Effects of Temperature and Photoperiod on the Larval Hibernation and Adult Aestivation of *Leptocarabus kumagaii* (Coleoptera: Carabidae)¹

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(Received June 10, 1987)

The effects of temperature and photoperiod on the larval development and gonad maturation of *Leptocarabus kumagaii* were studied. Larvae kept under diapause inducing photoperiod (12L–12D) completed their development either when photoperiod was changed to 14L–10D or 16L–8D at 15°C or when they experienced a period of low temperature (5°C) under 12L–12D. Egg production after adult emergence was promoted when photoperiod was changed from 16L–8D to 12L–12D or 14L–10D. At 12L–12D, oviposition started in 10–25 days and continued for 50 days, whereas at 14L–10D it started in 40–50 days and lasted for 150 days. Also, egg production was promoted at lower temperatures of 15 and 20°C but not at a higher temperature of 25°C. Under laboratory conditions, there was no difference in patterns of larval development and gonad maturation among a few local populations at different altitudes showing different seasonal cycles. Direct effects of temperature on the altitudinal variation of the seasonal cycle were suggested.

INTRODUCTION

Some insect species have two or more dormant phases during the life span of individuals. Autumn-breeding carabid beetles usually hibernate as larvae and aestivate as adults before the first reproductive season, and the adults sometimes undergo hibernation and aestivation repeatedly as they survive more than one year (e.g. THIELE, 1977). Diapause characteristics of these insects are complicated and have not been elucidated fully (THIELE, 1977; PAARMANN, 1979).

Leptocarabus kumagaii KIMURA et KOMIYA is one of such autumn-breeding carabids in the Kinki and Tokai districts of Honshu, Japan (SOTA, 1984, 1985 a, b). In previous experiments, I showed that larvae reared under long-day condition (16L–8D) developed directly into adults whereas under short-day condition (12L–12D) they did not pupate (SOTA, 1985 a). Thus in *L. kumagaii* a larval diapause may be induced by short-day conditions. Furthermore, the adult has a reproductive diapause during midsummer in lowland habitats (SOTA, 1984, 1985 a, b): the adult emergence occurs in June, but copulation and oviposition do not occur until September. With increasing altitude of the habitat, the adult emergence occurs later in the season, the length of aestivation

¹ This study was supported in part by a grant from the Nippon Life Insurance Foundation.

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period becomes shorter, and the reproduction starts earlier (SOTA, 1986). The altitudinal variation of seasonal cycle may be due to the direct effects of environmental factors such as temperature, or due to genetic difference in diapause characters (MASAKI, 1980).

This study aims at elucidating the effects of photoperiod and temperature on the larval development and egg production of *L. kumagaii*.

MATERIALS AND METHODS

Adult beetles of *L. kumagaii* used in this study were collected at the Botanical Garden of Kyoto University (alt. 60 m a.s.l.) in the Kyoto Basin and two sites of Mt. Kongo in Osaka, lower site (Mikumari; alt. 200 m) and upper site (near the mountain top; alt. 1,100 m) in 1984 and 1985. In the Kyoto Basin and the lower Mt. Kongo, newly-emerged adults appear from June to early July and reproduce from September to October. At the upper Mt. Kongo, new adults emerge from July and reproduce from August to October (SOTA, 1985 a, b, 1986).

Adults were caught with pitfall traps and reared in the laboratory. They were kept individually in plastic cups (12 cm in diameter; 10 cm in height) with a 2 cm deep soil layer for oviposition site, and minced beef and pieces of apple were supplied as food at 2–3 day intervals. Larvae were reared individually in plastic cups (8 cm in diameter; 5 cm in height) and fed on armyworms, *Pseudaletia separata*. Full-grown third-instar larvae were transferred into glass cylinders (6 cm in diameter; 10 cm in height) with a 5 cm deep soil layer in which they pupated.

To compare the development from egg to adult among the 3 populations, eggs were obtained from adults which were collected in late September and early October and allowed to oviposit under 12L–12D at 15°C. Larvae were kept under 16L–8D at 15°C, and the developmental period, pupal weight and adult body-length were recorded.

The effects of photoperiodic change and chilling on the larval development were studied with the Kyoto Basin population. Adults were collected in early October and allowed to oviposit under 12L–12D at 15°C. Eggs were collected every day and incubated at 15°C. After hatching, larvae were kept under 12L–12D at 15°C to induce diapause. Under this photoperiod, they did not make chambers for pupation in the soil, indicating that they were in diapause (SOTA, 1985 a). At the 10th day of the third instar, the larvae were divided into 2 groups. One group was transferred to 16L–8D or 14L–10D at 15°C. The other was chilled at 5°C and 12L–12D for 30, 65 or 90 days and then returned to 15°C and 12L–12D. After emergence, adults were kept under the same conditions as in the pupal stage for 50 days and then dissected to see the development of the gonads.

The effects of decreasing daylength on egg production were examined in the females from the lower and upper Mt. Kongo. Adults which had emerged in the laboratory and had been kept under 16L–8D at 15°C for 50 days were transferred to 14L–10D and 12L–12D. Eggs laid by the females were collected and counted every 5 days.

To examine the effects of temperature on egg production, newly-emerged adults collected in the Kyoto Basin in June were kept at 25°C under 16L–8D, conditions comparable to those in the field, for 20 days and then transferred to different temperatures of 15, 20 and 25°C under 13L–11D. Eggs were collected and counted every 5 days.

Table 1. Developmental period, pupal weight and adult body-length under 16L-8D at 15°C in *L. tumagaii* from three localities

Locality	N_1/N_2^a	Mean developmental period in days					Sex	Pupal weight, mg (mean $\pm$ SD)	Adult body-length, mm (mean $\pm$ SD)
		Egg	1st	2nd	3rd	pupa			
Kyoto Basin (alt. 60 m)	29/23	21.7	13.7	11.1	24.7	21.8	M	773.1 $\pm$ 52.3	32.4 $\pm$ 0.6
Lower Mt. Kongo (alt. 20 m)	34/24	21.4	15.3	9.5	26.9	24.0	F	761.6 $\pm$ 50.7	33.5 $\pm$ 0.8
Upper Mt. Kongo (alt. 1100 m)	35/22	20.9	15.3	10.5	27.0	23.8	M	760.5 $\pm$ 87.9	32.3 $\pm$ 1.1
							F	756.3 $\pm$ 82.1	33.1 $\pm$ 1.4
							M	724.7 $\pm$ 95.3	31.5 $\pm$ 0.9**
							F	735.3 $\pm$ 65.7	33.3 $\pm$ 0.9

^a N_1 : No. of 1st instar larvae reared, N_2 : No. of adults emerged.** Significantly different at $p < 0.05$ (*t*-test). Males and females were treated separately for pupal weight and adult body-length.

Table 2. Duration of 3rd instar and pupal periods in *L. kumagaii* from the Kyoto Basin population under changing photoperiod and temperature conditions

Group	Photoperiod and temperature after 10th day of 3rd instar period	N_1/N_2^a	3rd instar period in days (mean $\pm$ SD)	Pupal period in days (mean $\pm$ SD)
A	16L- 8D: 15°C	11/10	42.6 $\pm$ 4.4	23.4 $\pm$ 1.3
B	14L-10D: 15°C	10/9	44.5 $\pm$ 3.7	24.1 $\pm$ 0.4
C	12L-12D: 15°C	9/2	101.5 $\pm$ 6.4	24.0 $\pm$ 0.0
D	12L-12D: 5°C (30 days) $\rightarrow$ 15°C	10/8	84.4 $\pm$ 2.1	24.0 $\pm$ 0.0
E	12L-12D: 5°C (65 days) $\rightarrow$ 15°C	5/5	106.8 $\pm$ 7.8	23.8 $\pm$ 1.1
F	12L-12D: 5°C (90 days) $\rightarrow$ 15°C	15/10	124.6 $\pm$ 3.9	23.5 $\pm$ 1.1

^a N_1 : No. of 3rd instar larvae reared; N_2 : No. of adults emerged.

Larvae were kept under 12L-12D at 15°C from hatching until 10th day of the 3rd instar and then transferred to various rearing conditions.

RESULTS

Comparison of development among three populations

Table 1 shows the developmental period, pupal weight and adult body-length of laboratory offspring from the 3 localities. Their developmental parameters were similar although the mean developmental period was slightly shorter in the Kyoto Basin population than in the Mt. Kongo populations (*t*-test, $p < 0.05$). The mean pupal weight and adult body-length were not significantly different among populations (*t*-test, $p > 0.05$), except that the male body length was significantly shorter in the upper Mt. Kongo population than in other populations.

Effects of photoperiod and temperature on larval development

The developmental period under 12L-12D at 15°C was 14.3 ± 1.5 days for the 1st instar and 12.5 ± 2.0 for the 2nd instar ($N=60$; mean $\pm$ SD). For the 3rd instar, mortality was high in the larvae constantly kept under 12L-12D at 15°C (Table 2); only two larvae pupated after a considerably longer 3rd-instar period than under 16L-8D (Table 1).

Both change from short- to longer-daylength and chilling at 5°C resulted in pupation of most individuals (Table 2). The larvae transferred to 16L-8D or 14L-10D from 12L-12D pupated 30 days after the transfer. The mean length of their 3rd instar period was 43-45 days. The mean duration from the end of chilling to pupation decreased with increasing chilling period. The longest chilling of 90 days resulted in a low survivorship (67%). The length of pupal period was ca. 24 days in all groups.

Emerged beetles were kept under the same conditions as in the pupal stage and were dissected 50 days after emergence. No vitellogenesis occurred in the ovaries whereas testes contained many sperm bundles. As all beetles had well-developed fat bodies, they might have been in a reproductive diapause.

Effects of photoperiodic change on egg production

Figure 1 shows the oviposition patterns of females from the lower and upper Mt. Kongo populations, which were transferred from 16L-8D to 12L-12D or 14L-10D. Females transferred to 12L-12D started oviposition 10-25 days after the transfer and

Fig. 1. Oviposition patterns in *L. kumagaii* from the lower and upper Mt. Kongo populations at 15°C under 12L-12D (A) and 14L-10D (B) after the transfer from 16L-8D. Arrows indicate first oviposition by individual females and triangles death of individual females.

Fig. 2. Oviposition patterns in *L. kumagaii* from the Kyoto Basin population at 15, 20 and 25°C under 13L-11D after the transfer from 25°C, 16L-8D. Arrows indicate first oviposition by individual females and triangles death of individual females.

ceased it within 50 days. Thereafter, the beetles remained inactive until the end of the experiment. Most females transferred to 14L–10D began to oviposit 40–50 days after the transfer and continued oviposition as long as 150 days. Females laid more eggs (mean: 52–53) under 14L–10D than under 12L–12D (mean: 31–33). There was no substantial difference in ovipositional pattern between the two populations.

Effects of temperature on egg production

Figure 2 shows the oviposition pattern at different temperatures (15, 20, 25°C) under 13L–11D in females of the Kyoto Basin population after transfer from 16L–8D, 25°C. The females started oviposition 40 days after the transfer to 15 and 20°C and continued to oviposit for 80 days at maximum. The mean number of eggs laid by the females at 15°C was 51, about twice that at 20°C, whereas no eggs were laid at 25°C except a few laid by a single female after 65 days. However, some females which survived 60 days or longer had mature eggs in their ovaries.

DISCUSSION

Autumn-breeding carabids pass their first winter as larvae. Previous studies have shown that the larval development in some autumn-breeders is arrested at high temperatures, independent of photoperiod, and that cold is obligatory to complete it (thermic diapause: THIELE, 1969, 1977; THIELE and KREHAN, 1969; KREHAN, 1970; BRANDMAYR, 1983). The larval development of *L. kumagaii*, however, showed a long-day type photoperiodic response, and the larval diapause was completed either by an increased daylength at a constant temperature or by chilling under a constant photoperiod. Thus cold is not always necessary for *L. kumagaii*. It seems necessary to investigate the significance of low temperatures for various autumn-breeding carabid larvae (see also TAUBER and TAUBER, 1976; HODEK, 1983). In *L. kumagaii*, low temperature during winter may be effective for diapause maintenance, and the increasing daylength and temperature in spring may facilitate the post-diapause development. At higher altitudes the lower temperature early in the season seems to delay adult emergence.

L. kumagaii did not produce mature eggs when they were kept under constant photoperiodic conditions after emergence. The ovarian maturation of autumn-breeders so far studied showed long-day/short-day type responses (THIELE, 1969, 1977; PAARMANN, 1979). The present study on *L. kumagaii* is consistent with this. The ovarian maturation was influenced also by temperature: a high temperature (25°C) suppressed egg production whereas lower temperatures (15 and 20°C) promoted it (see also THIELE, 1969, 1977). As the mean temperature during midsummer is above 25°C at the lowland habitats of this species (Kyoto Basin and lower Mt. Kongo) but about 20°C at the upper Mt. Kongo (SOTA, 1986), such a difference in temperature seems to be related directly to the variation in the seasonal timing of reproduction at different altitudes. Thus the altitudinal variation of timing of reproduction may be mainly governed by the plasticity in gonad maturation in response to different temperature conditions, because there was no difference in oviposition pattern between the lower and upper Mt. Kongo populations in the laboratory. The plasticity in seasonal cycle of *L. kumagaii* may be of adaptive significance within its altitudinal range of distribution with variable seasonal conditions (SOTA, 1986).

Adults of *L. kumagaii* live for 2 or 3 years and reproduce repeatedly (SOTA, 1984).

The proportion of the old beetles in an adult population seemed to be higher at high altitudes than at low altitudes (SOTA, 1986). Adults which survived the first reproductive season enter hibernation and stay in the state of reproductive diapause until the following reproductive season. This reproductive diapause may be induced by certain short-day conditions during the course of reproductive period because the females transferred to 12L–12D re-entered reproductive diapause following a short period of egg production whereas those transferred to 14L–10D continued oviposition for a long time. Thus, photoperiodic control of the adult hibernation diapause in *L. kumagaii* needs further investigation.

ACKNOWLEDGEMENTS

I am grateful to Dr. A. TAKAFUJI for his critical reading of the manuscript. Thanks are also due to Drs. E. KUNO, T. INOUE and N. OHSAKI for their discussion and encouragement.

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