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Effect of temperature on the development of the Common Grass Yellow, *Eurema hecabe*

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Abstract

The developmental responses of insects to temperature are important considerations in gaining a better understanding of their ecology and life histories. Temperature-dependent phenology models permit examination of the effects of temperature on the geographical distributions, population dynamics, and management of insects. Measurements of insect developmental and survival responses to temperature pose practical challenges that depend on the chosen modality, variability among individuals, and high mortality rates near the lower and upper threshold temperatures. Different temperature levels can significantly affect larval development of *Eurema hecabe.* The development of *E. hecabe* reared on leaves of *Lespedeza cuneata* was investigated at three temperature regimes (20, 25, and 30°C), a relative humidity of 60%, and a light:dark photoperiod of 14:10 h. The developmental time from larva to adult was 34.3, 20.6, and 17.9 d at temperatures of 20, 25, and 30°C, respectively. Pupal rate was 47.6%, 47.6%, and 61.9% at temperatures of 20, 25, and 30°C, respectively. The developmental threshold temperature estimated from larva to pupae was 8.1°C with 381.7 degree-days. There is an increasing need for a standardized manual for rearing this butterfly species based on adequate knowledge of its ecology.

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Introduction

The pierid butterfly *Eurema hecabe*, which is common in southern Korea, is multivoltine, hibernates in the adult stage, and displays seasonal changes in wing pattern (the autumn morph diapauses, whereas the summer morph is non-diapausing) (Kato and Sano, 1987). Females mated in autumn survive winter and reproduce during the following spring without re-mating. The larval stage consists of five instars that feed mainly on *Lespedeza cuneata*.

One common approach to evaluating the effects of temperature on insect development is to monitor the duration of the various developmental stages. This permits the determination of two vital parameters of development: the thermal constant (K) and the minimum temperature of development (Tmin). The thermal constant is expressed as the number of degree-days (in °C) and provides an alternative measure of the physiological time required for the completion of a process or a particular developmental event (Damos and Savopoulou-Soultani, 2008).

Developmental rates decrease with a decrease in environmental temperature and development ceases at very low temperatures. Similarly, developmental rates increase with an increase in

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temperature up to a certain maximum, and subsequently decrease until reaching a minimum temperature at which development ceases.

The temperature-dependent developmental rate curve of an insect is an important feature of its life history (Taylor, 1981). Using degree-day accumulations to predict a wide variety of events such as egg hatch, adult emergence, or migratory flights may be feasible for insect rearing systems. Degree-day accumulations require a knowledge of both insect developmental responses to temperature and the lower developmental threshold (Woodson and Jackson, 1996).

Butterflies belong to the order Lepidoptera and can be used as indicators of habitat alteration because they are affected by anthropogenic activities, such as urbanization and changes to the natural landscape. Butterflies are also relatively easy to monitor and can therefore be used as an umbrella species when considering local habitat status, environmental conditions, biodiversity, and climate change (Blair, 1999; New, 1997). Butterflies are thought to be sensitive to changes in the environment that can lead to drastic changes in their numbers. These phenomena could justify further study into the changes that occur and the remediation that may be required. Climate change is predicted to lead to an increased mean temperature and more frequent climatic extremes (Pollard *et al*., 1997).

The relationship between temperature and development should be investigated to determine how best to regulate reproduction in the mass rearing of butterflies

Materials and methods

Insects. The larval stage of *E. hecabe* was fed *L. cuneata*. The insects were maintained at 25°C and a relative humidity of 65% under a light:dark (LD) photoperiod of 14:10 h. The hatched larvae were individually reared in Petri dishes. The containers used for rearing were small Petri dishes (35 × 10 mm) for the 1st and 2nd stages, medium Petri dishes (60 × 15 mm) for the 3rd and 4th stages, and large Petri dishes (100 × 40 mm) for the 5th stage.

Developmental time and survival. Temperature development studies of *E. hecabe* were conducted in environmental chambers (HB 302-2s-4, Hanbaek Science, Korea) at three constant temperatures (20, 25, and 30°C). Relative humidity and photoperiod were kept constant at 60% and LD 14:10 h, respectively. Larvae were collected from the leaves of *L. cuneata* and transferred individually to small Petri dishes containing moist filter paper. Laval development was monitored daily. A number of newly hatched 1st-instar larvae of *E. hecabe* were collected from the laboratory-reared colony. Individual larvae were isolated in Petri dishes (35 × 10 cm) containing the host plant and were placed in a randomized pattern within environmental chambers at set temperatures (15, 20, and 25°C with a variation of 5°C each) and photoperiod (LD 14:10 h).

Developmental rate and degree-day requirement. Developmental times at different temperatures were analyzed using the general linear model procedure for each instar separately, as well as for all instars combined. When the effects of temperature were significant ($p < 0.05$) F-values were derived.

Linear Model. For larvaal stage, the linear portion (20~30°C) of the developmental rate curve [R(T) = a + bT] was modeled using least squares linear regression, where T is temperature, and a and b are estimates of the intercept and slope, respectively. The base temperature threshold was estimated from the intersection of the regression line at R(T) = 0 and T_0 = -a/b. Degree-day requirements for each stage were calculated using the inverse slope of the fitted linear regression line (Campbell *et al*., 1974)1974.

Statistical Methods. Data were analyzed with analysis of variance (ANOVA) at $\alpha = 0.05$. Means were separated by the Tukey-Kramer honestly significant difference (HSD) test (Sokal and Rohlf, 1995)

Results and Discussion

The survival rates of *E. hecabe* at the three temperatures are presented in Fig. 1. The lowest percentage of pupation was recorded at 20°C, and the percentages steadily increased at 25 and 30°C. Differences in pupal weight were also significantly different ($F_{2,29} = 37.432$, $p < 0.0001$) (Fig. 2), and survival rates were 38.1, 47.6, and 57.1% at 20, 25, and 30°C, respectively. Emergence was significantly higher at 30°C than at 20°C. The optimal growth of *E. hecabe* occurred at 30°C. Low temperatures had an adverse effect on growth and caused high mortality in the immature life stages. Mortality was caused specifically by developmental issues, as immature *E. hecabe* were unable to

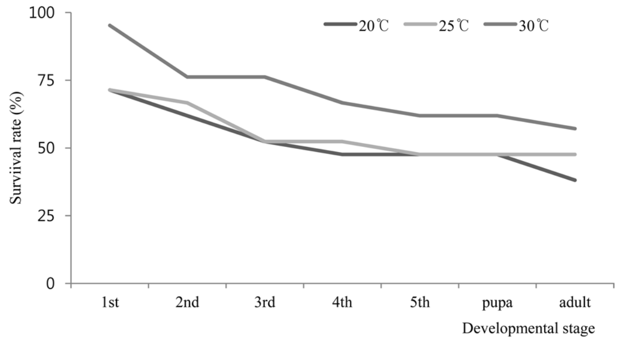

Fig. 1. Temperature response curves of survival rates at 20, 25, and 30°C.

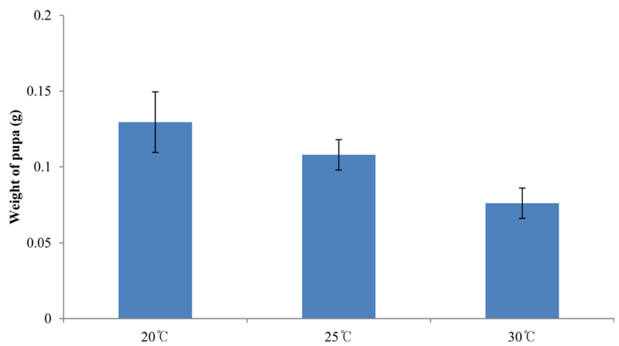

Fig. 2. Relationship between temperature and weight of pupa at 20, 25, and 30°C under long-day (LD 14:10 h) conditions.

pupate and complete the larval stage, or adults were affected by deformities (e.g., crippled wings). The developmental duration at three constant temperatures is presented in Table 1. The rate of development was positively correlated with temperature until the upper limit of 30°C was reached (r^2 = 0.786 – 0.996). At higher temperatures, development was completed at a faster rate.

The reciprocal of days required for each stage to develop at different temperatures was used to represent the developmental rate (1/d for development). The relationship between the developmetal rate (Y) and the rearing temperature (X) is represented by the regressionformula. The thermal constant is 1/a in Y = aX-b, and X is the developmental zero in Y = 0.

Larval development time decreased between 20 and 30°C, corresponding to a linear increase in development rate (Table 2), with a theoretical threshold value of $T0$ = 8.1, and a thermal constant K = 381.7 day-degrees. The developmental threshold of *E. hecabe* was 8.1°C, which is lower than the threshold reported for *Pieris rapae* (9.0°C) (Gilbert & Raworth, 1996).

According to (Lee *et al.*, 2012), the developmental duration of pre-adult stages of *E. hecabe* reared on the leaves of *L. cuneata* was 12.1 d at 25°C. Our results were comparatively higher than those of Lee *et al*. (2012). These findings could be

Table 1. Developmental duration (mean ± SE) of *Eurema hecabe* at various constant temperatures

Temp (°C)	1st instar	2nd instar	3rd instar	4th instar	5th instar	Pupae	1st instar to adult
20	3.9 ± 1.3a	4.4 ± 1.9a	3.3 ± 0.9a	4.6 ± 1.3a	9.1 ± 1.9a	10.1 ± 0.8a	34.3 ± 2.3a
25	2.1 ± 0.3b	2.6 ± 0.7b	2.2 ± 0.4b	2.7 ± 1.0b	5.5 ± 1.3b	5.7 ± 1.0b	20.6 ± 2.8b
30	1.8 ± 0.4b	1.9 ± 0.3b	1.9 ± 0.5b	2.6 ± 1.0b	4.6 ± 1.1b	5.5 ± 0.9b	17.9 ±1.8c

*Means within the same column followed by a different letter are significantly different at $p < 0.05$ (ANOVA followed by Duncan's new multiple range test). (1st instar: $F_{2,46} = 33.987$, $p < 0.0001$; 2nd instar: $F_{2,40} = 16.682$, $p < 0.0001$; 3rd instar: $F_{2,35} = 17.120$, $p < 0.0001$; 4th instar: $F_{2,32} = 11.165$, $p < 0.0001$; 5th instar: $F_{2,50} = 18.557$, $p < 0.0001$; pupae: $F_{2,27} = 74.227$, $p < 0.0001$).

Table 2. Mean developmental temperature (°C) thresholds ($T0$), thermal constants (K) in day-degrees above base, regression equations, and coefficients of determination (r^2) for the pre-adult stage of *Eurema hecabe*

stage	n	Developmental zero (T_0, °C)	Degree-days (K)	Linear regression equation[a]	r^2
1st instar	50	11.3	31.7	y = 0.0339x - 0.3821	0.9871
2nd instar	43	10.0	40.6	y = 0.0269x - 0.2685	0.9876
3rd instar	38	6.9	42.1	y = 0.0255x - 0.1758	0.9962
4th instar	35	6.9	57.2	y = 0.0202x - 0.1384	0.7857
5th instar	33	9.6	91.4	y = 0.0116x - 0.1117	0.9670
Pupa	33	7.2	118.5	y = 0.0087x - 0.0624	0.8112
1st instar to adult	30	8.1	381.7	y = 0.0027x - 0.022	0.9356

a Data for 20, 25, and 30°C

attributed to differences in the cultivation period of the host plant.

In the mass rearing of any insect, a well-organized work sequence must be planned; for example, adult rearing, egg collection, and rearing from egg to emergence (Koyama *et al*., 2004).

The influence of temperature during development and its effect on developmental stage of *E. hecabe* were evaluated. As expected, temperature showed a significant effect on developmental duration. The main advantage of maintaining pupae at 30°C seems to be reduced pupal developmental time, which favors adult emergence and is important in reducing infections and rearing costs. In contrast, higher pupal weight was significantly favored when pupae were allowed to develop at 20°C. Based on the present results, an environmental temperature of 30°C during pupal developmental time is recommended for *E. hecabe* mass rearing. Temperature showed a significant effect on pupal and larval development time.

Body size is also plastic and can change in response to different environmental conditions (Stern, 2001). 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 typically larger than those fed a poor-quality diet (Chapman, 1998). Given a particular growth rate, the final size of an insect should be proportional to its duration of growth. This means that either the developmental duration of an insect may be shortened at the cost of body size, or it may attain a larger size at the cost of prolonged development.

In insects, female lifetime reproductive success (mating ability in particular), is more closely correlated with body size (Honek, 1993) than the major components of male reproductive success are. Thus, in terms of fitness, larger females tend to have more attract.

As an insect larva grows, it normally progresses through a fixed number of instars. During its final instar, the larva attains a critical size that sets in motion the endocrine events that trigger metamorphosis.

Developmental thresholds and degree-day requirements can provide information about life stage events that might aid in developing efficient management strategies.

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