



Seasonal and geographical adaptations in the parthenogenetic stick insect, *Ramulus mikado* (Phasmatodea: Phasmatidae)*

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Abstract. Seasonal and geographical adaptations in terms of obligatory embryonic diapause in the parthenogenetic stick insect, *Ramulus mikado*, were studied. First and second instar nymphs were collected at locations at three latitudes in Japan and reared in the laboratory under a photoperiod of 16L : 8D or 12L : 12D at 25°C. Their eggs were kept at 30°C for 30 or 60 days after oviposition, but no eggs hatched. Hatching was observed more than 100 days after transfer from 30°C to 15°C. The long period between transfer and hatching indicate that eggs in an early embryonic stage of development enter diapause at high-temperatures. The time from oviposition to hatching of eggs laid by adults that originated from the three locations kept under constant conditions between 15 and 25°C were compared. In all these experiments, eggs laid by individuals originating from high latitudes took longer to hatch. The eggs of those originating from Okayama and Ehime did not hatch at 25°C. However, more than 80% of the eggs of those that originated from the northernmost population hatched. Hatching before winter was observed when the eggs of those that originated from the northern population were placed outdoors in Okayama, even when the maternal insects were reared under long-day conditions in the laboratory. These findings indicate that univoltine *R. mikado* enters diapause twice during embryonic development, which enables it survive adverse conditions in summer and winter, respectively. Furthermore, diapause intensity was lowest in insects that originated from the lowest latitude.

INTRODUCTION

Ramulus mikado (= *Baculum irregulariterdentatum*) is a parthenogenetic stick insect that occurs in East Asia. In Japan, *R. mikado* is found on Honshu, Shikoku and Kyushu islands (Okada, 1999). Recently, there have been outbreaks of this stick insect in Japan (Yano et al., 2021) and Korea (Lee et al., 2013, 2018).

Ramulus mikado is univoltine with obligatory embryonic diapause. Eggs are laid mainly in summer, after which individuals pass winter as pharate first-instar nymphs. At 20 and 15°C in the laboratory, most eggs hatch several months after oviposition, whereas at 25°C, many eggs remain in diapause (Yamaguchi & Nakamura, 2015). Moreover, eggs laid by females under a short-day photoperiod hatch significantly earlier than those laid by long-day females. Consequently, both maternal regulation of diapause intensity and rate of diapause development play important roles in maintaining and terminating diapause in *R. mikado* (Yamaguchi & Nakamura, 2015).

In several other stick insects, embryonic diapause also enables them to survive in periods of a year when con-

ditions are unfavourable (Bedford, 1978). In fact, some spend over a year as eggs (Bedford, 1978). For example, in *Didymuria violescens*, an Australian stick insect, eggs overwinter twice with a first and second diapause, which are terminated, respectively, in the first and second spring (Bedford, 1970; Readshaw & Bedford, 1971). In *Extatosoma tiaratum*, another Australian stick insect, diapause has been shown to be induced both during early embryonic development and again in the pharate first-instar nymph (Yoder & Denlinger, 1992).

In *R. mikado*, Yamaguchi & Nakamura (2015) suggest it is possible that there are two periods of diapause during embryonic development. However, the first diapause, which is predicted to occur in early embryonic development, has not been demonstrated experimentally in this species. In addition, the seasonal regulation of the univoltine life cycle in *R. mikado* differs from that in the tropical species described above, which overwinter twice within eggs. Consequently, the ecological significance of the first diapause in *R. mikado* is unclear, even if it exists. For this study, we assume that the first diapause is induced at an

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early developmental stage in order to survive summer, when eggs are laid early in the season. The first aim of this study is to show experimentally the existence of two periods of diapause in *R. mikado*.

Usually within a species, insects enter diapause prior to overwintering earlier at high than low latitudes. Therefore, diapause is induced under longer daylengths or at higher temperatures at high latitudes (Danilevsky, 1961). In addition, the initial intensity of diapause shows clear geographic trends (Masaki, 1999, 2002). In univoltine insects that enter diapause and overwinter, diapause intensity is often stronger in insects at low than at high latitudes. For example, there is a latitudinal cline in the intensity of embryonic diapause in the cricket, *Teleogryllus emma* in the Japanese Islands, decreasing from south to north (Masaki, 1965). If eggs laid by females of the southern populations terminate diapause in response to warm weather in autumn, then hatchlings would be killed by cold winter temperatures. Consequently, natural selection for more intense diapause is greater at low latitudes (Masaki, 1999, 2002). In summer diapause, the diapause intensity is often proportional to the length of the summer in the area from which they originated (Masaki, 1956; Grüner & Sauer, 1988; Ueda, 1978; Topp, 1990). Consequently, diapause is more intense in populations at low latitudes.

Parthenogenesis is frequently reported in phasmids. Some species are only parthenogenetic in part of their distribution. For example, *Phraortes illepidus* is unisexual in eastern and northern Japan, but bisexual in southwestern Japan (Nozaki et al., 2021). By contrast, *R. mikado* is unisexual through its distribution and males are very rare (e.g., Yano et al., 2021). It is likely that there is little geographical genetic variation in dormancy and unlikely that there is geographic variation in the intensity of embryonic diapause in clonal *R. mikado*. However, because of the well-regulated egg period in this species it is likely that diapause intensity will vary geographically. Eggs laid by females of the Okayama population kept under a short-day photoperiod of 12L : 12D hatch before winter when they are placed outdoors in early summer (Yamaguchi & Nakamura, 2015). Even when eggs that are laid by females under a long-day photoperiod of 16L : 8D are placed outdoors in June, a few hatched before winter. If the intensity of diapause in insects of the Okayama and southern populations is similar, then many eggs of the southern populations might hatch before winter because of their long warm autumns. Therefore, we assume that *R. mikado* is adapted to the local climate at each of the geographical locations studied. The second purpose of the present study is to determine whether there is geographic variation in embryonic diapause in parthenogenetic *R. mikado*.

This species was collected at three locations at different latitudes in Japan. We first exposed eggs at an early embryonic stage in their development to high temperature in order to determine whether summer diapause is induced. Specifically, eggs soon after oviposition were kept at 30°C for 1 or 2 months. Then they were transferred to 15°C. Summer diapause, which occurs under conditions

of high temperature and long daylength, is usually terminated by the lower temperatures and shorter day lengths experienced in autumn (Masaki, 1980). Consequently, the experimental transfer from 30°C to 15°C simulates the seasonal change in temperature from summer to autumn. If insects enter diapause only once as pharate first-instar nymphs, then exposure to high temperatures at early embryonic stages can enhance temperature-dependent pre-diapause development and shorten the time spent in the egg stage. In contrast, if summer diapause is also induced at early embryonic stages, then exposure to high temperature may prevent embryos from developing and thus prolong the period spent in the egg stage. Next, we kept eggs under constant moderate temperatures. The time spent as an egg and percentage that hatched recorded for three locations were compared in order to determine whether the intensity of embryonic diapause in *R. mikado* varies geographically. Some eggs were kept outdoors in Okayama. Then the time of hatching recorded for each the different locations was compared. Seasonal and geographical adaptations of the life cycle in *R. mikado* were inferred from the results.

MATERIALS AND METHODS

Collection and rearing of insects

First or second instar nymphs of *R. mikado* were collected in April and May 2011 in Takaoka city (36.7°N, 137.0°E) in Toyama prefecture, Okayama city (34.7°N, 133.9°E) in Okayama prefecture and Shikokuchuo city (33.9°N, 133.6°E) in Ehime prefecture, Japan. Insects were reared under a long-day (16L : 8D) or short-day (12L : 12D) photoperiods at 25 ± 1°C in groups of about 10 individuals in plastic containers (35 × 30 × 38 cm height). Both nymphs and adults were supplied with fresh leaves of cherry, *Prunus yedoensis*. After adult emergence, eggs were collected every other day. Groups of fewer than 100 eggs were placed on wet filter paper in petri dishes (90 mm diam.) and were used in the experiments. Water was supplied ad libitum via damp filter paper.

Effects of high-temperature on hatching

Within 48 h of oviposition, eggs were transferred to 30 ± 1°C under a 12L : 12D photoperiod. Sixty or 30 days later, they were transferred to 15 ± 1°C and the same photoperiod. As a control, some eggs were placed directly at 15°C and same photoperiod within 48 h of oviposition. The number of eggs hatching were recorded every other day. The experiments were discontinued 200 days after the transfer to 15°C.

Effects of constant temperature and photoperiod on hatching

Eggs within 48 h of oviposition were kept under 12L : 12D at 25 ± 1, 20 ± 1 or 15 ± 1°C. Every other day, the number of eggs that hatched were recorded until 200 days after oviposition, when the experiments were discontinued.

Hatching under outdoor conditions

Eggs within 48 h of oviposition were kept outdoors on the campus of Okayama University of Science (34.7°N, 133.9°E) between June 27 and October 10, June 20 and September 26 and July 4 and October 19, 2011, respectively, for those that originated from Toyama, Okayama and Ehime. The number of eggs that hatched were recorded every other day. The experiments were discontinued on June 14, 2012.

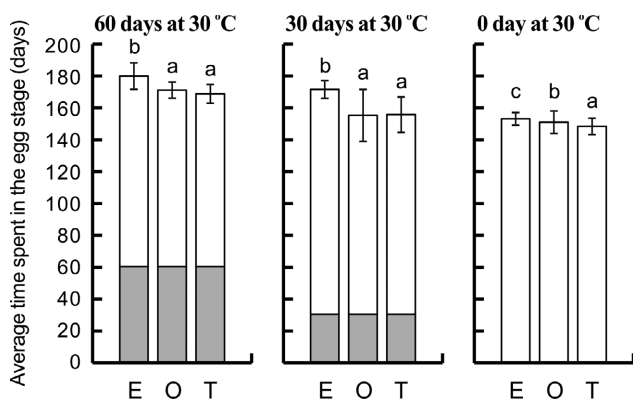


Fig. 1. Effects of high temperature on the time spent in the egg stage recorded for females of *R. mikado* from three geographic locations. Shaded areas are times spent at 30°C. Same letters above error bars denote no significant difference among geographical locations for each experimental condition (ANOVA/Tukey’s test, $p > 0.05$). E – Ehime; O – Okayama; T – Toyama.

Statistical analysis

Two-way ANOVA was used to compare the time spent in the egg stage in the different experimental treatments. For post-hoc tests, Ryan’s method was used. These statistical analyses were executed using ANOVA 4, available on the web (<http://www.hju.ac.jp/~kiriki/anova4/about.html>). Percentage hatching was arcsine transformed and analysed using ANOVA/Tukey’s test, inferring significance for $P \leq 0.05$ (Zar, 2010).

RESULTS

Effects of exposure to high temperature at an early stage in embryonic development

When eggs within 48 h of oviposition were kept at 30°C, none hatched within 60 or 30 days in the high-temperature treatment. Hatching was recorded more than 100 days after transfer to 15°C. Average period spent in egg stage, including the period of exposure to 30°C, was more than 150 days (Fig. 1). Two-way ANOVA (Table 1) revealed significant effects of the period at 30°C and geographical location on the total egg period ($p < 0.001$). A significant interaction between the period at 30°C and geographical location was also recorded ($p < 0.001$). Analyses of simple main effects showed a positive association between the length of the period at 30°C and length of the total egg period ($p < 0.001$). The egg period recorded for the Okayama individuals was significantly shorter than that for those from Ehime ($p < 0.001$). Furthermore, the egg period for those from Toyama was significantly shorter than those from Okayama ($p < 0.01$) or Ehime ($p < 0.001$).

Table 1. Results of two-way ANOVA of the effects of time exposed to 30°C and geographical location on the total time spent as an egg in *Ramulus mikado*.

| Sources of variation | df | MS | F | P |
|-----------------------------|----|----------|-------|------------|
| Period at 30°C | 2 | 29929.13 | 561.3 | <0.001 *** |
| Geographical location | 2 | 7972.41 | 149.5 | <0.001 *** |
| Period at 30°C × Population | 4 | 1124.75 | 21.1 | <0.001 *** |

*** $P < 0.001$

For each experiment, egg periods were compared among the three locations. In the experimental treatment with a 60-day exposure to 30°C, significant difference was found in the total egg period between those from Ehime and both of the northern locations ($p < 0.05$) (Fig. 1). No significant difference was found in the egg periods of those from Toyama and Okayama ($p > 0.05$). Furthermore, in the experimental treatment with a 30-day exposure to 30°C, the egg period for those from Ehime was significantly longer than that of either of the southern populations ($p < 0.05$). When eggs were placed directly at 15°C, no significant differences were found between any of the populations ($p < 0.05$).

Effects of constant temperature on hatching

The effects of geographical location and maternal photoperiod on egg period were analysed at constant temperatures (Table 2, Fig. 2). At 25°C, significant effects of geographical location and maternal photoperiod on egg period ($p < 0.001$) were found using two-way ANOVA. A significant interaction between geographical location and maternal photoperiod was also found ($p < 0.001$). Analyses of simple main effects showed that the egg period for those from Toyama was significantly shorter than that of those from either Okayama or Ehime ($p < 0.001$). In contrast, there was no significant difference between the southern populations ($p > 0.05$). Analyses of simple main effects also showed that the egg period under 16L : 8D was significantly longer than under 12L : 12D ($p < 0.001$).

At 20°C, significant effects of geographical location and maternal photoperiod on egg period ($p < 0.001$) were found using two-way ANOVA. Significant interaction between geographical location and maternal photoperiod was also recorded ($p < 0.001$). Analyses of simple main effects showed that the egg period was significantly different between any combination of two geographical locations ($p < 0.001$). Specifically, shorter egg periods were recorded for the high latitude locations. In addition, the egg period under 16L : 8D was significantly longer than that under 12L : 12D ($p < 0.001$).

Table 2. Results of two-way ANOVA of the effects of geographical location and maternal photoperiod on the time spent as an egg in *Ramulus mikado*.

| a) 25°C | | | | |
|--------------------------|----|-----------|----------|------------|
| Sources of variation | df | MS | F | P |
| Geographical location | 2 | 2403.76 | 75.102 | <0.001 *** |
| Maternal photoperiod | 1 | 13578.29 | 424.232 | <0.001 *** |
| Population × Photoperiod | 2 | 428.71 | 13.394 | <0.001 *** |
| b) 20°C | | | | |
| Sources of variation | df | MS | F | P |
| Geographical location | 2 | 116106.29 | 4150.002 | <0.001 *** |
| Maternal photoperiod | 1 | 21981.99 | 785.705 | <0.001 *** |
| Population × Photoperiod | 2 | 1054.57 | 37.694 | <0.001 *** |
| c) 15°C | | | | |
| Sources of variation | df | MS | F | P |
| Geographical location | 2 | 2455.6 | 64.428 | <0.001 *** |
| Maternal photoperiod | 1 | 34.8 | 0.913 | 0.339 |
| Population × Photoperiod | 2 | 382.4 | 10.033 | <0.001 *** |

*** $P < 0.001$

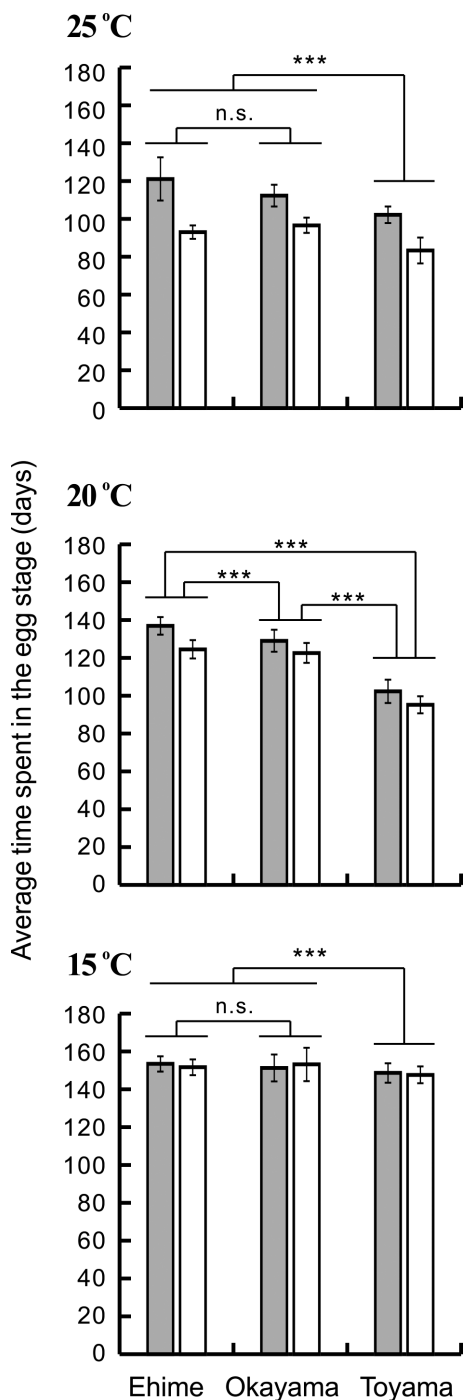


Fig. 2. Effects of temperature and maternal photoperiod on the average time spent in the egg stage recorded for *R. mikado* from three geographic locations. Shaded columns – 16L:8D; open columns – 12L:12D; *** – significant difference between geographical locations (two-way ANOVA, $p < 0.001$).

At 15°C, a significant effect of geographical location ($p < 0.001$) was recorded using two-way ANOVA, but no significant effect of maternal photoperiod ($p > 0.05$). Significant interaction between geographical location and maternal photoperiod was also recorded ($p < 0.001$). The egg period for those from Toyama was significantly shorter than that of either of the southern populations ($p < 0.001$).

Table 3. Effect of temperature and maternal photoperiod on the percentage of eggs of *R. mikado* that hatched.

| Maternal photoperiod | 16L:8D | | 12L:12D | |
|----------------------|----------------|-------------------------|----------------|-------------------------|
| | Number of eggs | Percentage that hatched | Number of eggs | Percentage that hatched |
| 25°C | | | | |
| Toyama | 198 | 88.9a | 192 | 86.5a |
| Okayama | 277 | 4.0b | 402 | 9.0b |
| Ehime | 226 | 4.0b | 209 | 10.0b |
| 20°C | | | | |
| Toyama | 190 | 89.5a | 188 | 85.1a |
| Okayama | 308 | 80.5a | 414 | 83.6a |
| Ehime | 243 | 83.5a | 212 | 70.8b |
| 15°C | | | | |
| Toyama | 207 | 87.9ab | 203 | 79.8bc |
| Okayama | 314 | 75.2cd | 429 | 66.4d |
| Ehime | 260 | 90.8a | 231 | 80.1bc |

Same letters indicate no significant differences among geographical locations at each temperature (ANOVA/Tukey's test, $p > 0.05$).

However, no significant difference was found between those from Okayama and Ehime ($p > 0.05$).

Effects of geographical location and maternal photoperiod on percentage hatching were also recorded (Table 3). For each experimental condition, eggs laid by more than 10 females were used in the experiments. When eggs of those from Toyama were kept at a constant temperature of 25°C, 88.9 and 86.5% of the eggs laid by females under 16L:8D and 12L:12D photoperiods hatched, respectively. In contrast, only 4.0–10.0% of the eggs of those from Okayama and Ehime hatched. The percentage hatching for those from Toyama was significantly higher than that of either of the southern populations (ANOVA/Tukey's test, $p < 0.05$). No significant difference was found between those from Okayama and Ehime ($p > 0.05$). When compared for each geographical location, no significant difference in the percentage hatching was recorded for the different maternal photoperiods ($p > 0.05$).

At 20°C, more than 70% of the eggs hatched, irrespective of geographic location and photoperiod. For the eggs laid by females under 16L:8D, no significant differences were recorded between the three locations ($p > 0.05$). For the eggs laid by females under 12L:12D, the percentage hatching for Ehime was lower than for either Toyama or Okayama ($p < 0.05$). When the percentage hatching was compared in terms of the maternal photoperiod, the only significant difference was recorded for Ehime.

At 15°C, 66.4–90.8% of the eggs hatched. Under both maternal photoperiods, the percentage hatching for Okayama was lower than for either Toyama or Ehime ($p < 0.05$). When the percentage hatching was compared in terms of the maternal photoperiod, the only significant difference was recorded for Ehime ($p < 0.05$).

Hatching under outdoor conditions

Eggs laid by the Toyama females under 16L:8D were placed outdoors from June 27 to October 10, 2011 (Fig. 3A). Of the eggs placed outdoors in late June, more than 85% hatched in that year (2011) and only a few eggs hatched the following year (2012). Of those eggs placed

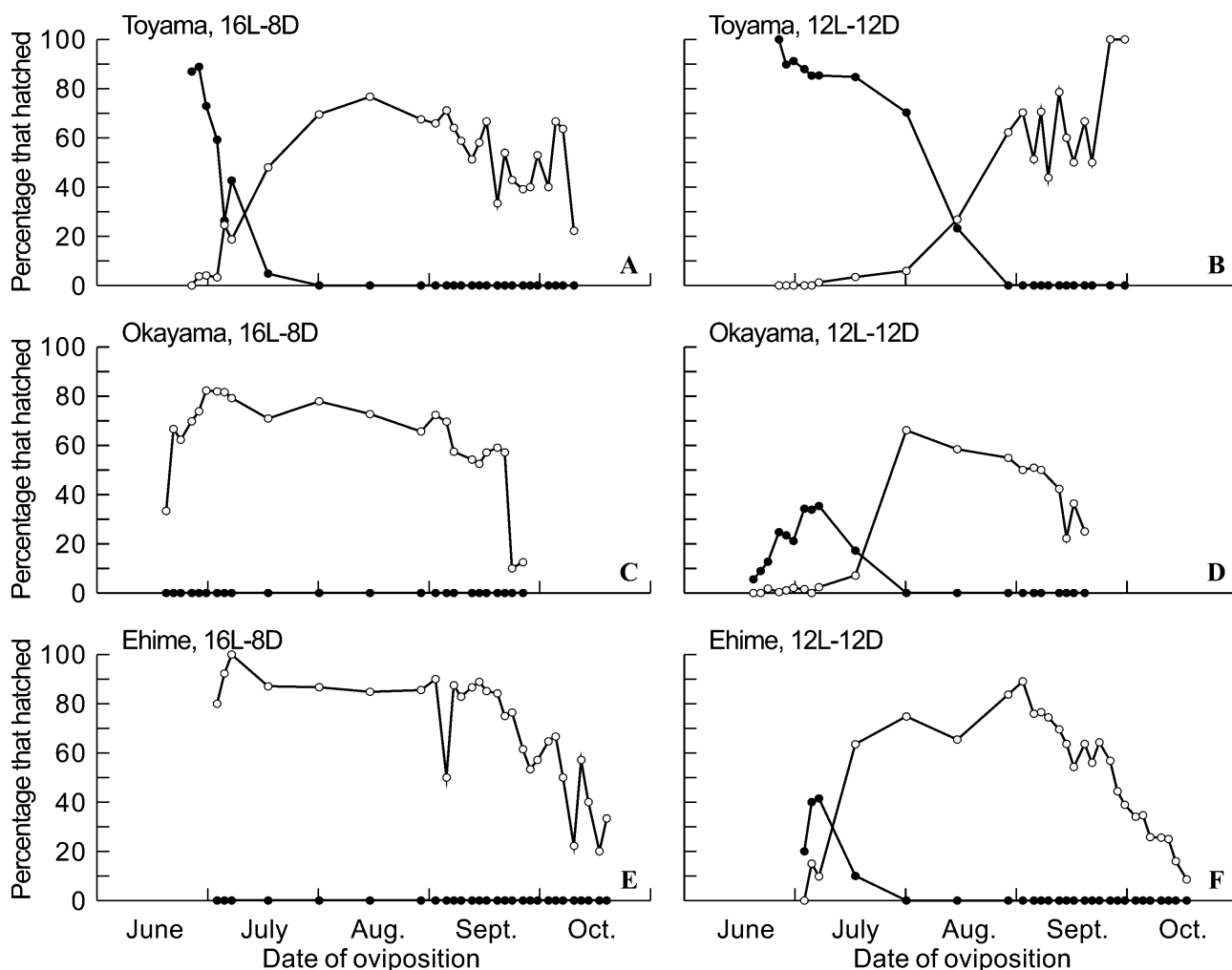


Fig. 3. Effects of maternal photoperiod and time of oviposition on the percentage that hatched recorded for *R. mikado* from three geographic locations. Eggs were placed outdoors in Okayama. Solid circles – eggs hatched in the year of oviposition (2011); open circles – eggs hatched the following year (2012).

outside in July, much lower percentages hatched in 2011 and were lower the latter they were placed outside. In contrast, the percentage that hatched in 2012 increased. For the eggs placed outside in August and later, hatching was only recorded in 2012. The median dates for hatching was late November when eggs were placed outdoors in late June (Fig. 4A), mainly December for those placed outside in early July and next spring for those put outside in mid-July and later. Eggs placed outside from August to mid-September hatched synchronously in mid-April. Thereafter, later dates of placing eggs outside are associated with later dates of hatching. These results indicate that the duration of diapause is not sufficiently long for preventing the Toyama eggs hatching before winter under the warmer climate conditions at low latitudes.

Hatching before winter was recorded also for eggs laid by Toyama females under 12L : 12D (Figs 3B, 4B). More than 80% of the eggs placed outdoors in June and July hatched in 2011. For the eggs placed outdoors on August 15, the percentage hatching in 2011 was almost identical

with that in 2012. Hatching was recorded only in 2012 for eggs placed outdoors in late August and later.

Eggs laid by Okayama long-day females did not hatch in 2011, irrespective of when they were placed outside (Fig. 3C). Synchronous hatching was recorded in April (Fig. 4C). Eggs laid by short-day females hatched in 2011 (Figs 3D, 4D). For eggs placed outside between June 20 and July 18, the percentage hatching in 2011 was greater than in 2012. Thereafter, the percentage hatching in 2011 decreased and none of those placed outside in August and September hatched in 2011.

Hatching of the eggs of Ehime females (Figs 3E–F, 4E–F) was similar to that of Okayama females. Specifically, eggs laid by long-day females did not hatch in 2011, but many of those laid by short-day females and placed outdoors in July hatched in 2011. The relation between the date of oviposition and hatching differed slightly from that recorded for Okayama females. Specifically, eggs laid by short-day Ehime females on July 18, the percentage hatching in 2012 was 63.5% (Fig. 3F), whereas for those of

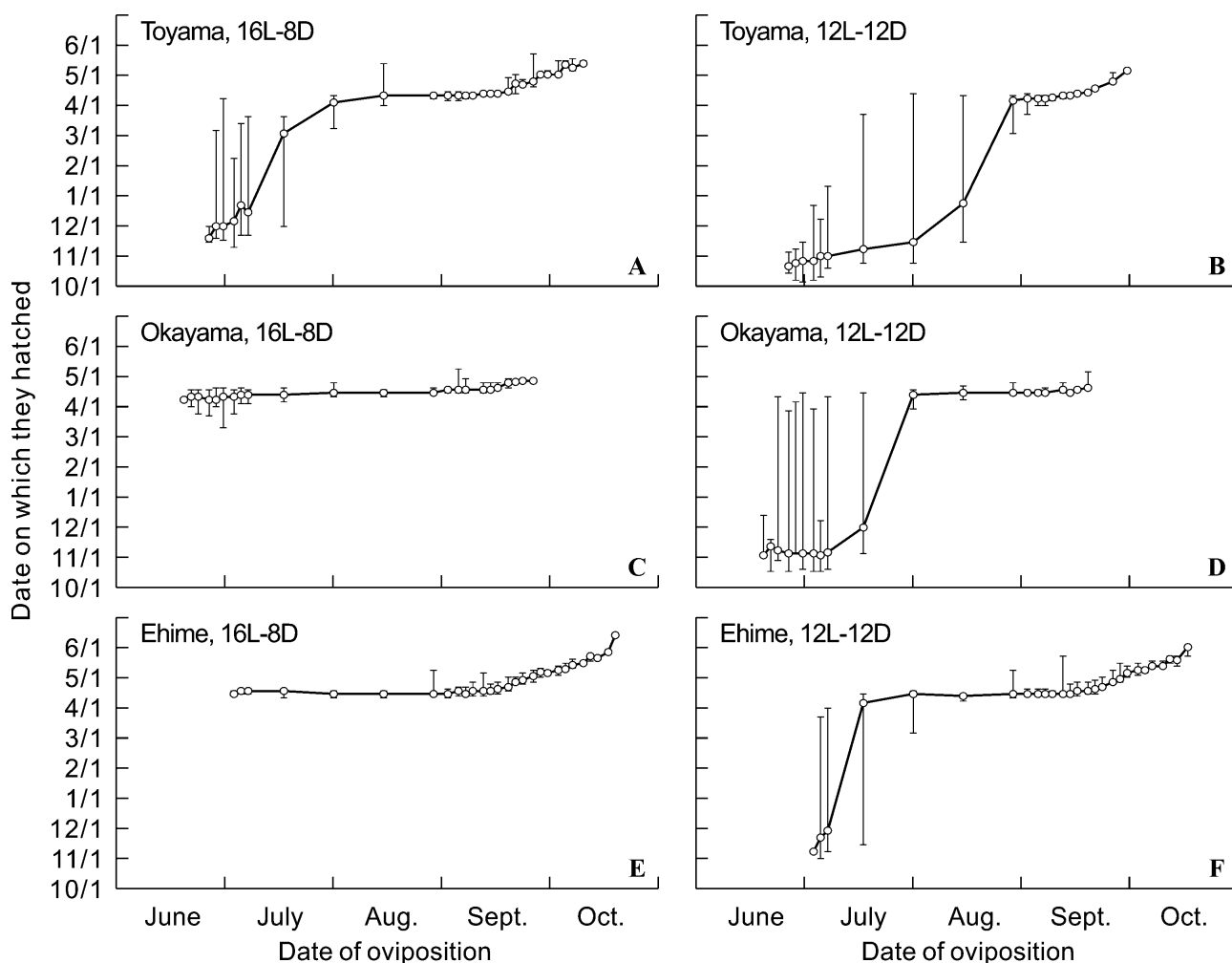


Fig. 4. Effects of maternal photoperiod and time of oviposition on the median date on which they hatched recorded for *R. mikado* from three geographic locations. Eggs were placed outdoors in Okayama. Vertical bars indicate the range in the dates on which they hatched.

Okayama females laid on the same date it was only 7.1% (Fig. 3D).

DISCUSSION

Possibility of two periods of diapause during embryonic development

Yamaguchi & Nakamura (2015) report that eggs of *R. mikado*, which are exposed to a low temperature of 5°C take longer to hatch than those exposed to 10°C. Based on that result, it is likely that there are two periods of diapause during the course of embryonic development. Specifically, even if the first diapause is terminated by 5°C, there is no development before the second diapause at this temperature (Yamaguchi & Nakamura, 2015). However, this experiment did not confirm the existence of the first diapause.

In the present study, we kept eggs at 30°C and then transferred them to 15°C. If insects enter diapause only once as pharate first-instar nymphs, then the exposure to high temperatures should enhance the pre-diapause development and shorten the period spent in the egg stage. However, a longer period at 30°C is associated with a longer period in the egg stage (Fig. 1). After a 60-day exposure to 30°C eggs still needed more than 100 days at 15°C for hatching.

The long egg period after the transfer to 15°C indicate that eggs at transfer were still in an early developmental stage. Consequently, the results reported here support the idea that during the egg stage there two periods when *R. mikado* can undergo diapause. Specifically, the first diapause occurs in an early embryonic stage and the second in pharate first-instar nymphs.

The occurrence of two periods of diapause in the egg stage is also reported for other phasmids (Bedford, 1970; Readshaw & Bedford, 1971; Yoder & Denlinger, 1992). In *E. tiaratum*, diapause is induced in early embryonic development and in the pharate first-instar nymph (Yoder & Denlinger, 1992). Based on the results of the present study, it is also likely that there are two periods when *R. mikado* can undergo diapause, as discussed above. However, the role of the first diapause in univoltine *R. mikado* differs from that in *E. tiaratum*, which overwinters twice as an egg.

In the almond seed wasp, *Eurytoma amygdali*, the environmental control of two periods of diapause in fully grown larvae differs in that the first diapause is terminated by the temperatures 16–19°C, whereas a higher temperature of 26°C delays diapause development (Tzanakakis &

Veerman, 1994; Margaritopoulos & Tzanakakis, 2006). Consequently, the first diapause of *E. amygdali* occurs during summer. Results of the present study indicate that, also in *R. mikado*, the first diapause is induced by a high temperature and terminated by transfer to a lower temperature (Fig. 1). Therefore, the temperature regulation of the first phase of embryonic diapause in *R. mikado* in nature is similar to that of the larval diapause in *E. amygdali*. The first diapause in *R. mikado* is similar to summer diapause.

When *R. mikado* eggs were exposed for 30 days to 30°C, egg stage at 15°C lasted 121–126 days. After exposure for 60 days to 30°C it was 109–120 days (Fig. 1). Consequently, the longer exposure to 30°C slightly shortened the egg stage at 15°C. During the course of diapause, a physiological process called ‘diapause development’ occurs, which results in the termination of diapause (Andrewartha, 1952; Hodek & Hodková, 1988; Hodek, 1996). The results of the present study indicate that diapause development during the first diapause in *R. mikado* eggs progresses even at 30°C, although the rate of diapause development is not high at this temperature.

Geographic variation in egg diapause

The present study revealed geographical variation in the egg diapause recorded in *R. mikado*. When eggs were kept under constant temperatures, the length of the egg stage differed for the different locations (Table 2, Fig. 2). Specifically, at the high latitude locations the egg stage was shorter. Similar geographical variation was also recorded when eggs were transferred from 30 to 15°C (Table 1, Fig. 1). Geographical variation in diapause intensity was revealed by comparing, under controlled experimental conditions, strains of insects collected from localities with widely different climatic conditions (Masaki, 2002). Consequently, differences in the length of the egg stage reflect differences in diapause intensity at different locations. Results of this study indicate that *R. mikado* from high-latitude locations undergo a less intense diapause.

The degree of geographical variation in embryonic diapause recorded for *R. mikado* is readily apparent from the results of keeping them outdoors in Okayama (Figs 3, 4). Eggs laid by long-day Okayama females hatched the next spring, whereas most of those laid by long-day Toyama females hatched before winter. This indicates the eggs laid by the Toyama females are adapted to cool northern conditions and enter a less intense diapause.

For eggs laid by short-day females, hatching in the year of oviposition was recorded for all three geographical locations (Figs 3, 4). However, the time of hatching in the year following oviposition occurred earlier for those laid by individuals from the more southern locations. This confirms that diapause intensity is lower in insects from northern locations than southern locations.

In many insects in the Northern Hemisphere, diapause intensity is greater in the warmer south than the cooler north, which prevents the untimely termination of diapause before winter (Masaki, 1999, 2002). In Toyama, which has the lowest temperatures of the three locations, insects start

oviposition later than at either of the southern locations. Furthermore, winter arrives earlier there than in either of the southern locations. Consequently, it is adaptive for the Toyama *R. mikado* to evolve a less intense egg diapause. Similarly, Ehime insects might have adapted to a warm climate. The length of the egg stage recorded for Ehime females is longer than that of those from the northern locations (Figs 1, 2). The long egg periods recorded for the Ehime females might prevent untimely hatching before winter and ensure the emergence of nymphs in warmer conditions the following spring.

Yamaguchi & Nakamura (2015) report that eggs of Okayama *R. mikado* remain in diapause for more than 200 days at 25°C. Furthermore, the present study revealed that most eggs of Okayama and Ehime females did not hatch at 25°C (Table 3). However, eggs of Toyama females successfully hatched during the experimental period. Consequently, diapause in eggs of Toyama females can be terminated by a moderate temperature of 25°C, whereas those of females from southern locations might require temperatures lower than 25°C for termination. Temperatures of a certain range are necessary for terminating winter diapause. In the false melon beetle, *Atrachya menetriesi*, the optimal temperatures for terminating egg diapause are between 5 and 10°C, although diapause development proceeds at low temperatures of 1–16°C (Ando, 1983). The present results indicate that temperature ranges for the termination of embryonic diapause in *R. mikado* differ for the different geographical locations. For the southern populations, it is low, which enables eggs to remain in diapause during a long warm period.

Hodek & Hodková (1988) and Hodek (2002) emphasized the importance of processes maintaining and terminating diapause. They listed insects for which low temperature is not a prerequisite for diapause termination. For such insects, low temperatures are often necessary for ‘healthy’ (Andrewartha, 1952) termination of diapause. The eggs of the Toyama *R. mikado* examined in the present study hatched at 25°C. Even for the southern locations, diapause is terminated at 20°C. Consequently, low temperatures are not a prerequisite for egg diapause termination in *R. mikado*. Low temperatures in winter may maintain diapause and synchronize hatching in spring.

The present study revealed that *R. mikado* can adapt to local climates. Nevertheless, it remains unclear when and how these geographical adaptations evolved in this parthenogenetic insect. Parthenogenesis in *R. mikado* might have occurred recently after its adaptation to local conditions.

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