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Effects of Temperature on the Development of Chinese Windmill Butterfly, *Atrophaneura alcinous* (Lepidoptera: Papilionidae)

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The Chinese windmill butterfly, *Atrophaneura alcinous*, is an important butterfly for exhibition in butterfly garden. The objective of this study was to determine the effect of temperature on *A. alcinous* in the laboratory. Development of *A. alcinous* reared on leaves of *Aristolochia contorta* was investigated at five constant the laboratory condition (20, 22.5, 25, 27.5 and 30°C) and at relative humidity of 60% with a photoperiod of 14:10 (L:D). Temperatures have been suggested as an important determinant of developmental rate, lifespan and mortality in invertebrates. As the temperature increased, the length of the developmental period gradually decreased. The developmental time (pupation) from egg hatching to pupation was respectively 25.8, 23.6, 19.6, 15.5, and 12.9 days at the temperatures of 20, 22.5, 25, 27.5 and 30°C. And pupation was respectively 40.0, 30.0, 63.4, 50.0, 23.3% at the temperatures of 20, 22.5, 25, 27.5 and 30°C. The developmental threshold temperature estimated for egg-to-pupae was 10.8, with a thermal constant of 230.4 degree-days. Therefore, the optimal developmental temperature for *A. alninous* was determined to be 25°C. To compare the effects of the total duration of chilling on the termination of diapause, larvae were subjected to a temperature of 8°C from 60 to 120 days. The rate of termination of diapause was significantly higher at 60 days compared to other incubation period.

Key words: *Atrophaneura alcinous*, *Aristolochia contorta,* Diapauses

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Introduction

The Chinese windmill butterfly *Atrophaneura alcinous* Klug (Papilionidae) feeds on the plant family Aristolochiaceae. Many plants in this family contain aristolochic acids, which are toxic alkaloids (Chen and Zhu, 1987). The host plant contains toxic substances that help the butterfly to maintain a high population density by allowing escaping the butterflies from predation. These butterflies may completely defoliate the host. Because the harmful substances are also feeding and oviposition stimulants for the butterfly, the starved larvae are frequently cannibalistic (Nishida and Fukami, 1989a, b). Adult male *Atrophaneura alcinous* emit a strong characteristic odor that is perceivable by humans, although the females of the insects are almost scentless. It has been shown that particular exocrine substances of certain male butterflies are sex pheromones and act as aphrodisiacs or arrestants, inducing the females to copulate (Myers and Brower, 1969; Pliske and Eisner, 1969; Lundgren and Bergström, 1975).

During the larval stages, the life cycle of this species depends on various environmental factors, such as photoperiod, temperature and food plants, as well as geographical factors, such as latitude and altitude (Kato, 2005). The rate of insect development depends upon the temperature to which the insects are exposed. The amount of heat required over time for an insect to complete specified aspects of development is termed the thermal constant (Andrewartha and Birch, 1954)

Many insect studies rely on laboratory-reared individuals. Providing information on how to successfully rear selected insects in the laboratory has long been a fundamental research goal (Cohen, 2003). A practical difficulty in working with butterflies is that many are active only part of the year. Moreover, their larval host plants are often seasonal. For this reason, artificial diets for insects are essential research tools. A wide range of commercial

diets are available, as well as published formulations. These diets have been developed to maximize insect growth and reproduction by meeting or surpassing the insects' minimum nutritional requirements. This study investigated the effects of temperature and diet on the developmental and life cycle of *A. alcinous* from Korea.

Materials and Methods

Experimental insects

Laboratory colonies of *A. alcinous* were established from adults collected at their overwintering sites on 15 May 2007 and 15 July 2008. For the larval stage the diet for the species is *Aristolochia contorta*. The insects were maintained at 25ºC and 65% relative humidity under a photoperiod of LD 16:8hrs. The hatched larvae were individually reared in Petri dishe. The containers used for rearing included small Petri dishes (35 × 10 cm) for the 1st and 2nd stages, medium Petri dishes (60 × 1.5 cm) for the 3rd and 4th stages, and large Petri dishes (100 × 40 cm) for the 5th stage.

Effects of temperature on developmental characteristics

The effect of constant rearing temperatures on the developmental period of *A. alcinous* was tested at 20, 22.5, 25, 27.5 and 30℃ (LD 14:10 hrs). *Aristolochia contorta* leaves were provided to hatched larvae. The larval period and the survival rate were then determined. Newly hatched larvae were placed on *A. contorta* plants under various temperatures. The larvae were examined daily, and food was added as needed. Observations were conducted daily to measure the survival and developmental time of each larva until the adult emerged. A linear model was applied to estimate the temperature-dependent development of *A. alcinous* on *A. contorta.*

Linear model

$$DD = d(T\text{-}DT)$$

In this model, DT is the developmental threshold temperature and (DD) is the effective temperature Campbell *et al*., 1974; Obrycki and Tauber 1982; Jarošik *et al*., 2002; Kontodimas *et al.*, 2004

Respirometry

The pupae of *A. alcinous* were allowed 5 hrs of acclimation in clear glass containers (30~60 ml) within the chamber. The rates of O_2 consumption were measured at 30 min intervals with a Micro-Oxymax System (Columbus Instruments, USA). The Micro-Oxymax System is a closed system with a reference chamber that recalibrates the sensors after each measurement and normalizes the rates of O_2 consumption to standard temperature (20ºC) and pressure (760 mm Hg). The air in the chambers was refreshed after each measurement to maintain constant O_2 and CO_2 concentrations. The O_2 uptake was expressed in units of mol/ml/g/min. Each value of oxygen uptake represents the mean of 3 replicates.

Diapause termination

To prepare diapausing insects, newly hatched larvae were individually placed in a Petri-dish (65 × 15 cm) with *A. contorta* and reared continuously under LD 8:16 hrs at 20ºC. Diapausing individuals at 5, 10, 15, 20, and 30 days after the final molt were used in the experiments to determine the sequence of diapause development during these periods. The diapausing larvae were chilled at 8ºC for 60, 70, 90 and 105 days and thereafter maintained at 25ºC. Throughout these treatments, the photoperiod was LD 16:8 hrs. The survival rate of the larvae after cold treatment was observed in association with the termination of diapause.

Statistical analysis

Differences in development and diapause were tested with an analysis of variance (ANOVA). If significant differences were detected, multiple comparisons were performed with a Tukey HSD multiple range test.

Results and Discussion

Effect of temperature on the development of *A. alcinous*

A. alcinous larvae were reared at various temperatures to determine the effects of temperature variation on development. The survival rates of *A. alcinous* at the 5 different temperatures used are shown in Table 1. These survival rates were 40.0, 30.0, 63.4, 50 and 23.3% at 20, 22.5, 25, 27.5 and 30ºC, respectively. The effect of these constant temperatures on the larval developmental time is summarized in Table 1 and Table 2. The mean duration of development, 12.9 days, was shortest at 30ºC. The duration of each instar was greater at relatively low temperatures than at higher temperatures (Egg: $F_{4, 87} = 12.247$, $p < 0.0001$; 1st instar: $F_{4, 63} = 35.412$, $p < 0.0001$; 2nd instar: $F_{4, 59} = 14.751$, $p < 0.0001$; 3rd instar: $F_{4, 58} = 7.987$, $P < 0.0001$; 4th instar: $F_{4, 57} = 13.214$, $p < 0.0001$; 5th instar: $F_{4, 57} = 7.347$, $p < 0.0001$; total larvae: $F_{4, 58} = 23.510$, $P < 0.0001$; full development (from egg to pupae): $F_{4, 57} = 46.162$, $p < 0.0001$). The developmental rate increased linearly with increasing temperature within the experimental temperature range (Table 2), as is generally the case in insects.

Table 1. Developmental characteristics of *A. alcinous* at different rearing temperatures

Temp. (°C)	Egg		Larvae					Total larval duration (days)	Pupation (%)
	Duration (days)	Hatching rate (%)	1st	2nd	3rd	4th	5th		
20	4.9±0.2^{b}	63.3^{b}	6.3±0.2^{d}	4.7±0.2^{d}	4.7±0.2^{b}	4.1±0.2^{b}	6.2±0.2^{b}	25.8±0.0^{c}	40.0^{a}
22.5	5.8±0.2^{b}	56.7^{b}	5.0±0.2^{c}	4.5±0.2^{cd}	4.3±0.2^{b}	3.8±0.3^{b}	6.2±0.2^{b}	23.6±0.0^{b}	30.0^{a}
25	3.3±0.2^{a}	86.7^{a}	3.7±0.3^{b}	3.7±0.3^{bc}	3.7±0.3^{ab}	3.2±0.3^{ab}	5.8±0.2^{a}	19.6±0.1^{b}	63.4^{b}
27.5	3.2±0.2^{a}	66.7^{b}	2.7±0.4^{a}	3.3±0.3^{b}	2.8±0.4^{a}	2.7±0.4^{a}	4.2±0.2^{a}	15.5±0.1^{a}	50.0^{b}
30	3.3±0.2^{a}	30.0^{c}	2.5±0.4^{a}	2.3±0.4^{a}	2.6±0.4^{a}	2.7±0.4^{a}	3.0±0.3^{a}	12.9±0.1^{a}	23.3^{a}

Hatching larvae of *A. alcinous* subjected to different temperatures (17.5, 20, 22.5, 25, 27.5 and 30°C). Values followed by the same letters within a column do not differ significantly (Tukey test following ANOVA, $p > 0.05$).

Table 2. Developmental threshold temperature (DT) and effective temperature (DD) for larval and pupal stages of *A. alcinous*

Developmental stage	Regression Equation[1)]	r^2	DT (°C)	DD (degree-days)
Egg	y = 0.0206x - 0.2973	0.81	14.4	44.8
1st instar	y = 0.0259x - 0.3674	0.97	14.2	38.5
2nd instar	y = 0.021x - 0.2362	0.86	11.2	48.1
3rd instar	y = 0.0187x - 0.1768	0.95	9.45	53.3
4th instar	y = 0.0144x - 0.0481	0.94	3.3	69.7
5th instar	y = 0.0168x - 0.2076	0.80	12.3	106.0
Egg to pupation	y = 0.0031x - 0.0334	0.93	10.8	230.4

[1)] y=ax+b, where y is developmental rate and x is temperature.

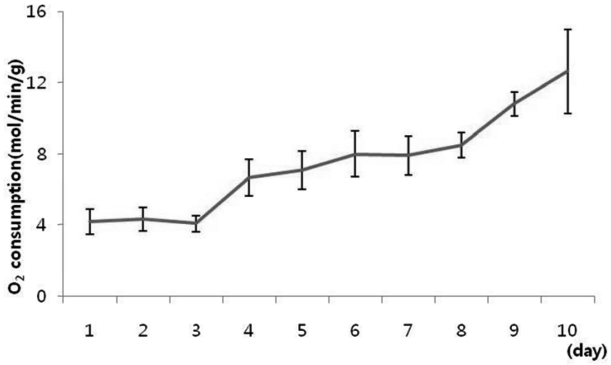

Fig. 1. Oxygen consumption by developing pupae of *Atrophaneura alcinous* from pupation until final diapause. The pupae were reared at LD 16:8 h and incubated at 25°C.

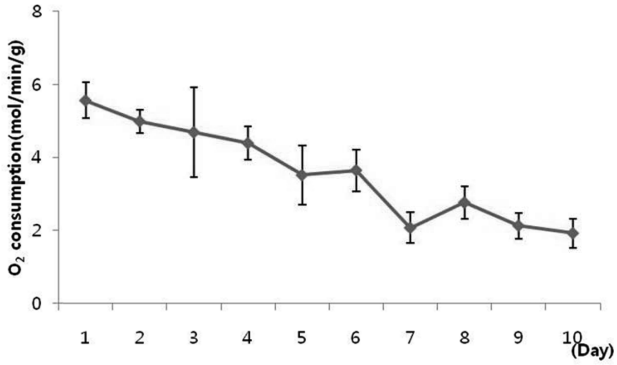

Fig. 2. Oxygen consumption by developing pupae of *Atrophaneura alcinous* from pupation until final diapause. The pupae were reared at LD 8:16 h and incubated at 20°C.

The lower developmental threshold temperature was estimated to be 10.8°C, and the estimated thermal constant was 230.4 degree-days for the larvae (Table 2). The coefficients of determination (r^2) exceeded 0.850 except for the egg and the 5th-instar larvae.

Diapause termination

The oxygen consumption of pupae was compared with that of non-diapausing pupae at the same stage, and the oxygen consumption of pupae in the final diapause stage was compared with that of pupae at the beginning of that stage (Fig. 1, Fig. 2). Usually, oxygen consumption by diapausing insects is lower than that of the corresponding active stage. There are, however, considerable differences between species. Generally, the diapausing stages of insects consume between 5 and 70% as much oxygen as the corresponding active stages (Hayes *et al*., 1968; Adamek and Fischer, 1985). Thus, a decrease in oxygen

Table 3. Development of *Atrophaneura alcinous* after storage at low temperature for diapause termination

Day	Days from diapause termination to emergence (days)	Emergence rate (%)	Crippled wing (%)
60	18.3±6.3[b]	80.7[a]	21.1[a]
75	10.0±1.6[a]	57.1[c]	25.0[a]
90	10.5±1.4[a]	71.6[b]	9.5[a]
105	9.7±0.2[a]	73.2[ab]	12.2[a]

Values followed by the same letters within a column do not differ significantly (Tukey test following ANOVA, P > 0.05).

consumption is sometimes used as evidence for the incidence of diapause (Gehrken, 1985).

Studies on the developmental control of diapause termination have been performed using butterfly species belonging to different families, including *Papilio xuthus*, *P. polyxenes* (Papilionidae) and *Pieris rapae* (Pieridae). To investigate the environmental cues affecting the diapause termination of *A. alcinous*, hatching larvae reared under short day conditions at 20°C were transferred and allowed to terminate diapause at different temperatures. Chilling has been shown to accelerate the termination of diapause in certain species (Lees, 1955). The effect of exposure to low temperature on the rate of diapause development of the Chinese windmill butterfly was examined. The effect of chilling at different temperatures on diapause termination is shown in Table 3. Diapausing larvae were chilled for 60 days at 8°C and thereafter subjected to LD 16:8 h at 25°C (Table 3). The most suitable chilling temperature was 60 days ($F_{3,7}$ = 19.840, p < 0.001). In conclusion, temperature had significant effects of *A. alcinous*. Optimal developmental temperature for the *A. alcinous* was 25°C.

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