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Larval growth and the effect of temperature on head capsule size in *Atrophaneura alcinous* (Lepidoptera: Papilionidae)

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Abstract

The size of head capsule is one of the most important factors for identifying developmental stage. In order to understand the developmental characteristics of the butterfly *Atrophaneura alcinous*, we examined fluctuations in larval head capsule size under three different temperature conditions (20, 25, and 30°C) and 60% humidity. As a result, larvae developed to the fifth instar at all three temperatures. The head capsule size of larvae tended to be larger at the lowest temperature and smaller at the highest temperature. The development rate showed a regular change, consistent with Dyar's rule regarding head capsule size development. Furthermore, the development of head capsule size was found to correspond to a second degree polynomial regression better than to a linear regression. On the basis of these results, it would appear to be possible to perform an accurate assessment of instar status during the development of *A. alcinous*.

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Introduction

Body size is one of the most important life history characteristics of an organism. Its effects on fitness are well documented and have been widely studied both theoretically and empirically (Roff, 1992). Body size is also plastic: it can change under various environmental conditions (McShea, 1998). For example, insects that develop at higher temperatures are generally smaller than those that develop at lower temperatures (Atkinson, 1994), and well-fed organisms are generally larger than those fed a low quality diet (Chapman, 1998).

Dyar (1890) proposed the first mathematical classification criterion to determine the numbers of insect instars. This relationship can be described by a simple linear equation, $y = a + bx$. However, Hansen *et al*. (Hansen, Owens, & Huddleston, 1981), concluded that the development of the head capsule was better described by a second-degree polynomial equation, $y = a + bx + cx^2$. In both expressions, y represents the head capsule width, and x represents the instar.

The goal of this study was to determine whether development of the head capsule in the Chinese windmill butterfly (*Atrophaneura alcinous*) follows Dyar's rule, and to study the effect of temperature on the size of the head capsule.

Materials and Methods

Experimental insects

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Before starting bioassays, a number of *A. alcinous* were subjected to different temperatures. Newly hatched first-instar larvae were individually placed in Petri dishes (35 mm diameter and 10 mm height), in which they were supplied with food plant (*Aristolochia manshuriensis*). After molting to the third instar, each larva was moved to a medium-sized Petri dish (60 mm diameter and 10 mm height), in which it remained until the end of the fourth instar. Upon molting to the final (fifth) instar, larvae were transferred to large Petri dishes (100 mm diameter and 40 mm height), where they pupated. Freshly eclosed adults were released into a greenhouse where they were provided with a 10% sugar solution and *A. manshuriensis* as an oviposition substrate.

Larval development was examined at the following constant temperatures: 20, 25, and 30°C. Larvae were checked daily and the head capsules that were collected and stored in groups depending on temperature and instar. Head capsule width was measured using a Carl Zeiss V12 (Germany) microscope equipped with an ocular micrometer. Dyar's constant (head capsule width at instar x + 1/head capsule width at instar x) was calculated for each of the larval molts. Data on head capsule width were processed using the computer program SPSS 19.0. The effect of temperature was assessed by analysis of variance, with a significance level of 5%. Means were analyzed using a least significant difference multiple range test. Frequency distributions were determined using the same computer program. Adjustment of the data to linear and second-degree polynomial equations was performed using Excel software.

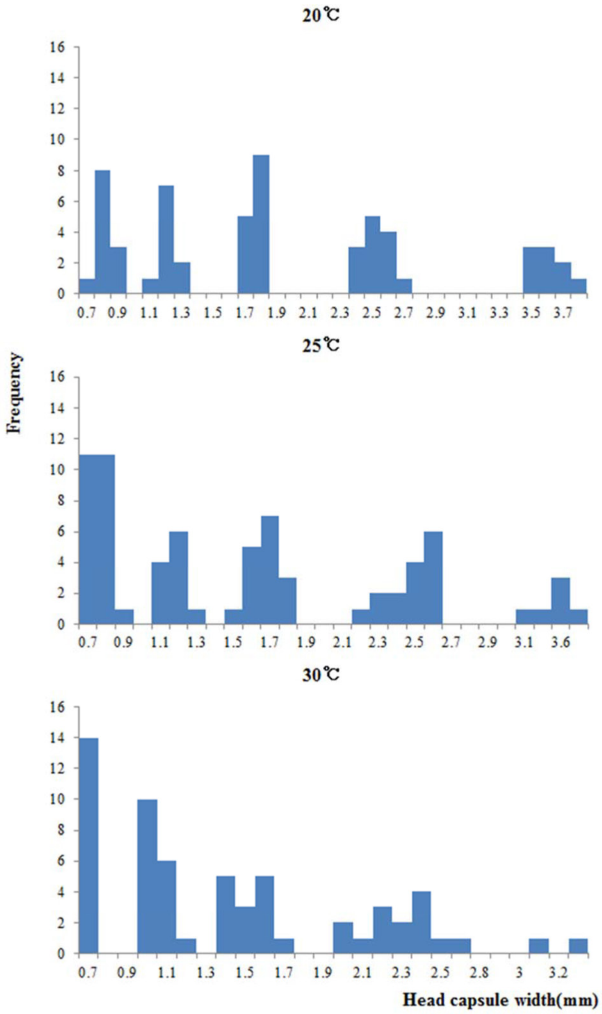

Fig. 1. Frequency distribution of the head capsule widths of larval instars of *Atrophaneura alcinous* at different temperatures

Results

The means of head capsules increased from the first to the fifth instar and were significantly different from one another. The frequency distribution of the head capsule width of the larval stages also showed five distinct groups corresponding to the five larval instars (Fig. 1). Head capsule widths ranged from 0.71 to 3.66 mm. Peaks were detected at 0.80, 1.22, 1.75, 2.51, and 3.66 mm. However, only the distribution of the first instar was discrete; the distributions of the remaining instars exhibited different degrees of overlap, which increased with instar progression (Table 1.)

Temperature had a notable affect on the size of *A. alcinous* head capsules. When head capsule width data for the same instar were compared according to temperature, significant differences were found for all instars ($p < 0.05$; Table 1). The largest head capsules were recorded at 20°C, whereas the smallest were recorded at 30°C. However, temperature had no influence on the growth ratio (Dyar's constant).

Growth ratios remained constant, ranging from 1.40 to 1.56, and no clear changing tendencies were detected. The average growth ratio was 1.46, irrespective of temperature. Data were depicted by linear and second-degree polynomial equations. High adjusted correlation coefficients were obtained in all cases ($R^2 > 0.958$), although the second-degree polynomial equation provided a better fit to the data ($R^2 > 0.998$). The head capsule width allowed us to distinguish the five larval instars without overlap of values. Significant differences were

Table 1. Mean head capsule width (mm) of *Atrophaneura alcinous* larvae reared at different temperatures

Temp. (°C)	Larval instar				
	L1	L2	L3	L4	L5
20	0.80±0.04a	1.22±0.04a	1.75±0.05a	2.51±0.09a	3.66±0.12a
25	0.75±0.05b	1.17±0.06b	1.67±0.07b	2.47±0.13a	3.48±0.22a
30	0.71±0.05b	1.04±0.06c	1.51±0.08c	2.29±0.17b	3.21±0.10b

Means within a column followed by the same letter are not significantly different at α = 0.05 (ANOVA and LSD) L_1: $F_{2,43} = 6.439$, $p < 0.005$; L_2: $F_{2,43} = 37.363$, $p < 0.0001$; L_3: $F_{2,43} = 28.890$, $p < 0.0001$; L_4: $F_{2,42} = 9.150$, $p < 0.0001$; L_5: $F_{2,17} = 4.220$, $p < 0.05$.

Table 2. Range of head capsule widths and growth ratios obtained for each instar at different temperatures

Temp. (°C)	Instar	n	Range (mm)	Dyar's constant
20	L1	13	0.72–0.88	-
	L2	13	1.15–1.27	1.52
	L3	13	1.68–1.82	1.43
	L4	13	2.36–2.67	1.43
	L5	9	3.49–3.90	1.46
25	L1	26	0.63–0.86	-
	L2	16	1.06–1.30	1.56
	L3	16	1.52–1.78	1.43
	L4	15	2.22–2.61	1.48
	L5	6	3.12–3.66	1.41
30	L1	17	0.68–0.73	-
	L2	14	0.96–1.16	1.46
	L3	14	1.40–1.66	1.45
	L4	14	2.03–2.67	1.51
	L5	2	3.14–3.27	1.40

revealed by the analysis of variance. The growth ratio of head width in consecutive instars was determined by dividing the mean head capsule width of the respective instar stage with that of the previous instar stage. When all five larval instars were considered, the head capsule width of *A. alcinous,* in relation to the total number of instars recorded, only followed Dyar's rule. Non-overlapping distributions were detected among the five instars. From the third instar onward, the head capsule widths of females were larger than those of males, and for both sexes, the growth ratio decreased and showed overlapping distributions when larvae had seven or eight instars (Table 2).

Discussion

Temperature is the most important factor affecting development in insects because they are poikilothermic organisms (Bale *et al*., 2002), and most metabolic reactions are temperature-dependent (Salvucci, Hendrix, & Wolfe, 1998, 1999). However, whereas the increase in body weight is constant, hardened structures, such as larval head capsules, grow discontinuously (Davidowitz, D'Amico, & Nijhout, 2003). The head capsule hardens rapidly after each molt, and thus it

Table 3. Parameters and adjusted correlation coefficients of the linear and second-degree polynomial regression equations describing the instar–head capsule width relationship at different temperatures

Temp. (°C)	Linear equation [a)]	R^2	Second-degree equation [a)]	R^2
20	y = 0.7014x-0.1146	0.958	$y = 0.121x^2 - 0.0246x + 0.7324$	0.998
25	y = 0.6766x-0.1188	0.967	$y = 0.1053x^2 + 0.0446x + 0.6185$	0.999
30	y = 0.6246x-0.1211	0.961	$y = 0.1051x^2 - 0.0061x + 0.6147$	0.999

can only grow during this short period of time a process that is also temperature-dependent. Therefore, it is expected that larger head capsule widths will be obtained in larvae reared at low temperatures and smaller widths will be obtained under higher rearing temperatures.

The instar–head capsule width relationship can be expressed in terms of regression models. The close fits recorded in all cases provides further evidence that the number of instars in *A. alcinous* remained the same at each of the temperatures tested. As revealed by the higher adjusted correlations, second-degree polynomial equations provided a closer fit than those obtained with linear regression equations.

These results indicate that temperature affects head capsule width but not the number of instars in *A. alcinous*. *A. alcinous* follows Dyar's rule because its number of instars does not fluctuate, and there are no overlaps between the head capsule widths of successive instars. These results can be used to determine the instar of field-collected larvae of *A. alcinous,* and thereby aid field biology research. In many insect species, the number of instars varies, and this is generally related to the nutritive value of the host plants (Dyar, 1890).

The results we obtained indicate that temperature appears to have no effect on the number of instars in *A. alcinous*. Since the measurement results were distributed in distinct groups, the number of instars was clearly indicated.

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