Diapause and Tolerance to Extreme Temperatures in Tropical, Subtropical and Temperate Populations of the Ladybird Beetle *Epilachna vigintioctopunctata*

Sih Kahono  Balai Penelitian dan Pengembangan Zoologi, Puslitbang Biologi, LIPI, Jl. Raya Bogor Jakarta Km 46, Cibinong 16911, Indonesia
Motoko Tokunaga  Zoological Institute, Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan
Masahito T. Kimura  Laboratory of Ecology and Genetics, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan
Koji Nakamura  Laboratory of Ecology, Faculty of Science, Kanazawa University, Kanazawa 920-1192, Japan
Haruo Katakura  Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan

**Abstract**  Diapause and tolerance to extreme temperatures were compared among tropical, subtropical and temperate populations of *Epilachna vigintioctopunctata* to understand how they adapt to environmental conditions. The tropical population had no diapause, while the temperate population entered reproductive diapause at a short daylength. Females of the tropical population matured fastest while those of the temperate population matured slowest. The tropical population was the least tolerant to both heat and cold, while the temperate population was the most tolerant to these stresses.

**Key words:** diapause / tolerance to heat and cold / *Epilachna*.

The phytophagous ladybird beetle *Epilachna vigintioctopunctata* (Fabricius) is thought to have no dormancy in aseasonal humid tropical regions, because all developmental stages are found throughout the year (Nakamura et al., 1988, 1990; Inoue et al., 1993). This species does, however, enter reproductive diapause in response to short daylengths to pass the winter in temperate regions (Kono, 1979, 1980, 1986). The first aim of this study is to ascertain this expectation by monitoring the seasonal change of reproductive conditions of females in a tropical population and also by checking its photoperiodic response.

The second aim is to compare heat and cold tolerance of tropical and temperate lowland populations of this species to understand how they adapt to environmental temperature conditions. Cold and heat tolerance has been well studied in temperate insects (Tauber et al., 1986; Lee & Denlinger, 1991; Leather et al., 1992), but little information has been gathered on the temperature tolerance of tropical insects. It is generally thought that tropical lowland insects are less tolerant to
cold but more tolerant to heat than temperate ones. However, Chen et al. (1992) and Kimura et al. (1994) observed that tropical species of Sarcophaga and Drosophila are not always more tolerant to heat than their temperate relatives. In addition, Kimura (1982, 1988) and Kimura et al. (1994) reported that drosophilid flies show no geographic variation in cold tolerance within species, although winter temperatures vary considerably in their distribution ranges. Thus, heat and cold tolerance of insects does not always reflect the temperature conditions of their environments.

MATERIALS AND METHODS

Epilachna vigintioctopunctata is widespread in warm temperate to tropical regions from Asia, through Oceania to Australia, and is notorious for the serious damage it causes to solanaceous crops and weeds (Dieke, 1947; Richards, 1983; Katakura et al., 1988; Shirai and Katakura, 1999). Recent studies, however, indicated that this nominal species in East and Southeast Asia comprises at least two biological species that differ in karyotypes, mitochondrial COI gene sequences, and crossability (Kobayashi et al., 2000). In the present material examined, beetles from Indonesia and those from Japan belonged to different biological species. Hence the present study should be regarded as an analysis of variation across two closely related forms, rather than an analysis of infraspecific variation, although we hereafter treat all the studied populations as a single species E. vigintioctopunctata.

Field study
Epilachna vigintioctopunctata was collected on solanaceous plants in and near Bogor, Indonesia (6°S in latitude) from January 1995 to May 1996, and females were examined for ovarian conditions. In the present study, females with ovaries with mature oocytes (stage 13 in Kono, 1980) were classified as mature, those lacking mature oocytes but with hardened elytra as immature, and those with soft elytra as teneral. Females were also examined for sperm storage (Katakura et al., 1994) to discover whether they had mated or not. Eight to 15 females (mean = 11.4) were dissected in each sampling (in total, 405 individuals were examined).

Laboratory experiments
Experimental stocks originated from tropical (Bogor, Java, Indonesia: BG, mean monthly temperature from 25.3 to 26.8°C), subtropical (Naha, the Ryukyus, Japan: NH, 26°N, average mean monthly temperature 22.4°C) and temperate areas (Hiroshima, Japan: HR, 34°N, 16.2°C). The BG stock was maintained for approximately one year on planted black nightshade (Solanum nigrum) under laboratory conditions (14 h light: 10 h dark at 26°C). The NH and HR stocks were collected from potato fields just before experiments. Parental beetles were fed on potato leaves at 25°C under a long daylength (15 h light: 9 h dark) and allowed to oviposit. Eggs on leaves were placed in culture cases and reared under a short (12 h light: 12 h dark) or long daylength (15 h light: 9 h dark) at 25°C. After eggs hatched, fresh leaves were supplied every two or three days.

Ovarian development was examined 16 days after adult eclosion. Ovaries with no sign of vitellogenesis were classified as undeveloped (stage 1 in Kono, 1980), those with developing oocytes as developing (stage 2-12), and those with mature oocytes as mature (stage 13).
Heat and cold tolerance were also examined at the 16-day adult stage. Beetles in culture cases were placed at 37 or 0°C without acclimation, and survival was monitored thereafter. At 37°C, fresh leaves were supplied everyday, but no leaves were supplied at 0°C. In experiments at 0°C, survival was checked after beetles were maintained 1 h at 25°C, because they often fell into a stupor. After the check, they were returned to 0°C.

**RESULTS AND DISCUSSION**

In and near Bogor, mature females were collected throughout the year (Fig. 1). In addition, females of the BG stock matured within 16 days after eclosion irrespective of the photoperiod (Table 1). Thus, the Bogor population of *E. vigintioctopunctata* did not have dormancy.

In the Japanese stocks (NH and HR), ovarian development was slightly retarded at a short daylength (Table 1). The difference in ovarian development was examined with the Mann-Whitney U test by giving ranks to ovarian stages: 1 (undeveloped), 2 (developing) and 3 (mature). In the HR stock, the effect of the photoperiod was significant (P<0.05), though it was not in the NH stock (P>0.05). Kono (1986) observed a more distinct photoperiodic response in a Kyoto (35°N) population of this species. The cause of this difference was not clear. It may be attributable to the difference in geographic origin of experimental stocks or rearing conditions (e.g., foods or moisture). Further study is needed to answer these questions.

Among the stocks studied, the BG stock was least tolerant to heat and cold and the HR stock was the most tolerant to these stresses (Table 2), and the difference among the three stocks was statistically...
Table 1. Ovarian conditions of females of three populations of *E. vigintioctopunctata* maintained for 16 days under short (12 h light: 12 h dark) and long (15 h light: 9 h dark) daylength conditions at 25°C. N, number of individuals examined; M, fraction of individuals with mature ovaries; D, that with developing ovaries; U, that with undeveloped ovaries.

<table>
<thead>
<tr>
<th>Population</th>
<th>Day length condition</th>
<th>12 L</th>
<th>15 L</th>
<th>12 L</th>
<th>15 L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>M</td>
<td>D</td>
<td>U</td>
</tr>
<tr>
<td>Bogor</td>
<td>12</td>
<td>1.00</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Okinawa</td>
<td>15</td>
<td>0.40</td>
<td>0.33</td>
<td>0.27</td>
<td>0</td>
</tr>
<tr>
<td>Hiroshima</td>
<td>16</td>
<td>0</td>
<td>0.69</td>
<td>0.31</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Survival time (days, mean with SD; sample size in parentheses) at heat (37°C) and cold (0°C) conditions of adult beetles of three populations of *E. vigintioctopunctata*. Results for males and females are pooled.

<table>
<thead>
<tr>
<th>Population</th>
<th>Rearing condition</th>
<th>12L</th>
<th>15L</th>
<th>12L</th>
<th>15L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>heat</td>
<td>cold</td>
<td>heat</td>
<td>cold</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12L</td>
<td>15L</td>
<td>12L</td>
<td>15L</td>
</tr>
<tr>
<td>Bogor</td>
<td>0.50±0.00 (28)</td>
<td>0.86±0.49 (28) **</td>
<td>3.52±4.62 (23)</td>
<td>2.40±1.61 (32)</td>
<td></td>
</tr>
<tr>
<td>Okinawa</td>
<td>1.40±0.50 (25)</td>
<td>1.48±0.47 (20)</td>
<td>11.43±8.95 (29)</td>
<td>6.06±3.01 (25)</td>
<td></td>
</tr>
<tr>
<td>Hiroshima</td>
<td>2.60±0.74 (29)</td>
<td>2.19±0.76 (26) *</td>
<td>18.10±5.66 (33)**</td>
<td>9.18±5.66 (33)**</td>
<td></td>
</tr>
</tbody>
</table>

*: **: The difference between short (12L) and long (15L) day conditions are statistically significant at 5 and 1 %, respectively (Mann-Whitney U test).

significant in all the four rearing conditions (Kruskal-Wallis test, P<0.01). It is noteworthy that the tropical population was less tolerant to heat than the temperate population. Chen *et al.* (1992) and Kimura *et al.* (1994) observed that temperate insects are often more tolerant to heat and cold than tropical relatives. The temperature encountered in summer in the temperate regions is not very different from that encountered in the tropical regions, and this is likely to be reflected in the temperature tolerance of insects from these regions.

It appeared that individuals reared at a short daylength were significantly (Mann-Whitney U test, P<0.05) more tolerant to cold and heat than those reared at a long daylength in the HR stock (Table 2). It is well known that diapausing insects are more tolerant to environmental stresses than nondiapausing ones (Taubert *et al.*, 1986). On the other hand, no or inverse effects of photoperiods were observed in the tropical (BG) and subtropical (NH) stock.

At the 16 day adult stage, the percentage of females with matured ovaries was highest in BG but lowest in HR even under long-day conditions (Table 1), suggesting that BG females developed ovaries fastest and HR ones the slowest. Thus, the rate of ovarian development seemed to be negatively correlated with tolerance to extreme temperatures among the stocks studied, although the correlation was not statistically significant. It is not known whether or not these two traits, ovarian development and temperature tolerance, are connected by trade-off, but it is possible that tropical populations mature rapidly at the expense of tolerance to heat and cold. Kimura (1982, 1988) observed in several species of *Drosophila* that cold tolerance does not vary geographically within the species in spite of considerable variation in winter temperature in their distribution ranges and considered that a trade-off between cold tolerance and some other adaptive traits may have prevented the development of geographic variation in cold tolerance.
Diapause and tolerance to extreme temperatures of the ladybird beetle

ACKNOWLEDGEMENTS We wish to express our sincere thanks to the following persons: Dr. S. Wirjoatmodjo and Dr. A. Budiman (former and present Directors of Puslitbang Biologi, LIPI) for their encouragement and giving S. Kahono an opportunity to study this subject; Dr. Yoichi Shirai (National Agro-Environmental Science) who allowed us to use facilities in his laboratory; Dr. Takayoshi Nishida (Kyoto University), Dr. Masako Yafuso (University of the Ryukyus) and Dr. Susumu Nakano (Hiroshima Shudo University) who kindly collected beetle stocks used in the present study. The present study was carried out with the permission of LIPI, and received import permission of Yokohama Plant Protect Station of the Ministry of Agriculture, Forestry and Fisheries (No. 5Y2866), and was financially supported by the International Scientific Research Program of the Ministry of Education, Science, Sports and Culture of Japan (Nos. 05041086, 08041141).

REFERENCES


Received Dec. 25, 1998
Accepted Jan. 12, 1999

Sih Kahono, 徳永元子, 木村正人, 中村浩二, 片倉晴雄 ニジュウヤホシテン
トウの熱帯, 亜熱帯, 温帯個体群の休眠と温度抵抗性

ニジュウヤホシテントウの熱帯, 亜熱帯, 温帯産個体群を用いて, 休眠および高・低温ストレスに対する抵抗性を比較した。熱帯個体群は休眠せず, 温帯個体群は短日条件下で生殖休眠に入った。また, 熱帯個体群の雌はもっとも短時間で成熟し, 温帯個体群の雌は成熟にもっとも時間をかかった。さらに, 熱帯個体群は高温, 低温に対する抵抗性がもっとも強く, 温帯個体群はこれらのストレスにもっとも強くかった。
