 0.0001$). A comparison of air temperatures at the height of 28 climbing larvae (39.0 ± 0.4°C) and at the nearest host-plant height (43.0 ± 0.6°C) indicated significantly cooler temperatures near climbing larvae (paired t test: $df = 24$, $t = -12.014$, $P < 0.001$).

The observed proportion of red larvae at Freeman Ranch increased over time in both 2003 and 2004 (Fig. 2). In 2003, the proportion of red larvae increased steadily and only red larvae were observed after May 19. In 2004, the proportion of red larvae also increased over time and reached 100% on May 22. However, black larvae appeared again in June, coinciding with a period of below-average temperatures. The relationship between the larval color and temperature was explored using 5-day averages for maximum daily temperature. Temperature predicted the occurrence of red and black larvae and the frequency of red larvae was associated with warmer temperatures (Fig. 3) (whole model: $\chi^2 = 116.261$, df = 3, $P < 0.0001$, $R^2 = 0.4752$); the effect of maximum temperature was significant (Wald $\chi^2 = 27.59$, df = 1, $P < 0.0001$), year and the year by temperature interaction were not significant ($P > 0.1$).

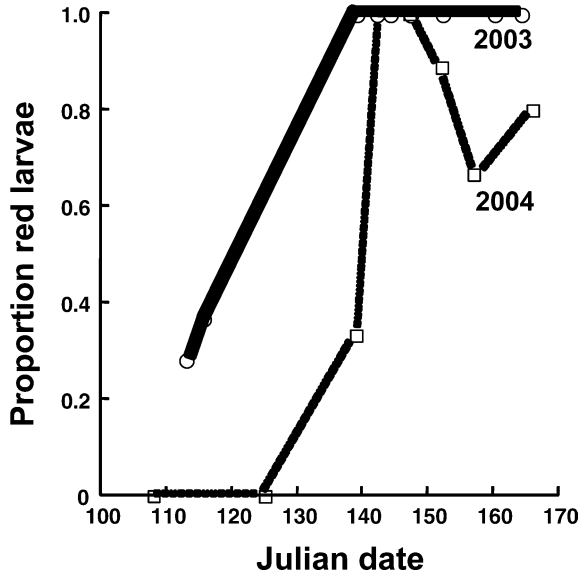

Fig. 2 Proportion of pipevine swallowtail larvae (*Battus philenor*) at the Freeman Ranch, TX field site observed exhibiting the red phenotype from April to June. Data from field surveys conducted in 2003 (*open circle*) and 2004 (*open square*)

The effect of temperature on larval color and performance

The common garden experiments clearly demonstrated the role of temperature in the determination of larval color. All larvae reared at 24 and 30°C exhibited the black larval phenotype, regardless of family or population of origin. All larvae reared at 36°C exhibited the red larval phenotype regardless of family or population. These findings are consistent with the hypothesis that larval color in *B. philenor* is a phenotypically plastic response to temperature and that the geographical variation in the observed larval color is not attributable to heritable differences between populations.

In terms of larval performance, measured as: time to pupation (days) and weight at pupation (mg), there were significant effects of temperature, family, and population. (Table 1). Temperature and population both affected the time to pupation. Post hoc tests were performed with the Tukey–Kramer HSD method ($\alpha = 0.05$). Larvae reared at 24°C grew slower than larvae reared at 30 and 36°C, though the 30 and 36°C treatments were not different (Fig. 4a). Larvae from the California population took 3–5 days longer to pupate than did the larvae from Texas over all the temperature treatments and there was no interaction between population and temperature (Fig. 4a, Table 1a). In addition, a significant family effect and a significant temperature by family effect were detected indicating the existence of among-family variation in performance in response to temperature.

Weight at pupation varied significantly with temperature and between populations and there was a significant interaction between temperature and population (Table 1b). Larvae from California had higher pupal weights at the two lowest temperature treatments. However, at 36°C, there was no difference between the weights of California and Texas larvae at pupation (Fig. 4b). There was a marginally significant family effect on pupal weight, as might be expected from the analysis of time to pupation, but there was no evidence that families from the two populations responded differently to the temperature treatments.

When Texas larvae from five families (17 total larvae) were subjected to a constant 40°C treatment, none molted to the second instar. These larvae lived less than 6 days and appeared to experience heat torpor. The 40°C treatment is clearly above (or equal to) the maximum critical temperature at which *B. philenor* is capable of sustained growth and development.

The effect of larval color on body temperature

The experiment where temperature probes were placed inside larvae indicated that larval color has an effect on larval body temperature when exposed to field conditions. Over the 14 min of each experimental trial, black larvae obtained higher temperatures compared to red

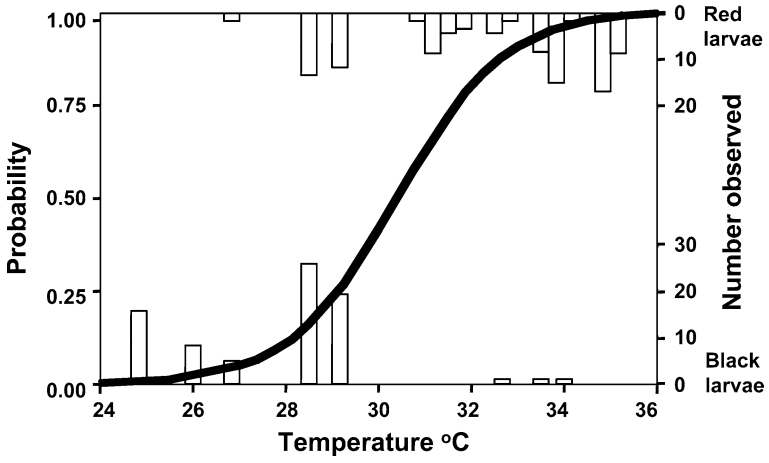

Fig. 3 The probability of the red larval phenotype over the range of maximum daily temperatures. Temperatures are 5-day means of maximum daily temperatures recorded at the San Marcos, TX NOAA weather station. *Bar graphs* indicate the number of individual larvae observed at given temperatures that are included in the model. Observations of larvae in the field were made from April 24 to June 12, 2003 and April 17 to June 14, 2004

larvae (repeated measures ANOVA, $F_{1,9} = 7.7998$, $P = 0.0210$, Fig. 5). By the end of the experiment, black larvae obtained an average body temperature of 44.91 ± 1.16°C, whereas red larvae obtained a body temperature of 41.87 ± 1.46°C. On average, these temperatures correspond to 6.85 ± 1.15°C and 3.81 ± 1.04°C above ambient temperatures for black and red larvae, respectively.

Discussion

The results of this investigation indicate that the climbing behavior and the larval color polyphenism observed in *B. philenor* may be adaptive strategies to avoid extreme high temperatures in the microhabitat where larvae feed. Larvae were found to climb on vegetation to heights more than 25 cm above the soil surface, which is often more than 10 cm above the average height of *A. erecta*. Mean temperature at the height of climbing larvae was significantly cooler than those recorded at the height of the host-plant, which was higher than 40°C on average and thus higher than the temperature at which *B. philenor* can sustain growth. Climbing allows larvae to escape from high temperatures at their host-plants and may also increase convective cooling once they move away from the soil surface. Similar climbing behavior has been observed in arthropods from more extreme environments than those of the Hill Country of south Texas. Ward and Seely (1996) demonstrated that tenebrionid beetles in the Namib desert climb bushes to avoid soil surface temperatures that exceed the beetles' critical thermal maximum of 50°C. At the Freeman Ranch study site, other arthropods including grasshoppers (Acrididae), katydids (Tettigoniidae), jumping spiders (Salticidae), and scorpions (Scorpiones) have also been observed climbing vegetation on particularly warm days, which may also serve a thermoregulatory function (CCN, personal observation).

Our observations of larval color in the field and in the laboratory are consistent with the hypothesis that the observed color polymorphism is a plastic response to temperature. The proportion of red larvae in the field increased in both years of observation and was predicted by increasing daily temperatures. The decrease in the

Table 1 Analysis of variance in larval performance measured as (a) time to pupation (days) and (b) weight at pupation (mg)

Source	df	SS	Error MS[a]	*F*	*P*
A					
Population (a)	1	208.2176	(c)	53.04	< 0.001
Temperature (b)	2	1496.1174	(e)	92.68	< 0.001
Family (Population) (c)	7	27.4780	(f)	2.98	0.009
Population X Temperature (d)	2	24.2616	(e)	1.50	0.256
Family (Population) X Temperature (e)	14	112.9958	(f)	6.13	< 0.001
Error (f)	69	90.8826			
B					
Population (a)	1	0.5305	(c)	18.10	< 0.004
Temperature (b)	2	0.9466	(e)	29.96	< 0.001
Family (Population) (c)	7	0.2049	(f)	2.09	0.057
Population X Temperature (d)	2	0.3884	(e)	12.29	< 0.001
Family (Population) X Temperature (e)	14	0.2205	(f)	1.13	0.356
Error (f)	69	0.9696			

[a] Source of denominator for F ratio

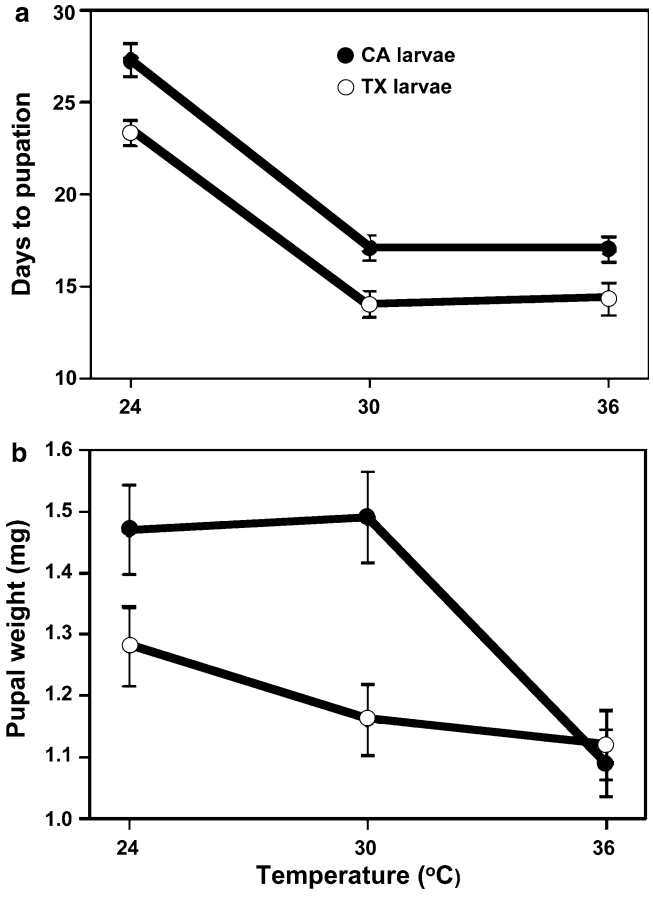

Fig. 4 The effect of temperature on **a** days to pupation and **b** weight at pupation for larvae from California (CA larvae, *filled circle*)and Texas (TX larvae, *open circle*). Symbols are mean ± SE

proportion of red larvae observed late in 2004 corresponded with a period of below-average temperatures, further highlighting the link between temperature and larval coloration. Based on the common garden experiments, the critical temperature threshold for the red phenotype appears to lie between 30 and 36°C. Additionally, the polyphenism in *B. philenor* is reversible and not dependent on larval stage. Larvae transferred from warmer to cooler temperatures, and back again, change color upon molting at the new temperature (unpublished data). This reversibility might allow the larvae to adapt to temporally variable conditions as may be evidenced by the decrease in the proportion of red larvae observed during and after unseasonally cool temperatures in June, 2004 (Fig. 2). The apparent discrepancy between the threshold temperatures observed in the lab and those estimated from field observations (Fig. 3) most likely results from larvae experiencing higher microclimate temperatures than are recorded at the NOAA weather station.

The geographic variation in larval coloration observed across North America is likely attributable to microclimate or habitat differences between populations or localities rather than heritable differences. The *Aristolochia* host-plants of many *B. philenor* populations are often associated with shaded, riparian or woodland habitats (Pfeifer 1966; 1970). This is the case for *A. californica*, the host of the California population studied here. No red larvae have been observed in the Stebbins Cold Canyon Reserve, CA population over the last 7 years of study (JAF, personal observation), despite the results reported here that the larvae from this population are capable of producing the red phenotype. Thus, the absence of red larvae in California indicates that this population does not experience sustained temperatures above the threshold required for the production of red larvae. In contrast, the host of the Texas population studied here, *A. erecta*, is unusual in that it does not occupy riparian or woodland habitat. Instead, *A. erecta* occurs in upland areas such as open, Juniper-Live Oak savanna habitat at the Freeman Ranch study site (Barnes et al. 2000). These plants do not commonly experience shade. Consequently, the Texas caterpillars probably experience higher microhabitat temperatures compared to caterpillars from California and therefore exhibit the red phenotype. By extension, populations in the southeastern USA, which use *A. macrophylla* in forested or riparian habitats, may also be capable of producing the red phenotype but may rarely experience the high temperatures required for its production. In Arizona and New Mexico where the red phenotype is commonly encountered, host-plants also tend to occur

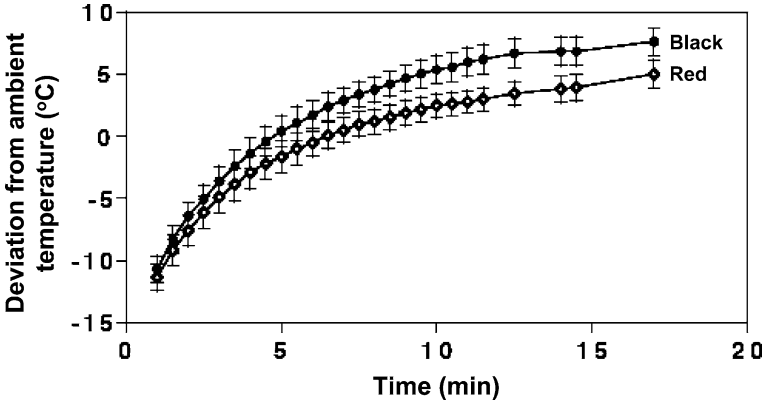

Fig. 5 Effects of larval color on internal body temperatures in *Battus philenor*. Body temperatures (mean ± SE) of 11 pairs of black (*filled circle*) and red (*open diamonds*) larvae exposed to sunlight for 17 min

in warmer microhabitats that exceed the threshold temperature for the production of the red phenotype.

The observation that the California larvae are capable of producing, but do not characteristically exhibit, the red phenotype implies that there is little cost associated with the maintenance of this plasticity. Alternatively, there may be some cost of plasticity but there has not been sufficient time for selection to operate on this trait in California. Data from mitochondrial DNA indicate that the disjunct California population may represent a recent colonization of the area (Fordyce and Nice 2003) thus lending some support to this alternative explanation.

The adaptive benefit of this phenotypic plasticity may be realized late in the season as temperatures increase at the south Texas study site. High temperatures approach 40°C (the highest maximum temperature recorded at the NOAA weather station approximately 10 km east of Freeman Ranch over the course of this study was 39°C). Larvae reared from hatching at 40°C fail to develop and die after several days. Thus it appears that temperatures near 40°C are metabolically challenging for *B. philenor* larvae. Larvae appear to experience heat torpor at these high temperatures. Observations of internal body temperatures of size-matched red and black larvae exposed to full sunlight clearly indicate that color alone can affect larval temperatures, where red larvae maintain lower temperatures. This is consistent with studies of other caterpillars demonstrating that darker coloration facilitates energy absorption (Casey 1993; Fields and McNeil 1988; Hazel 2002). The development of the red phenotype may be a mechanism to avoid internal temperatures above the thermal maximum temperature for growth and development in *B. philenor* or to maintain body temperatures in the optimum range to facilitate maximum growth rate. Additionally, larval orientation behavior that could minimize exposure to solar radiation may work in concert with color for efficient thermoregulation. The maintenance of maximum growth rate may be critical for insect larvae susceptible to larval predators or parasites (Nylin and Gotthard 1998). Slower growth may have a considerable impact on larval mortality by increasing either their exposure to natural enemies (Feeny 1976; Clancy and Price 1987; Williams 1999) or their susceptibility to abiotic factors (Fordyce and Shapiro 2003).

Other potentially adaptive explanations should not be ignored. The red phenotype may provide benefits beyond the thermoregulatory functions investigated here. The red phenotype may, for example, provide enhanced aposematism. This may be important if the suite of potential predators varies over the season and is correlated with the daily temperature increase or the climbing behavior may expose larvae to different predators. Thus, multiple agents of selection may shape the larval color polyphenism in these larvae.

While no among-family or between-population variation in larval coloration was detected, there were significant differences in larval performance among families and between populations. Larvae from California took longer to reach pupation at all temperature treatments and had significantly higher pupal weights at all but the highest temperature treatment. Unlike the Texas larvae, the larvae from California seem to perform relatively poorly at 36°C. This indicates that there may be between-population variation in tolerance of high temperatures that may reflect adaptation to the local thermal environments, with the Texas population capable of withstanding higher temperatures.

The larval color plasticity in *B. philenor* is seemingly different from other cases of polyphenism in butterflies and moths in the sense that this involves an apparent reduction in black pigmentation in response to warmer temperatures. Most studied cases have emphasized plasticity in response to ambient thermal conditions in the Lepidoptera that involves an increase in black coloration in response to cooler conditions. Seasonal polyphenism in adults is most often manifested as increased melanic scaling on the wings in cooler seasons (winter or spring) (Shapiro 1976), which has been demonstrated to augment warming, allowing these animals to maintain body temperatures above ambient temperatures (e.g., Watt 1968; Kingsolver and Wiernasz 1991). Similarly, larval plasticity is viewed as increased production of melanin in cooler seasons (e.g., Hazel 2002). Whether the ''cool season'' phenotype or the ''warm season'' phenotype is the default, or ancestral, state remains an open question. For most of the species of *Battus*, the black larval phenotype is the rule, with *B. philenor* being the exception (Tyler et al. 1994), suggesting that the polyphenism and red phenotype are derived. In his examination of South American pierid butterflies of the *Tatochila sterodice* group, Shapiro (1980a; 1980b; 1984) argued convincingly that the light (estival) adult phenotypes are derived. Determining which larval phenotype is derived will contribute to our understanding of the evolutionary development of the polyphenism in *B. philenor* and perhaps other butterflies.

The reported cases of plasticity in adults or larvae are most often under the control of photoperiod (Shapiro 1976; Hazel 2002). The larval color polyphenism in *B. philenor* is clearly not regulated only by photoperiod given that the common garden experiments were conducted in complete darkness. Furthermore, results from larvae reared in the lab under ambient photoperiod indicate that photoperiod does not play a critical role in determining larval color (unpublished data).

The results of our observations and experiments support the hypothesis that larval climbing behavior and color polyphenism are adaptive responses to high temperature microhabitat conditions. Geographic variation in larval color is not attributable to heritable differences alone. By seeking out cooler microhabitats and exhibiting phenotypic plasticity in larval coloration, *B. philenor* is able to maintain body temperatures that may permit maximum performance under local, microhabitat conditions.

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