

Research Article

Effects of temperature on the phenology and fitness traits of *Pachliopta aristolochiae* (Lepidoptera: Papilionidae) in Dhaka, Bangladesh

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ABSTRACT

Butterflies are highly sensitive to climate change, especially to variations in temperature, and are therefore considered important bioindicators of environmental change. In this study, an attempt was made to determine the effects of temperature (32 °C, 29 °C and 20 °C) on the phenology, development, and fitness traits of *Pachliopta aristolochiae* (Lepidoptera: Papilionidae) under laboratory conditions (16L:8D photoperiod; 78±2 RH) from May 2023 to February 2024. The results revealed clear temperature-dependent effects on the butterfly's developmental and fitness traits. The larval survival rate was significantly higher at 32 °C and 29 °C than at 20 °C ($F = 11.86$; $df = 2$; $P = 0.05$). Relative hatchability of eggs was highest at 32°C (88.89 ± 6.0%), followed by 29 °C (66.67 ± 12.2%) and 20 °C (15.38 ± 10.0%). The incubation period was 3.37 ± 0.81 days at 32 °C, followed by 3.75 ± 0.46 days at 29 °C and 4 ± 0.25 days at 20 °C. Moreover, the total developmental period was 33.34 ± 4.33 days at 32 °C, 36.19 ± 6.52 days at 29 °C, and 42.4 ± 2.45 days at 20 °C. Thus, temperature variation significantly altered the rate of metabolism and growth of the butterfly. The results suggest latitudinal adaptations of *P. aristolochiae* to the temperature of Dhaka city and regional thermal acclimation.

Introduction

Climate change is a major anthropogenic disturbance to natural ecosystems, causing a rise in extreme temperature events that animals must adapt to survival (Diffenbaugh et al., 2005; Sala et al., 2000; Thomas et al., 2004; IPCC, 2014). Temperature is one of the most important ecological factors for ectothermic species, as they must tolerate frequent thermal fluctuations (Parmesan and Yohe, 2003; Parmesan, 2006; Overgaard et al., 2008). Butterflies are important bio-indicators for monitoring population changes in the context of global warming (Singh and Sondhi, 2016; Kremen, 1992; Schulze et al., 2010; Wood and Gillman, 1998). They are highly

sensitive to temperature, changes in microclimate, and habitat (Erhardt, 1985; Shobana et al., 2012; Sakuratani, 1991). Butterflies are particularly sensitive to environmental variation and maintain a positive relationship with host-plant diversity, habitat complexity, topography, and climate (Leps and Spitzer, 1990; Molina and Palma, 1996). In addition, under global warming scenarios, many butterfly species will become extinct due to changes in their environment and habitat (Braby et al., 2013; Whitworth et al., 2018). Butterflies' phenological activities depend on regional temperature variation (Diamond et al., 2014; Dennis et al., 2016). The

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phenology of many butterflies is changing dramatically in response to temperatures (Walther et al., 2002; Parmesan and Yohe, 2003).

Butterflies in the Papilionidae family represent a stunning assemblage and are globally distributed (Rajeswari and Jeyabalan, 2015). Members of this family are ecologically important pollinators and help maintain ecosystem health (Bashar, 2013, 2014; Morris et al., 1995). Most swallowtail butterflies are highly host-specific, with a narrow and species-specific selection of larval host plants (Nakayama and Honda, 2004). *Pachliopta aristolochiae* is particularly notable for its incredible adaptations, large body size and complex interactions with its host plants (Bashar, 2014). This butterfly species survives by adapting to extreme temperatures (Anderson et al., 2012). Tropical insects face constant threats from habitat degradation and climate change (Weiss et al., 1988). Butterfly egg size also changes with temperature, leading to increased mortality rates (Fischer et al., 2003). In addition, the ongoing climate change and increasing temperature variability pose serious threats to species with narrow thermal tolerance (New et al., 1995; Piyaphongkul et al., 2012; Kingsolver et al., 2015). Alam et al. (2017) investigated the phenology of *P. aristolochiae*, with special emphasis on female oviposition behavior and pupation strategy in relation to host plant quality. The biology and larval activities of *P. aristolochiae* are directly dependent on its association with host plants (Alam et al., 2014). Moreover, successful and effective conservation of these butterflies depends on a sound understanding of their phenology and temperature interactions. However, it remains unclear how this butterfly will acclimate to the ongoing increase in temperatures in Dhaka city. To the best of our knowledge, no data exist on the effects of temperature on *P. aristolochiae* in Bangladesh and information is required from a management perspective to understand the specific vulnerability of butterflies to rising temperatures. Monitoring butterfly population changes in the context of anthropogenic climate change is vital (Dennis et al., 2016). This change is thought to present a significant threat to biodiversity and has

received relatively little attention. Therefore, the objectives of the present study were to evaluate the effects of various temperature ranges (low, medium, and high) on the phenology, developmental duration, fitness traits, body weight, and fecal production (as a measure of metabolic rate) of *P. aristolochiae*. This research aims to understand how temperature affects urban butterflies to support effective conservation and biodiversity management.

Materials and methods

Study area and study period

The study was conducted in the Entomology Research Laboratory, Department of Zoology, University of Dhaka, from May 2023 to February 2024. The samples of *Pachliopta aristolochiae* (eggs, larvae and pupae) were collected once a week during the study period, along with the host plant (*Aristolochia indica*), from the Germplasm Center of the Zoological Garden (GPC), University of Dhaka (latitude 23.726899° N, longitude 90.401542° E).

Sample collection and preparation

The butterfly samples were identified following the methods of Bashar (2013, 2014) and the host plant was identified according to Ahmed et al. (2008). Collected adults were preserved for wing venation and morphometric identification following the methods of Bashar (2014) and Alam et al. (2014, 2017). Observation of adult females and their egg-laying activities on the host plant was conducted regularly, following the methods of Jermy and Szentesi (1978) and Kumar et al. (2024). The eggs were collected immediately after oviposition (within one day). The eggs remained attached to the host plant leaves, which were collected by cutting the stems and the cut ends were immediately wrapped with soaked cotton (Fig. 1a, b). Water was sprayed on the eggs to prevent desiccation, according to the methods of Arju et al. (2015a, b). Collected larvae with host plant stems (Fig. 1c, d, e, f) were immediately transferred into a 500 mL beaker containing water-soaked cotton to prevent desiccation. Temperature and humidity at the GPC were recorded regularly using a Clock

and Hygro-thermometer (Brannan, England) during the study period.

Temperature selection and preparation for rearing

The collected eggs with stems were placed in Petri dishes with some water-soaked cotton (Fig. 1 a,b) and observed during the incubation period under a portable binocular stereo microscope (EuromexEduBlue series) following the methods of Arju et al. (2010). To determine the effects of rearing temperature on butterfly phenology and fitness traits, samples were kept in a special three-layered butterfly rearing cage (39.62 cm × 35 cm × 30 cm) following the methods of Arju et al. (2015a). A specialized round larval rearing box (15.5 cm × 7 cm) was placed inside the rearing cage. The rearing box was perforated with some pores (4-5) at the bottom so that the stems of the host plants were attached properly and connected to water to keep the stems alive. Adequate amounts of young *A. indica* shoots were supplied regularly at 24-hour intervals as larval food. Each rearing box contained two to three larvae of the same instar (Fig. 1.e, f). Four larval rearing boxes were prepared for the fourth-, third-, second- and first-instar larvae individually. Two emergence rearing boxes (52.5 cm × 45 cm × 90 cm), made of mosquito net and iron rods and with dry stems of the host plants, were placed at different angles were used for pupation according to Alam et al. (2017). All samples were transferred to a climate chamber (BIOBASE) under a 16-hour Light and 8-hour Dark Cycle and at 65% RH at any one of the 32 °C, 29 °C, and 20 °C. The rearing temperatures were selected based on minimum to maximum survival rates during the pre-treatments. Adequate numbers of replicates n = 14, n =16 and n =15 were prepared for all developmental stages for different rearing temperatures.

Effects of temperature on the phenology of *P. aristolochiae*

The developmental duration (days) of each developmental stage was recorded individually for each larval instar (1st to 4th), pre-pupal and pupal stage. Larvae were observed regularly at 8-hour intervals to provide food and identify molting. Fresh host-plant leaves were supplied as food at 2-day intervals for the first instar and one-day intervals for the second instar.

Old foliage was removed regularly by following the methods of Rafi et al. (1999) and Koda and Nakamura (2010, 2012). Damp sponges were kept in the emergence box to maintain sufficient humidity.

Effects of temperature on the development of *P. aristolochiae*

Another similar set of butterfly rearing experiments was conducted to evaluate the effects of rearing temperatures on development. The larval period of an instar was recorded from the time of first larval appearance to the larval molt to the next instar following the methods of Zalucki et al. (2002). Larval morphological changes, body size and coloration were observed by following the methods of Miah et al. (2015) and Arju et al. (2015a). Longevity of larval stages was also recorded. The number of surviving larvae was counted and compared across the rearing temperatures. Larvae were considered dead when they became sluggish, changed body color from greenish to brownish, remained motionless, and ceased feeding. Mortality in different categories of larvae was recorded twice (morning and evening) daily. A total of 14 replicates for 20 °C, and 15 and 13 replicates for 32 °C, and 29 °C, were prepared, respectively.

Effects of temperatures on the hatchability of *P. aristolochiae*

To investigate the effects of temperature on egg hatchability, the number of newly hatched larvae from collected eggs was counted at 24-hour intervals (Fig. 1. a) across all replicates according to the methods of Geister et al. (2009).

Effects of temperatures on body weight and metabolism of *P. aristolochiae*

A total of 15 replicates for each of the three rearing temperatures were prepared separately to observe body weight and daily fecal output at 24-hour intervals after each molt (Fig. 1. g, h). The treated larvae were provided with 20 g of fresh, and young leaves of the host plant regularly. The body weight and fecal output were recorded using a digital balance (KERN ADB 220-4). After supplying the young leaves, water was sprayed on the leaves to prevent desiccation and maintain leaf turgidity, following the methods of Alam et al. (2014) and Arju et al. (2015a).

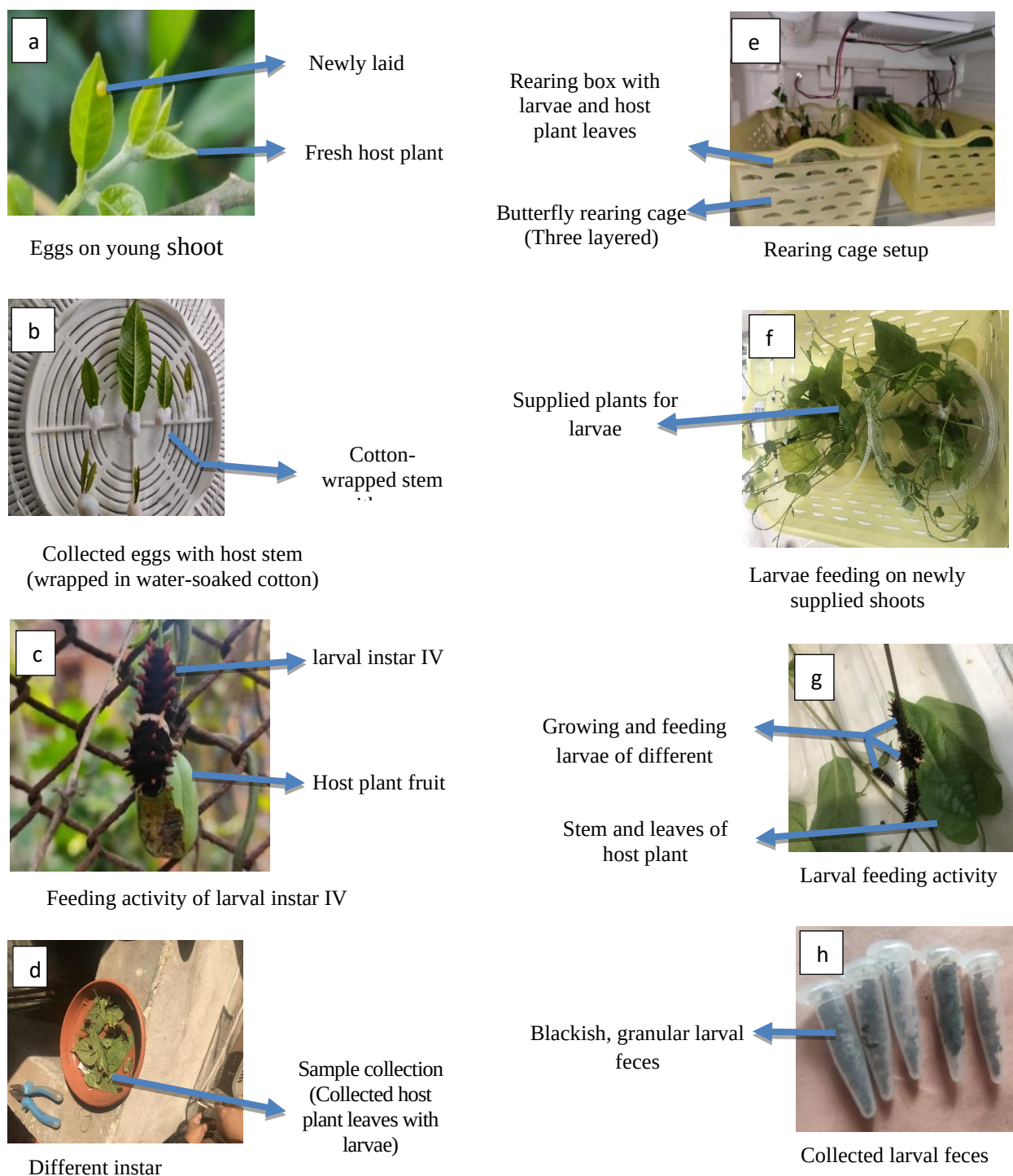


Fig. 1. Butterfly rearing at different developmental stages under laboratory conditions.

a) The newly collected eggs of *P. aristolochiae* on the young leaves of *Aristolochia indica*; **b)** Prepared eggs with host plant stem and shoot for rearing; **c)** Mature larval instar IV actively feeding on fruits of host plant; **d)** Larvae of different instars with host plant leaves; **e)** Butterfly rearing cage containing larval rearing boxes; **f)** Larval feeding potential observation with newly supplied food; **g)** Feeding larvae of different instars; **h)** Collected larval excreta (feces).

Statistical Analysis

The mean developmental period of the egg, larval and pupal stages at each temperature was computed and the standard error was determined. Mean values for mortality, duration of development (days), body weight and amount of feces for all replicate larvae at different rearing temperatures were calculated. Differences in egg hatchability across rearing temperatures were analyzed using t-tests. ANOVA was used to analyze hatchability and mortality and to compare the effects of rearing temperature as a fixed factor. The collected data were analyzed statistically using Minitab and Microsoft Excel. Descriptive analysis and inferential analysis were conducted to evaluate differences among variables (temperature) with significant P value <0.05.

Results and discussion

Effects of temperature on the phenology of *Pachliopta aristolochiae*

The results revealed that the phenology of *P. aristolochiae* consists of four developmental stages (egg, larva, pre-pupa and pupa) across all rearing temperatures (Fig. 3). The total developmental period was the longest at 20 °C, lasting approximately 42.4±2.45 days and gradually shortened as temperatures increased, reaching 36.19±6.52 days and 33.34 ± 4.33 days at 29 °C and 32 °C, respectively. The duration of the egg stage decreased

significantly as temperature increased. At 20 °C, a longer developmental period was associated with higher mortality across stages. At 20 °C, the developmental periods for the egg, I, II, III, and IV instar larval stages, pre-pupa and pupa were about 4 ± 0.45 days, 2.5 ± 0.5 days, 5.25 ± 0.75 days, 6.45 ± 1.34 days, 8.45 ± 1.1 days, 2± 0.75 days and 13.75±0.58 days, respectively. At 29 °C, the developmental periods of the II, III, and IV instar larvae gradually decreased to 4.43±0.82 days, 5.22± 0.71 days, and 6.67±0.91 days, respectively compared with 20 °C. Moreover, at 32 °C, the developmental periods for the third- and fourth-instar larval stages were shorter, measuring 4.12 ± 0.46 days and 5.75± 0.69 days (Fig. 2). The pre-pupal and pupal stages were prolonged by approximately 2 days at 20 °C. The larval stage duration decreased significantly as temperature increased. Barua and Slowik (2007) stated that the life cycle of *P. aristolochiae* was completed within 30 days during summer, with an incubation period of 4 days, larval duration of 20 days, and pupation period of 12 days. Koda and Nakamura (2010) reported that the pre-pupal stage was 4.71 ± 0.18 days at 20 °C, compared with 1.63 ± 0.15 days at 25 °C and 2.06 ± 0.19 days at 30 °C. In addition, adult emergence from the pupal stage was very low at lower temperatures. Significant differences were observed in the pre-pupal periods between 20 °C and higher temperatures.

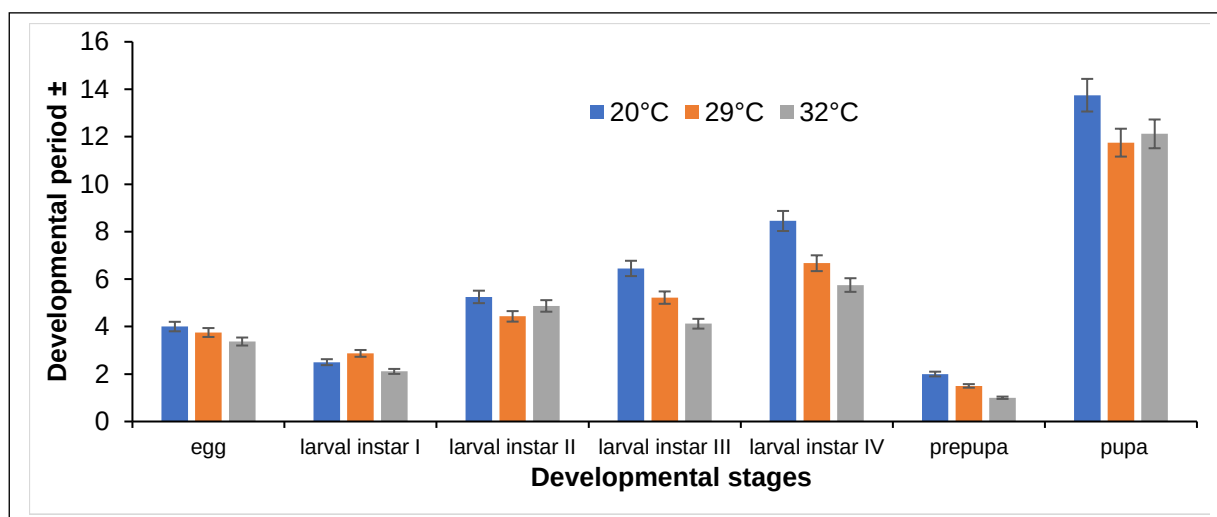


Fig. 2. Effects of temperatures (20 °C, 29 °C and 32 °C) on the developmental stages of *P. aristolochiae*.

Temperature is arguably the most important abiotic factor influencing butterfly life history. It limits survival and affects metabolic processes, including energy use, nutrient procurement, development, and growth rates (Kennelly et al., 2017).

In the *Plutellaxylostella* (Lepidoptera: Plutellidae), rearing temperatures (10°C -30 °C) affected growth and development rates. Rearing at lower temperatures increased the development time and slowed the growth rate.

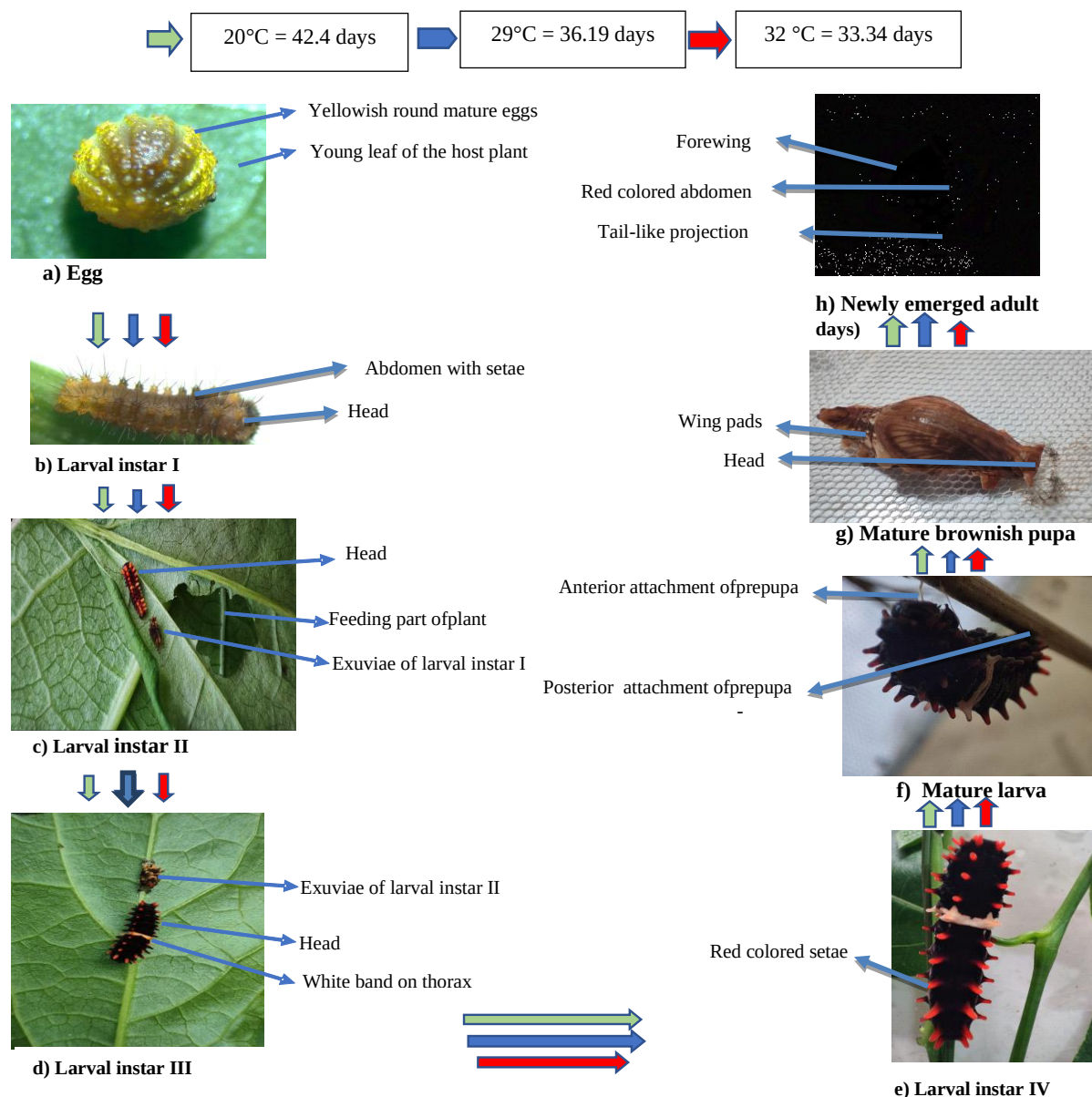


Fig. 3. Phenology of *P. aristolochiae* with *A. indica* under laboratory conditions.

a) Mature laid eggs on young leaf b) Pale brown larval instar I c) Newly molted larval instar II d) Blackish body banded with white and reddish setae of larval instar III e) Full grown, voracious feeder larval instar IV on the stem of host plant f) Mature larva before pupation attached with dry stem of plant g) Mature brownish pupa h) Newly emerged adult.

Thus, at sub-optimal temperatures, greater food resources are required during the developmental period (Garrad et al., 2015). Higher temperatures increase the metabolism rate of butterfly larvae during their developmental periods. With a higher metabolic rate, butterfly larvae use more energy (Kingsolver et al., 2015). However, the effects of slightly raised temperatures on butterfly population dynamics are difficult to predict. A small temperature rise is more likely to increase the metabolic rate. Additionally, higher temperatures may adversely affect larval development (Klockmann et al., 2016, 2017). At 32 °C, the total developmental duration of *P. aristolochiae* was 33.34 ± 3.8 days from egg to pupa. Conversely, 29 °C resulted in a longer total developmental period of 36.19 ± 3.83 days, indicating that temperature influences the overall time required for development and that these species are adapted to high temperatures. Islam et al. (2019) found that lower temperatures prolong the developmental period of *Papilio demoleus*, while

higher temperatures accelerate it. Kumar et al. (2024) reported that larval duration ranged from 11.18 to 16.49 days and 13.15 to 14.88 days, respectively, for *P. demoleus* and *P. polytes* at higher temperatures (32 °C) whereas it was prolonged at low temperature (23 °C).

Effects of temperature on the survival of *P. aristolochiae*

The mortality rate at the beginning and final stages of development was higher than at the intermediate stages (instar I, instar II, pre-pupa and pupal stage). Mortality was higher at 20 °C. Very few pre-pupae survived to become pupae at 20 °C, indicating that this temperature is below the optimum that is required for the normal growth, development, and metamorphosis of *P. aristolochiae*. However, the lowest survival rate of the IV instar larvae and pupal stage was recorded at 29 °C, suggesting that such temperatures may reduce the metabolic rate of the final stage larva, which requires maximum energy, thereby potentially causing mortality.

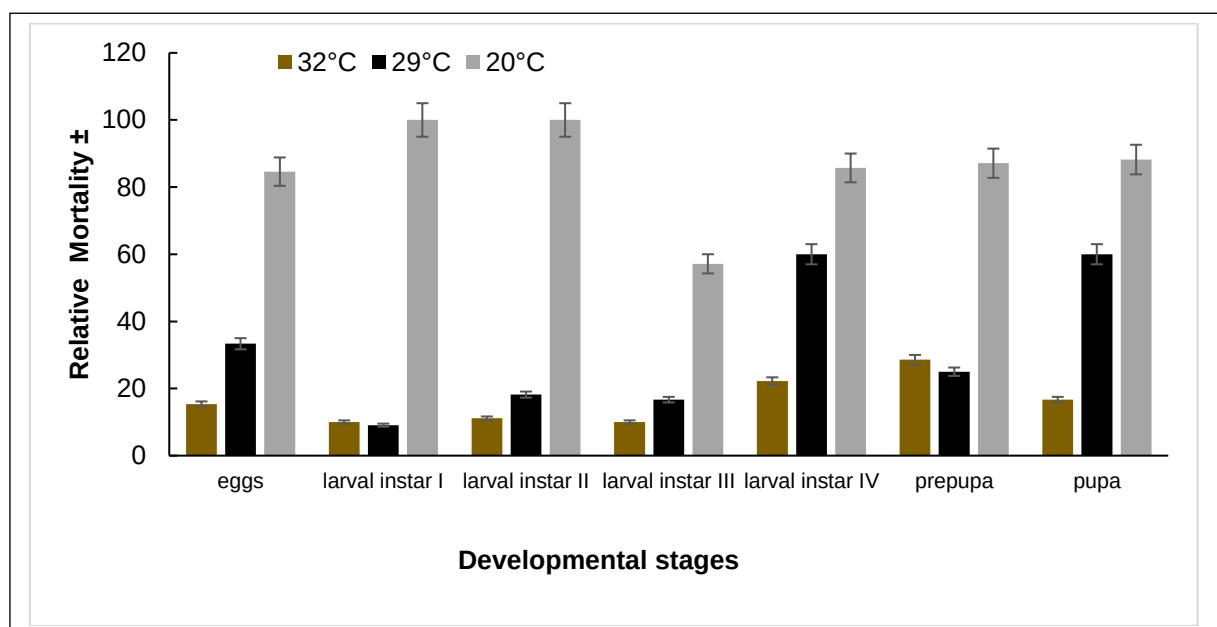


Fig. 4. Effects of rearing temperatures on the survival of *P. aristolochiae* during development.

The survival rate was comparatively higher across all developmental stages at 32 °C. Temperature, from lower to higher, was significantly correlated with the mortality across stages ($F = 46.8$; $df = 2$; $P < 0.05$). The results highlighted the generally higher temperature acclimation capacity of *P. aristolochiae*, suggesting that gradually increasing temperature tolerance could mitigate heat shock during summer, especially in hot summers. The survival rate of eggs was significantly lower (84.62 ± 2.74) at 20 °C, and mortality was lowest (15.38 ± 1.73) at 32 °C. The lowest mortality rates for all developmental stages were recorded at 32 °C and 29 °C (Fig.4). Temperature significantly affects butterfly survival (Klockmann et al., 2016).

Effects of temperature on the incubation period and hatchability of *P. aristolochiae*

The average incubation period of eggs was 4 ± 0.25 days, with the lowest egg hatchability rate of 15.38% and no pupation occurring at 20 °C. However, at 29 °C, the incubation period was 3.75 ± 0.46 days with a higher hatchability rate of 66.67% and a pupation rate of 25%. Arju et al. (2015a) found that the average incubation period of the Pierid butterfly *Eurema hecabe* was 5.7 ± 0.6 days at this temperature. The lowest survival rates from the first to third instar of *E. hecabe* were recorded at low temperatures, with 18.8% at 15 °C while the highest was 76.9% at 20 °C. At 32 °C, the incubation period of eggs was 3.37 ± 0.81 days, with the highest hatchability rate of 88.89% and a pupation rate of 40% (Fig. 3). Moreover, a significant difference in hatchability was found among the three rearing temperatures ($F = 34.46$; $df = 2$; $P < 0.05$). Results of this study reveal that in tropical regions, egg hatchability of *P. aristolochiae* was highest at 32 °C and lowest at 20 °C (Table 1 and Fig. 5).

Table 1. The incubation period, hatchability rate of *P. aristolochiae* at different temperatures.

Temperature (°C)	No. of eggs	Incubation period (day $\pm$ SD)	Hatchability (% $\pm$ SD)
20	13	4.00 ± 0.25	15.38 ± 10.0
29	15	3.75 ± 0.46	66.67 ± 12.2
32	27	3.37 ± 0.81	88.89 ± 6.0

The shortest developmental periods for eggs and larvae were 4.0 and 15.8 days, respectively, at 30 °C, and the durations of the egg and larval stages increased significantly as temperature decreased (Koda and Nakamura, 2010). The mean incubation period of the Lycaenid butterfly, *Lampides boeticus*, was 4 ± 0.17 days at 29 °C, and around 50% of the eggs hatched (Miah et al., 2015).

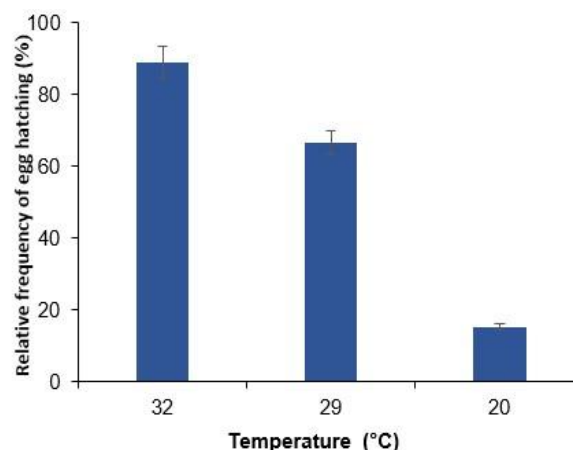


Fig. 5. Effects of rearing temperatures on the hatchability of eggs of *P. aristolochiae*.

Strong adverse effects of high temperatures have been reported on both egg-hatching success and larval survival by many previous studies (Piyaphongkulet et al., 2012; Kingsolver et al., 2015; Klockmann et al., 2017). Low temperatures also adversely affected egg survival in *Shijimiaeoidea divinusbarine* (Lepidoptera: Lycaenidae), since this species is distributed in warmer regions. Different effects were found when reared across a range of rearing temperatures (15 °C, 17.5 °C, 20 °C, 25 °C, 30 °C, and 35 °C) under a long-day photoperiod (16L:8D). Egg hatchability peaked at 20 °C (88.0%), declining sharply at higher temperatures (30 °C: 30.0%, 35 °C: 0.0%). The duration of incubation decreased significantly as the temperature increased to 30 °C in *Lycaeidesargyrognomon* (Koda and Nakamura, 2012). Similar results were observed in other members of Papilionidae family. The incubation period was longer at lower temperatures, measuring 3.39, 3.06, and 2.82 days, respectively, at

23, 28 and 32 °C for *P. demoleus*, and the incubation periods were 3.83, 3.61 and 3.08 days, respectively, at 23, 28 and 32 °C for *P. polytes*, as reported by Kumar et al. (2024). Egg size is a life history trait, as it is simultaneously a maternal and a progeny character. Variation in insect egg size represents temperature-mediated plasticity. Temperature is the most important environmental factor influencing reproduction (Fox et al., 1997). Smaller eggs are laid at higher temperatures, whereas larger eggs are laid at lower temperatures (Steigenga and Fischer, 2009). Egg survival was lower at 26-36 °C (49%), though larvae developed faster and adults attained a higher body mass within this temperature range (Huang et al., 2024).

Effects of temperature on body weight & fecal weight

The mean weight of the instar II larvae did not vary with rearing temperatures. However, body weights for the second through fourth instars were lower at 20 °C than at 29 °C and 32 °C. The weights of the 3rd and 4th instar larvae were 1.62 ± 0.054 g and 1.88 ± 0.059 g at 32 °C, respectively (Fig. 6).

The larvae started to gain weight rapidly from instar II, and the rate of increase was higher at 29 °C and 32 °C.

However, despite the variations, higher temperatures (29 °C and 32 °C) were not significantly correlated with higher larval weights ($F = 0.08$; $df = 1$; $p > 0.05$). Food consumption increased gradually in both amount and frequency across successive larval stages (Miah et al., 2015). Larval weight and food consumption were positively correlated in *Catopsilia crocale* (Pieridae), with maximum consumption (1.37 g) recorded during the final larval stage (Arju et al., 2015a).

The larvae excreted a large amount of black, granular feces during development. Fecal production did not vary across the three rearing temperatures during early development (instars I to II) but gradually increased after the third instar molt.

The total feces excreted was 9.37 ± 0.089 g at 29 °C and 6.92 ± 0.084 g at 20 °C, both of which varied from the amount (13.24 ± 0.132 g) recorded at 32 °C (Fig. 7). However, the difference in fecal production between 29 °C and 32 °C was not statistically significant ($F = 1.62$; $df = 1$; $P > 0.05$).

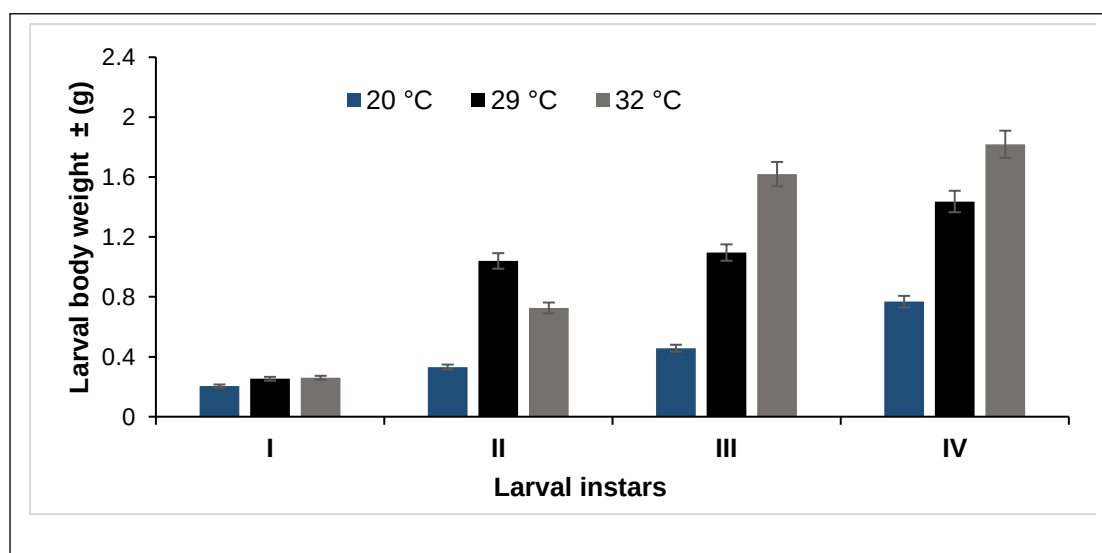


Fig. 6. Effects of rearing temperatures on the body weight (g) of *P. aristolochiae*.

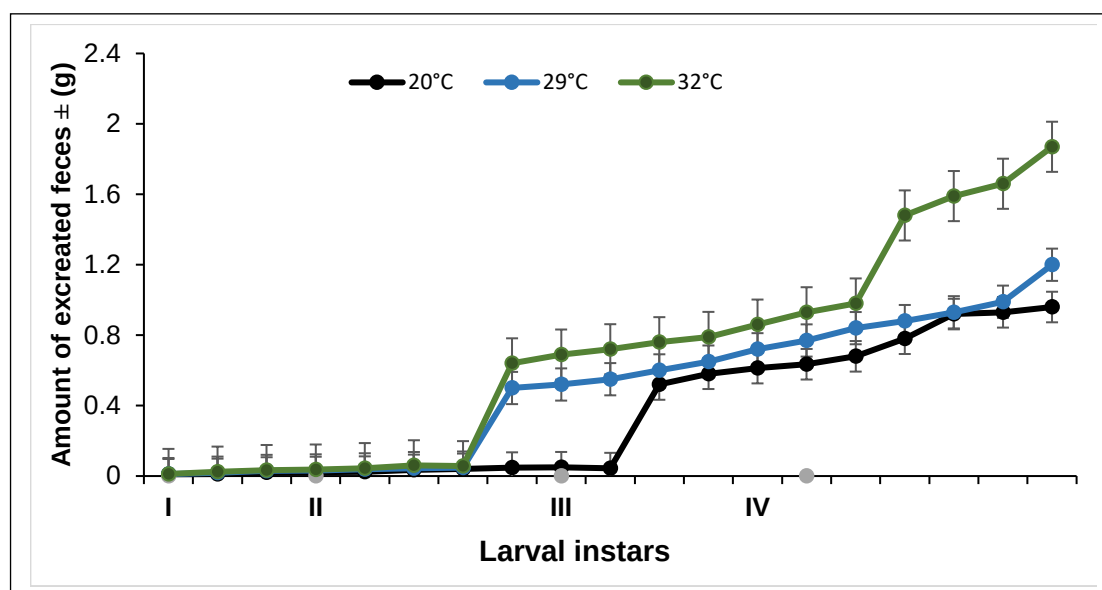


Fig. 7. Amount of excreted feces (in gram) after each molting of *P. aristolochiae* at 20 °C, 29 °C and 32 °C.

The fecal amount of *Danaus chrysippus* was lower at 27 °C than at 32 °C (Mathavan and Pandian, 1975), indicating that a higher temperature range is required for growth and development. Arju et al. (2015a, b) observed that fecal output increased with developmental stage, from the first instar (0.04 ± 0.001 g) to the final instar larva (0.347 ± 0.1 g) of *Catopsilia crocale*. Temperature significantly affects butterfly body weight and size during developmental stages. In the present study, the increased fecal output at 32 °C indicates elevated feeding activity and metabolic demand. In *Lycaides argyrognomon*, higher temperatures accelerated development. Higher temperatures enhance metabolism by speeding up growth and development (Angilletta and Dunham, 2003; Jones et al., 1982). Such conditions in tropical regions may drive the physiological and evolutionary adaptations of *P. aristolochiae*. Even small deviations from optimal temperatures can substantially affect survival and population dynamics (Koda and Nakamura, 2012).

Conclusion

The present study demonstrates that temperature ranges between 29 °C and 32 °C are favorable for

the survival, hatchability, and development of *P. aristolochiae*, highlighting its adaptation to tropical thermal environments. Temperatures of 29 °C and 32 °C improved survival, hatchability, body weight, and metabolic rate, whereas temperatures below the optimum (20 °C) led to higher mortality and slower growth. These findings contribute valuable insights into the phenological responses of tropical butterflies and provide important baseline data for predicting the impacts of future climate warming on butterfly populations and for developing effective conservation strategies.

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Authors contribution

Nousheen Parven: Validation, investigation, review, editing and funding acquisition; Refath Tasnin: Data analysis, writing- original draft and funding acquisition; Kashfia Bintey Rabbi: Writing-original draft, review and editing, Md. Abul Bashar: Conceptualization and objective selection; Aleya Begum: Conceptualization and objective selection.

Conflict of interest

The authors declare no conflict of interest.

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