Effects of temperature on the development and survival of an endangered butterfly, *Lycaeides argyrognomon* (Lepidoptera: Lycaenidae) with estimation of optimal and threshold temperatures using linear and nonlinear models

Keiko KODA and Hiroshi NAKAMURA
Education and Research Center of Alpine Field Science, Faculty of Agriculture, Shinshu University, Minamiminowa, Nagano, Japan

Correspondence: Keiko Koda, Education and Research Center of Alpine Field Science, Faculty of Agriculture, Shinshu University, Minamiminowa, Nagano 399-4598, Japan.
Email:insect2@shinshu-u.ac.jp

Abstract

The effects of temperature on the development and survival of *Lycaeides argyrognomon* were examined in the laboratory. The eggs, larvae and pupae were reared at temperatures of 15, 17.5, 20, 25, 30 and 33ºC under a long-day photoperiod of 16-h light and 8-h darkness. The survival rates of the first-third instars ranged from 40.0 to 82.4%. The mortalities of the fourth instar were lower than those of the first-third instars. The development time of the overall immature stage decreased from 78.33 days at 15 ° C to 21.07 days at 30ºC, and then increased to 24.33 days at 33ºC. The common linear model and the Ikemoto-Takai model were used to estimate the thermal constant (K) and the developmental zero (T_0). The values of T_0 and K for the overall immature stages were 10.50ºC and 418.83 degree-days, and 9.71ºC and 451.68 degree-days by the common model and the Ikemoto-Takai model, respectively. The upper temperature thresholds (T_{max}) and the optimal temperatures (T_{opt}) of the egg, the first-third instars and the overall immature stages were estimated by the three nonlinear models. The ranges of T_{opt} estimated were from 30.33ºC to 32.46ºC in the overall immature stages and the estimates of T_{max} of the overall immature stages by the Briere-1 and the Briere-2 models were 37.18ºC and 33.00ºC, respectively. The method to predict the developmental period of *L. argyrognomon* using the nonlinear models was discussed based on the data of the average temperature per hour.

Key words: Briere model, developmental time, developmental zero, hatchability, Ikemoto-Takai model, Janisch model, survival rate, thermal constant.

INTRODUCTION

Lycaeides argyrognomon (Bergsträsser) is a grassland lycaenid butterfly with a wingspan range of 20 to 30 mm. This butterfly is distributed in Japan, the Korean Peninsula, Northeastern China, Europe and North America. The Japanese population was classified into a subspecies, *L. argyrognomon praeterinsularis* (Verity) (Shirozu 2006). Though many leguminous plants are reported as host plants of *L. argyrognomon* larvae in Europe and North America (Takahashi 2007), in Japan larvae feed on mainly *Indigofera pseudo-tinctoria* Matsum. (Fabaceae) and there are a few exceptional reports that eggs and larvae were found on *Hedysarum vicioides* Turcz (Fabaceae) (Fukuda *et al*. 1984).

Recently, the habitats grown with *I. pseudo-tinctoria* have rapidly decreased in Japan (Tashita *et al*. 1999). Large populations of this butterfly had been found throughout Nagano Prefecture, but it has now become extinct in the northern part of Nagano Prefecture (Tashita *et al*. 1999). Habitats in the banks of rivers in Shizuoka Prefecture are decreasing rapidly due to river improvement or vegetation changes (Hagiwara *et al*. 2009). Thus, this butterfly was designated Vulnerable (VU) by the Ministry of the Environment (2007) and Near Threatened (NT) by Nagano Prefecture (2004).

Now, many butterflies inhabiting grasslands and the banks of rivers are in crisis and are threatened with extinction. Therefore, preservation activities and ecological research are being carried out to conserve these endangered butterflies, e.g. *Shijimiaeoides divinus* (Fixsen) (Murata & Nohara 1993; Hama 2007; Koda & Nakamura 2010), *Melitaea protomedia* Ménétriès (Nanba 2009) and *Zizina otis emelina* (de l'Orza) (Kobayashi *et al*. 2009). However, there are few ecological and physiological reports on *L. argyrognomon* in Japan (Omura *et al*. 2009; Koda *et al*. 2010) except for the life cycle (Fukuda *et al*. 1984).

This butterfly is multivolitine and overwinters in the egg stage. There are three generations each year in Ina City and Suwa City (Koda *et al*. 2010) and two generations in Shiojiri City (Fukuda *et al*. 1984) of Nagano Prefecture. On the other hand, it is known that there are five generations in Shizuoka Prefecture (Fukuda *et al*. 1984). At the habitats of *L. argyrognomon*, grasses including *I. pseudo-tinctoria* have happened to be cut down all together using a mowing machine. For the conservation of this butterfly, it is important to know correctly the number of generations and the appearance time of adults and larvae in the habitat.

Recently the effects of temperature on the development of the two butterflies, *Pelopidas jansonis* (Butler) (Inoue 2009) and *Celastrina sugitanii* (Matsumura) (Komeyama & Hoshikawa 2007) have been reported in the sight of climatic adaptation. In these reports, the developmental zero and the thermal constant are calculated from the regression line of the rearing temperature and the developmental rate expressed as the reciprocal of the number of days required for the development of a stage.

In this study, the developmental rates of the eggs, larvae and pupae of *L. argyrognomon* were examined at different constant temperatures to clarify the effect of temperature on the development of this butterfly and to estimate the developmental zero and the thermal constant using linear models, and the optimal temperature and the threshold temperatures using nonlinear models. Then, we tried to discuss how to estimate the appearance time of adult butterflies at the habitat using the weather data.

MATERIALS AND METHODS

Insects

The adult females of *L. argyrognomon* were originally collected at the some spots of the Otagiri River in Komagane City, Nagano Prefecture, Japan in June 2008. These females were fed on sucrose solution of 2.0 % as nectar in a cylindrical cage (220 mm in diameter, 150 mm in height) made of polyester cloth. The cage was kept at room temperature under a photoperiod of 16-h light and 8-h darkness (16L:8D) in the Insect Ecology Laboratory of Education and Research Center of Alpine Field Science, Faculty of Agriculture, Shinshu University. For oviposition, females were put in a cylindrical cage (200 mm in diameter, 150 mm in height) which covered the branch of *I. pseudo-tinctoria* growing naturally on our campus. We used the eggs laid by nine females.

Rearing methods

Within one day of oviposition, the eggs were placed on a damped filter paper in Petri dishes (90 mm in diameter, 10 mm in height) and kept in incubators under 16L:8D. These Petri dishes were set at different constant temperatures of 15, 17.5, 20, 25, 30 and 33ºC. The number of newly hatched larvae was counted daily.

Newly hatched larvae were placed in Petri dishes with leaves of *I. pseudo-tinctoria* cultivated on our campus and kept in incubators under 16L: 8D. These Petri dishes were set at different constant temperatures of 15, 20, 25, 30 and 33ºC. Ten or fewer larvae were reared together in a Petri dish until the end of the third instar. The fourth instar larvae were individually transferred to larger Petri dishes (90 mm in diameter, 40 mm in height). Fresh leaves of *I. pseudo-tinctoria* were given at intervals of two or three days. After pupation, each pupa was placed into a new Petri dish of the same size with filter paper. The mortality and development of the larvae and pupae were examined daily.

Estimation of developmental zero and thermal constant

The developmental rate is the reciprocal of the developmental period in days (D). Based on the law of total effective temperature, $D\ (T-T_0) = K$, the common linear equation, $1/D = (1/K)T - T_0/K$, has been used to describe the relation between the developmental rate and temperature (T) ; where T_0 is the developmental zero and K is the thermal constant. The parameters T_0 and K can be estimated as the value of x (temperature axis) at y (developmental rate axis) = 0 and as the reciprocal of the slope of the regression line, respectively.

In addition to the common linear model, the Ikemoto-Takai linear model derived from the law of total effective temperature can obtain more reliable estimates of T_0 and K (Ikemoto & Takai 2000). This linear formula is as follows: $(DT) = K + T_0 D$, which represents a linear line with the x axis = D and the y axis = DT (Ikemoto & Takai 2000). The parameters T_0 and K can be obtained as the slope and the intercept of the Ikemoto-Takai model. In this study, we used the two linear models to estimate T_0 and K.

Estimation of optimal temperature and upper temperature threshold

The linear models only fit well within the suitable temperature range of insect development (Campbell *et al.* 1974). Therefore, in addition to the two linear models, we used the three nonlinear models to describe the relation between temperature and the developmental rate. The nonlinear models were the following empirical models: the Briere-1 model,

$$\frac{1}{D} = a \times T \times (T - T_{min}) \times \sqrt{T_{max} - T}$$

the Briere-2 model

$$\frac{1}{D} = a \times T \times (T - T_{min}) \times (T_{max} - T)^{\frac{1}{m}}$$

where T_{min} is the lower temperature threshold, T_{max} is the upper temperature threshold and, a and m are empirical constants (Briere *et al.* 1999). The Briere-1 and Briere-2 models estimate these temperatures as constants and can also estimate the optimal temperature (T_{opt}) at which the rate of development is highest. T_{opt} of the Briere-2 model is equal to

$$\frac{\left\{2mT_{max}+(m+1)T_{min}+\sqrt{4m^2{T_{max}}^2+(m+1)^2{T_{min}}^2-4m^2T_{min}T_{max}}\right\}}{4m+2}$$

and when m=2, the equation shows T_{opt} of Briere-1 model.

And we used the Janisch model

$$\frac{1}{D} = \left\{\frac{D_{min}}{2} \times \left[e^{k(T-T_{p})} + e^{-\lambda(T-T_{p})}\right]\right\}^{-1}$$

where D_{min} and T_p is the developmental period (D_{min}) in the optimum (T_p) (Janish 1932). We estimated T_{opt} by

$$\frac{1}{k+\lambda} \times \left\{ln\frac{\lambda}{k} + (k+\lambda)T_p\right\}$$

where k and λ are empirical constants.

Statistical Analysis

The effects of temperature on the developmental periods were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer test. Differences in the survival rates at various constant temperatures were analyzed using the χ^2 test. These statistical analyses were conducted using XLSTAT Version 2010.6.03 (Addinsoft). The parameters of the nonlinear models were estimated using the JMP statistical programs (v.8.02: SAS Institute 2009).

To assess the performance of the mathematical models, we used two criteria: the coefficient of determination (R^2) and the adjusted coefficient of determination (R^2_{adj}) (Rezaei & Soltani 1998).

RESULTS

Relation between survival and temperature

Table 1 showed the survival rates of four developmental stages at the six different temperatures. The hatchability of the eggs ranged from 80.0 to 100.0 %. There were no significant differences among temperatures (χ^2 test , $P > 0.05$). The survival rate of the first-third instars ranged from 40.0 to 82.4%. The lowest value of 40.0% was obtained at 33ºC, which was significantly different from the 82.4% at 30ºC (χ^2 test, $P < 0.05$). The survival rate of the fourth instar ranged from 70.6 to 100%. The mortalities of the fourth instar were lower than those of the the first-third instars. Deaths were scarcely observed after the fourth instar. There were no significant differences in the survival rates of the fourth instar among different temperatures (χ^2 test, $P > 0.05$). The survival rate of the pupal stage ranged from 50 to 100%. The lowest value of 50.0% was obtained at 15ºC, which was significantly different from the 91.7% at 20ºC (χ^2 test, $P < 0.05$).

Relation between development and temperature

The development periods of the eggs, larvae, pupae and overall immature stages are summarized in Table 2. Periods of the egg stage ranged from 16.00 days at 15ºC to 3.00 days at 30ºC and 33ºC. The duration of the egg stage decreased significantly as the temperature increased to 30ºC (Tukey-Kramer test, $P < 0.05$) and then did not decrease further at 33ºC. The development period of the first-third instars ranged from 29.00 days at 15ºC to 9.00 days at 30ºC. Period of this stage at 33ºC was longer than that at 30ºC. Period of the fourth instar ranged from 17.33 days at 15ºC to 2.67 days at 33ºC. The duration of the fourth instar decreased as the temperature increased.

The developmental period of the pupae ranged from 17.67 days at 15˚C to 4.83 days at 33ºC. The duration of the pupal stage decreased significantly as the temperature increased to 30ºC (Tukey-Kramer test, $P < 0.05$). Period of the overall immature stage decreased from 78.33 days at 15˚C to 21.07 days at 30ºC and then increased to 24.30 days at 33ºC.

Developmental zero

The developmental zero (T_0) and the thermal constant (K) of the immature stages are shown in Table 3. The values for the egg, first-third instar and overall immature stages at 33ºC were omitted from the targets for line fitting because the development periods of these stages at 33ºC did not become shorter than that at 30ºC (Table 2). On the other hand, all data sets of the fourth instar larvae and pupae from 15 to 33ºC were included for line fittings. Both of the linear models showed an acceptable fit to the data for every stage judging from the high values of R^2 and $R^2_{adj.}$, and $P < 0.05$.

The common linear model tended to give higher estimates of T_0 and lower estimates of K than the Ikemoto-Takai model at every developmental stage (Table 3). The values of T_0 of the egg and fourth instar stages were larger than 10ºC, but those of the first-third instar and pupal stages were smaller than 10ºC. The thermal constants of the overall immature stage were 418.83 and 451.68 degree-days as determined by the common linear model and the Ikemoto-Takai model, respectively.

Optimal temperature and upper temperature threshold

The relations between the developmental rate and the rearing temperature of the fourth instar and the pupal stages were linear within the temperature range (15-33ºC) examined in this study. So, only the data of the egg, the first-third instar and the overall immature stages were used for the three models fitting. The curves of the developmental rates fitted by the three nonlinear models are shown in Fig.1. These models fitted the data points well judging from the high values of R^2 and R^2_{adj} (Table 4).

Table 4 shows the values of three parameters, the lower temperature threshold (T_{min}), the upper temperature threshold (T_{max}) and the optimal temperature (T_{opt}), estimated by the three models. The Janisch model cannot estimate the values of T_{min} and T_{opt}. The ranges of the estimates of T_{opt} were from 28ºC to 33ºC, though the value of the egg stage obtained by Briere-1 was 37.55ºC. The estimates of T_{max} obtained by the Briere-2 model were about 33ºC, but the values by the Briere-1 model were much larger than

33ºC .

DISSCUSSION

In the present study, the hatchability of *L. argyrognomon* eggs was greater than 80% at all rearing temperatures. On the other hand, the hatchability of *S. divinus barine* that inhabits grassland in Nagano Prefecture becomes lower at higher temperatures (e.g. 30% at 30ºC) (Koda & Nakamura 2010). *S. divinus barine* is univoltine, and the egg stage occurs only in June when the average temperature is 19.5ºC in the habitat of Nagano Prefecture. On the other hand, the eggs of *L. argyrognomon* have to grow in the hot summer season because it is multivoltine. So this butterfly may have tolerance to high temperatures.

The duration of the egg stage of this butterfly was reported to be 3-4 days in summer in October in Shizuoka City of Shizuoka Prefecture (Fukuda *et al.* 1984), where the average temperature in August from 1971 to 2000 was 26.8ºC (JMA 2011). Table 2 shows that the developmental periods of the egg stage are 3-5 days at 25-30ºC. This result corresponds well with the reports on the developmental days of this butterfly in Shizuoka Prefecture (Fukuda *et al* 1984).

The values of T_0 and K estimated by the Ikemoto-Takai model were lower and larger than those of the common linear model (Table 3). This result was consistent with the reports on *C. pomonella* by Aghdam *et al.* (2009) and *Pelopidas jansonis* (Butler) by Inoue (2009). For the following discussion, we tentatively used the estimates of the overall immature stage obtained by the common linear model. The values of T_0 and K of *L. argyrognomon* as determined by the common linear model were 10.50ºC and 418.83 degree-days from egg to adult emergence.

We observed that the occurrence peak of the overwintering generation of *L. argyrognomon* adults was on June 15 in 2010 in Ina City (K. Koda & H. Nakamura, unpublished). If female adults that emerged on June 15 would lay eggs on June 17 of this year, the first generation adults could be expected to appear on July 21, based on the data for the daily average temperature over 10.5ºC in 2010 (JMA 2011) and the thermal constant of 418.83 degree-days for the full development. Then, the second and third generations could be calculated to appear on August 19 and September 19, respectively. Therefore, the thermal constant obtained from the common linear model could allow *L. argyrognomon* to produce four generations per year.

It is known, however, that this butterfly produces three generations in Ina City (Koda *et al*. 2010). Actually we observed that the occurrence peaks of the first and the second generation adults were on July 30 and on September 7, respectively, and the adult butterflies of the third generation did not appear in 2010 (K. Koda & H. Nakamura, unpublished). This may be explained by the fact that the total effective temperature from 8 September to 31 December in 2010 was 396.2 degree-days, which was smaller than the thermal constant (418.83 degree-days) for the full development of *L. argyrognomon*.

The present study made clear that the developmental rate at temperatures higher than 30ºC decreases in the egg, first-third instar and overall immature stages (Table 2). As the temperature of summer in Ina City almost exceeds 30ºC in the daytime, it may well be that the development of this butterfly is influenced by high temperature. So, the daily effective temperature is not equal to $T - T_0$, but may be calculated by the formula $a(T - T_0)$ ($0 =< a <1$) when the daily maximum temperature exceeds 30ºC. It is necessary to reflect the decrease of the developmental rate by high temperature on the calculation of the total effective temperature.

One method is to modify the linear model using the nonlinear model at high temperature zone. For example, from the estimated equation of the Janisch model, we can calculate the developmental rate as $1/D = 0.045$ at the temperature 32ºC over T_{opt}

(30.33ºC) and this value is equivalent to 28.4ºC below T_{opt}. Then the effective temperature at 32ºC may be estimated 17.9 degree-days when T_0 = 10.5ºC.

The daily average temperatures of August in 2010 in Ina City did not exceed 30ºC (JMA 2011). Supposing that T_{opt} is 30.33ºC by the Janish model (Table 4), in case of using the daily average temperature, the total effective temperature of *L. argyrognomon* can be calculated as the accumulated value of T - T_0 without modification of the linear model. Therefore if an egg was laid on August 1 in 2010, the adult butterfly was expected to emerge at 10:00 a.m. on August 28. However, there were 25 days when the daily maximum temperatures were over 30.33ºC in August this year in Ina City (JMA 2011). So we used every average temperature date per hour to calculate the total accumulated effective temperature. In case of correcting the average temperatures per hour which exceeded T_{opt} by the method mentioned above, the adult butterfly is expected to emerge at 8:00 a.m. on August 29, which was about one day later than the case of using the daily average temperature. This result means that this method has evaluated the influence on the development of *L. argyrognomon* by high temperature in the daytime.

Another method is that the developmental rate is calculated using a nonlinear model based on temperature data and the insect is considered to complete the full development when the accumulated value of the developmental rate is equal to one. The anticipation of the adult emergence was performed by this method using the same temperature data above-mentioned. The emergence time of the adult butterfly was calculated at 4:00 a.m. on August 27 in case of using the daily average temperature. On the other hand, when the average temperature per hour was used, the adult butterfly was expected to emerge at 11:00 p.m. on August 28, which was about two days later than the time using the daily average temperature. It may be said that this method can evaluate the delay effect on the development of *L. argyrognomon* by high temperature more correctly than the first method, and that the prediction of the development using a nonlinear model is a simple and logical method.

The occurrence peak of the second generation adult in 2010 was in fact on September 6, which was ten days later than the emergence day expected by the two methods. The following two points can be considered as this reason. 1) The period from the occurrence peak of the first generation adult (July 31) to that of the second generation is not necessarily consistent with the developmental period from the egg to the adult emergence of this butterfly. 2) The temperature of Automated Meteorological Data Acquisition System (AMEDAS) in Ina City may differ from that of the habitat of *L. argyrognomon*.

As this butterfly inhabits at the dry riverbed and the field ridge where direct rays hit and become high temperature in the daytime, it may be exposed to higher temperature than the observation station of AMEDAS in Ina City. It is necessary to investigate the developmental period and the temperature data from egg to adult at the habitat and to verify the method of prediction of the seasonal prevalence of occurrence using a nonlinear model. Moreover, it is important to describe the relation of temperature and the developmental rate using the more appropriate nonlinear model based on the enough data in both the number of insects and temperature.

ACKNOWLEDGMENTS

We thank Prof. Dr H. Kitahara for advice on the rearing method of *L. argyrognomon*. This study was partly supported by the Fujifilm Green Fund Public Trust (FGF).

REFERENCES

Aghdam HR, Fathipour Y, Radjabi G, Rezapanah M (2009) Temperature-dependent development and temperature thresholds of Codling Moth (Lepidoptera: Tortricidae) in Iran. *Environmental Entomology* **38,** 885-895.

Briere JF, Pracros P, le Roux AY *et al.* (1999) A novel rate model of temperature-dependent development for arthropods. *Environmental Entomology* **28,** 22-29.

Campbell A, Frazer BD, Gilbert N, Gutierrez AP, Mackauer M (1974) Temperature requirements of some aphids and their parasites. *Journal of Applied Ecology* **11,** 431-438.

Fukuda H, Hama E, Kuzuya T *et al.* (1984) *The Life Histories of Butterflies in Japan*. III. Hoikusya, Osaka. (In Japanese.)

Hagiwara Y, Yamashita M, Sawada H (2009) Invasion status of *Eragrostis curvula* in the *Lycaeides argyrognomon praeterinsularis* habitat along the Abe River, and the effects of its removal. *Japanese Journal of Conservation Ecology* **14,** 25-35. (In Japanese with English summary.)

Hama E (2007) Conservation of the habitats of *Shijimiaeoides divinus barine*, liberated at Azuminoshi, Nagano. *The Nature & Insects* 42(7), 27–31. (In Japanese.)

Ikemoto T, Takai K (2000) A new linearized formula for the law of total effective temperature and the evaluation of line-fitting methods with both variables subject to error. *Environmental Entomology* **19,** 671-682.

Inoue T (2009) Effects of temperature on the development of overwintering pupae of *Pelopidas jansonis* (Butler) (Lepidoptera, Hesperiidae). *Transactions of the Lepidopterological Society of Japan* **60,** 196-202.

Janisch E (1932) The influence of temperature on the life-history of insects. *Transactions of the Entomological Society of London* **80,** 137-168.

Japan Meteorological Agency (JMA) (2011) *Weather, Climate & Earthquake Information*. [Cited 13 April 2011.] Japan Meteorological Agency, Available from:http://www.jma.go.jp/jma/menu/report.html

Kobayashi T, Kitahara M, Suzuki Y, Tachikawa S (2009) Assessment of the habitat quality of the threatened butterfly, *Zizina emelina* (Lepidoptera, Lycaenidae) in the agro-ecosystem of Japan and implications for conservation. *Transactions of the Lepidopterological Society of Japan* **60,** 25-36.

Koda K, Furui H, Osuga H, Nakamura H (2010) Seasonal change and diurnal activity of *Lycaeides argyrognomon*（Lepidoptera:Lycaenidae）at the bank of the Mibu River in Nagano Prefecture *The Annals of Environmental Science Shinshu University* **32,** 65-68. (In Japanese.)

Koda K, Nakamura H (2010) Effects of temperature on the development and survival of an endangered butterfly, *Shijimiaeoides divinus barine* (Leech) (Lepidoptera: Lycaenidae). *Entomological Science* **13,** 29-34.

Komeyama S, Hoshikawa K (2007) Rapid growth at lower temperatures by the larvae of *Celastrina sugitanii* (Lepidoptera, Lycaenidae). *Transactions of the Lepidopterological Society of Japan* **58,** 245–251.

Ministry of the Environment (2007) *Threatened Species. Japan Integrated Biodiversity Information System*. [Cited 14 December 2010.] Available from: http://www.env.go.jp/press/file_view.php?serial=9945&hou_id=8648 (In Japanese.)

Murata K, Nohara K (1993) Decline and conservation of *Shijimiaeoides divinus* (Matsumura) in Kumamoto Prefecture. In: Yata O, Ueda K (eds) *Decline and Conservation of Butterflies in Japan* *Ⅱ*, pp. 151-159. Lepidopterological Society of Japan, Osaka. (In Japanese with English summary.)

Nagano Prefecture (ed.) (2004) *Red Data Book Nagano Edition*. Nagano Prefecture, Nagano. (In Japanese with English summary.)

Nanba M (2009) Ecology and conservation of *Malitaea proromedia* in Ombara

Highland, Okayama Prefecture. In: Mano T, Fujii H (eds) *Decline and Conservation of Butterflies and Moths in Japan* 6, pp. 39-48. (In Japanese.)

Omura H, Watanabe M, Honda K (2009) Cuticular hydrocarbons of larva and pupa of Reverdin's blue, *Lycaeides argyrognomon* (Lycaenidae) and its tending ants, *Transactions of the Lepidopterological Society of Japan* **60,** 203-210.

Rezaei A, Soltani A (1998) *An introduction to applied regression analysis.* Isfahan, University of Technology Isfahan, Iran.

SAS Institute (2009) JMP : a guide to statistical and data analysis, version 8.02. SAS Institute, Cary, NC.

Shirozu T（2006）*The Standard of Butterflies in Japan.* GAKKEN, Tokyo. (In Japanese.)

Takahashi M (2007) A problem on the food plant of *Plebejus argyrognomon* (Bergsträsser, 1779) (Lycaenidae) in Japan and the vicinity. *Gekkan-Mushi* **439**, 18-21. (In Japanese.)

Tashita M, Nishio N, Maruyama K (1999) *Distribution and Variation of the Butterflies in Nagano, Japan.* Bun-ichi, Tokyo. (In Japanese.)

Figure legend

Figure1 Fitting three nonlinear models to the observed values of the developmental rates (1/*D*) of egg stages (A-C), 1st-3rd instar stages (D-F) and overall immature stages (G-I) at rearing constant temperature (ºC) of *Lycaeides argyrognomon*. □ : observed data.

Table 1　Survival rate of each developmental stage of *Lycaeides argyrognomom* at different temperatures

Temperature (℃)	Number of individuals					Survival rate (%)			
	Egg	1st instar	4th instar	Pupa	Adult	Egg	1st−3rd instar	4th instar	Pupa
15	20	16	7	6	3	80.0	43.8	85.7	50.0
17.5	20	20	−	−	−	100.0	−	−	−
20	25	25	17	12	11	100.0	68.0	70.6	91.7
25	18	16	11	10	10	88.9	68.8	90.9	100.0
30	20	17	14	14	14	85.0	82.4	100.0	100.0
33	17	15	6	6	6	88.2	40.0	100.0	100.0

Table 2 Developmental periods of eggs, larvae, pupae and overall immature stage of *Lycaeides argyrognomom* at different temperatures

Temperature (℃)	Egg		Larva				Pupa		Overall immature stage	
			1st–3rd instar		4th instar					
	Number of samples	Mean ± SE* (days)	Number of samples	Mean ± SE* (days)	Number of samples	Mean ± SE* (days)	Number of samples	Mean ± SE* (days)	Number of samples	Mean ± SE* (days)
15	16	16.00±0.00^{a}	6	29.00±0.00^{a}	6	17.33±1.41^{a}	3	17.67±1.20^{a}	3	78.33±2.19^{a}
17.5	20	13.05±0.11^{b}	–	–	–	–	–	–	–	–
20	25	8.84±0.07^{c}	12	21.00±0.00^{b}	12	11.00±0.00^{b}	11	11.18±0.23^{b}	11	51.18±0.23^{b}
25	16	5.00±0.00^{d}	11	10.73±0.19^{c}	10	5.30±0.15^{c}	10	7.70±0.15^{c}	10	28.70±0.15^{c}
30	17	3.00±0.00^{e}	14	9.00±0.00^{d}	14	4.00±0.00^{cd}	14	5.07±0.16^{d}	14	21.07±0.16^{d}
33	15	3.00±0.00^{e}	6	13.83±0.17^{e}	6	2.67±0.21^{d}	6	4.83±0.17^{d}	6	24.33±0.21^{e}

*: Values followed by a different letter in the same column are significantly different (ANOVA, Tukey–Kramer test, $P < 0.05$)

–: no data.

Table 3 Linear equation, developmwntal zero (T_0) and thermal constant (K) of *Lycaeides argyrognomom* analyzed with the two linear models

Linear model	Developmental stage		Linear equation (Fitting equation)	T_0(℃)±SE	K(degree-days)±SE	R^2	R^2_{adj}	P
Common	Egg		$1/D = 0.0183T - 0.2359$	12.91±1.13	54.70±6.08	0.9643	0.9524	0.003
	Larva	1st—3rd instar	$1/D = 0.0055T - 0.0524$	9.50±2.22	181.50±28.46	0.9531	0.9297	0.023
		4th instar	$1/D = 0.0169T - 0.2233$	13.21±1.99	59.17±8.99	0.9353	0.9137	0.007
	Pupa		$1/D = 0.0089T - 0.0832$	9.35±1.42	112.21±9.31	0.9798	0.9731	0.001
	Overall immature stage		$1/D = 0.0024T - 0.0251$	10.50±1.35	418.83±42.80	0.9796	0.9694	0.010
Ikemoto and Takai	Egg		$1/D = 0.0160T - 0.1918$	11.95±1.11	62.32±11.55	0.9740	0.9653	0.002
	Larva	1st—3rd instar	$1/D = 0.0056T - 0.0552$	9.80±2.06	177.51±39.65	0.9114	0.8670	0.045
		4th instar	$1/D = 0.0146T - 0.1721$	11.82±1.34	68.65±13.08	0.9620	0.9492	0.003
	Pupa		$1/D = 0.0085T - 0.0749$	8.77±0.89	117.07±9.23	0.9692	0.9589	0.002
	Overall immature stage		$1/D = 0.0023T - 0.0224$	9.91±1.40	442.74±69.95	0.9603	0.9404	0.020

Table 4 Three parameters: lower temperature threshold (T_{min}), optimal temperature (T_{opt}), upper temperature threshold (T_{max}) estimated by the nonlinear models, the values of constants and two criteria: coefficient of determination (R^2), adjusted R^2 (R^2_{adj}) to assess the fitness of the model

Model	Parameters	Developmental stages		
		Egg	1st–3rd instar	Overall immature stages
Briere-1	T_{min} (℃)	10.94	10.59	9.83
	T_{opt} (℃)	37.55	28.83	30.92
	T_{max} (℃)	45.33	34.42	37.18
	α	1.36E-04	8.48E-05	2.72E-05
	R^2	0.977	0.961	0.979
	R^2_{adj}	0.962	0.922	0.959
Briere-2	T_{min} (℃)	8.32	5.09	2.10
	T_{opt} (℃)	32.92	30.80	32.46
	T_{max} (℃)	33.00	33.05	33.00
	α	4.98E-04	1.26E-04	5.49E-05
	m	167.26	6.23	29.29
	R^2	0.995	0.983	0.996
	R^2_{adj}	0.989	0.933	0.985
Janisch	T_{opt} (℃)	31.62	28.92	30.33
	K	0.45	0.39	0.33
	$Dmin$	3.37	10.17	23.99
	λ	0.12	0.12	0.11
	T_p	33.92	31.34	32.87
	R^2	1.000	0.993	0.999
	R^2_{adj}	0.999	0.973	0.996

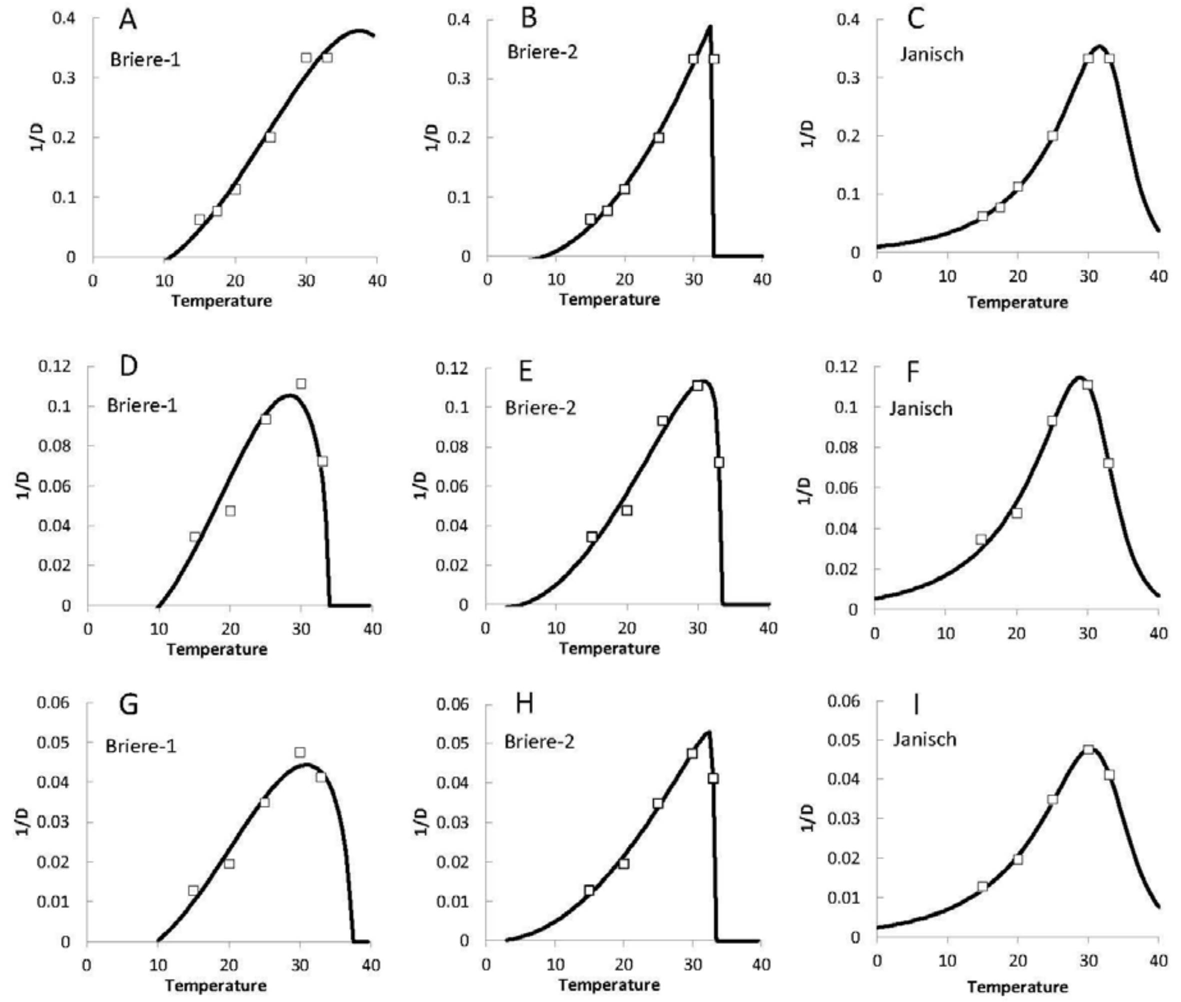

Fig.1 Fitting three nonlinear models to the observed values of the developmental rates (1/*D*) of egg stages (A-C), 1st-3rd instar stages (D-F) and overall immature stages (G-I) at rearing constant temperature (°C) of *Lycaeides argyrognomon*. □ : observed data.