Diapause Characteristics of the Emma Field Cricket, *Teleogryllus emma*

Nam Jung Kim*, Seong Jin Hong, and Seong Hyun Kim

Applied Entomology Division, Department of Agricultural Biology, National Academy of Agricultural Science, Suwon 441-100, Korea

(Received 22 September 2011; Accepted 15 November 2011)

The diapause of *Teleogryllus emma*, the Emma field cricket, was investigated to study the ecological characteristics of the species. Changes in the volume, oxygen consumption, and water content of *T. emma* eggs were followed from oviposition. An increase in volume, oxygen consumption and water uptake occurred from 7 to 8 days following oviposition. The oxygen consumption of the eggs increased slowly for 7 days following oviposition, but then decreased until 15 days following oviposition. These results showed that a physiological change at diapause initiation affected the volume, water content, and oxygen consumption of the *T. emma* eggs. An experimental investigation of egg hatching showed that the eggs could be stocked at 10°C for 40 days with a 14 day pre-period after laying and yield, 62.1% hatch-ability under these conditions. Maintaining the temperature at approximately 10°C was favorable for hatching. Another experiment on egg hatching showed that the storage of eggs at 10°C from 40 to 180 days would ensure satisfactory, hatching capacity.

Keywords: Cricket, Diapause, Egg, Hatching

Introduction

Insects are currently perceived not only as practical resources for agriculture and the biotechnology industry but also as genetic resources with an infinite amount of potential. Moreover, interest in these potential uses has gradually increased. Research on obtaining and breeding various insects (Kim and Seol, 2003; Seol and Kim, 2001; Yoon et al., 2000) is fundamental to find, mass rear and preserve lineages of useful insect resources.

Crickets have been kept in captivity for thousands of years because many people consider the singing of the adult males to be pleasant. More recently they have been kept by many people as a live food source for a great variety of carnivorous animals. They are also an excellent bait for fishing. However, they can also be kept purely for the joy of viewing because they are small, attractive animals with a real charm of their own. Field crickets (*Gryllinae*, especially *Gryllus* spp.) have many advantages for use in field and laboratory studies of insect ecology and behavior (Bertram, 2002; Choo and Choi, 1983; Doherty and Storz, 1992; Fitzpatrick and Gray, 2001; Gray, 1999).

Field crickets spend the winter as eggs laid in the soil. These eggs hatch in late spring or early summer, and tiny immature crickets (nymphs) emerge. The nymphs resemble the adults but adults but are smaller and lack wings. The adults mate and lay eggs during the late summer before succumbing to old age or freezing temperatures in the fall. These crickets undergo an embryonic diapause and produce one generation per year. The nymphs hatch in late spring or early summer and mature in autumn. The adults lay diapause eggs in damp soil (Bae, 1998).

Insects generally require adequate environmental conditions to maintain normal physiological function (Bauer, 1976; Hoy, 1975; Tsurumaki et al., 1999). The most important environmental conditions for artificial rearing are temperature and photoperiod. However, relatively little is known about the developmental effects of these factors, particularly in *T. emma*. Previous studies (Alexander and Bigelow, 1960; Bigelow, 1960a, b, 1962; Ohmachi and Masaki, 1964, 1965, 1966, 1978) provide valuable information on the effects of photoperiod on cricket development. In addition, the cold temperature condition of the egg does not precisely reflect the actual temperature conditions of the overwintering habitat.

This paper describes experiments performed to study egg diapause termination in the rearing of *T. emma* for the
The eggs were then placed on the mat and kept at 25 °C. Changes in the morphological characteristics of the eggs during prediapause were examined each day. After the cold treatment, the eggs were held at 28 °C, 30 °C, 32.5 °C, 35 °C, or 37.5 °C and until day 110. The nymphs that hatched were examined daily at the same time. The Oasis mat was moistened frequently with water so that the hatching of the eggs would not be affected by the environment inside the rearing container.

**Materials and Methods**

**Insects**

*T. emma* were collected from fields in the Suwon region of Korea during September 2002. The crickets were reared in the laboratory at 28 ± 1 °C and 60% R.H. under a 16 h light, 8 h dark photoperiod. The *T. emma* were reared in the laboratory for over a year before individuals were used in the experiments (Kim et al., 2005). The hatched nymphs were individually reared in plastic containers under the conditions described above. A small plastic container (9.0 cm in diameter and 1.5 cm height) was used for rearing the 1st through the 4th stages, and a large plastic container (15.0 cm in diameter and 2.5 cm height) was used for rearing the 5th through the 9th stages. The 1st through the 4th stages were fed an artificial diet of wheat bran and water in a container with flower foam and an Oasis® mat (4 cm × 2 cm). From the 5th through the 9th nymph stages, wheat bran mixed with 40% fish meal and distilled water was provided in a small container with artificial cotton at the bottom (4 cm × 2 cm × 2 cm).

**Changes in the morphological characteristics of the eggs during prediapause**

Egg-forming is a method developed by Kim et al. (2005). An Oasis® oviposition mat was cut to 70 mm × 50 mm × 25 mm, sufficiently moistened with water and placed in a plastic container (47 cm × 32 cm × 29 cm) for four hours. The eggs were then placed on the mat and kept at 25 °C during the experiment.

To investigate the gross morphological characteristics of the eggs, their length, width, and weight were measured from the first day to the fifteenth day of the experiment. The length and width were determined using three repeated measurements on thirty eggs. The weight was measured with three repetitions on one hundred eggs. The interior of the eggs was observed with TEM.

**Measurement of oxygen uptake and water content**

The basic oxygen uptake technique described by Kim (1987) was used. The oxygen uptake of eggs (usually one egg) was measured in a volumetric system into an O₂ uptake resin (Daiyo Scientific Industrial Co., Tokyo, Japan). A total of 1000 eggs were placed in the 20 ml main chamber of each of the vessels. CO₂ gas was absorbed with a strip of filter paper saturated with 0.5 ml of 20% potassium hydroxide. The oxygen uptake tester was placed in a room maintained at 25 °C, and the chambers containing the eggs were submerged in a water bath maintained at 25 °C. Readings were taken at intervals of 30 min for a period of 3–4 h. All of the eggs were kept at 25 °C until they were transferred for measurement. Oxygen uptake was expressed as µl/ml eggs/h. Each value for the oxygen uptake represents the mean of 3 replicate observations. The water content was measured using a Denver Instrument Mark 2 HP.

**The hatching capacity of the eggs during prediapause**

To examine hatching capacity, the eggs, were given a cold treatment at 10 °C for 50 days according to the egg stage from the first day to the fifteenth day. Following the cold treatment, the eggs were held at 28 °C. The nymphs that hatched were examined each day. After the cold treatment, the Oasis® mat was sufficiently moistened with water so that the hatching of the eggs would not be affected by the environment inside the rearing container.

**Conditions for the cold treatment of the eggs**

To determine hatching capacity in relation to the temperature and length of the cold treatment, 500 eggs were put into Petri dishes (150 mm × 25 mm) and covered with dirt, which was sufficiently moistened using a water spray. After the eggs were held at 28 ± 1 °C for 7 days or 14 days, they were moved again into growth chambers at 7.5 °C and 10 °C, respectively, and stored. After handling, the nymphs were examined everyday in the 28 °C rearing room on the 40th, 60th, and 90th day. The group size for each handling was 500 eggs, and three rep.

**The effects of temperature on the diapause termination of the eggs**

After the eggs were held at 28 °C for eight days, they were moved to a growth chamber at 10 °C and given a cold treatment from the tenth day to the 365th day. A hatching capacity experiment was performed by investigating the hatched nymphs each day after they were moved to 28 °C following the cold treatment. After oviposition, the eggs were put in growth chambers at 20 °C, 22.5 °C, 25 °C, 27.5 °C, 30 °C, 32.5 °C, or 35 °C and until day 110. The hatched nymphs were examined everyday at the same time. The Oasis® mat was moistened frequently with water so that the hatching of the eggs would not be influenced by moisture loss.

**Statistical analysis**

Differences in development, reproduction, and diapause were tested with an analysis of variance (ANOVA). If significant differences were detected, multiple comparisons were made using Tukey's HSD multiple range test (P = 0.05).
Results and Discussion

Changes in the morphological and physiological characteristics of the eggs during prediapause

The morphological characteristics of the eggs by stage are shown in Fig. 1. The length, width, and weight of the eggs changed very little for the first 6 days and then increased abruptly on the seventh and eighth days. On the following 2 days, the length, width, and weight of the eggs remained the same. After the eleventh day the length, width, and weight of the eggs decreased slowly. Moreover, almost no morphological changes occurred after the eleventh day. Transmission electron microscopy showed the interior of the eggs (Fig. 2). These results showed that on the eighth day after oviposition, the exochorion and the endochorion were clearly separated. Furthermore, the results of statistical analysis of the gross morphology of *Teleogryllus emma* eggs according to eggs stage after oviposition showed highly significant differences \((P < 0.0001)\). The analysis, confirmed that the eggs increased in volume and weight on the seventh and eighth days after oviposition.

The rate of oxygen consumption (given as microliters of oxygen per 1 g of *Teleogryllus emma* per hour) for each day of embryonic development is shown in Fig. 3. The oxygen consumption for the first 3 days was low and then increased abruptly and continued to increase steadily for 4 days, reaching an average value of 121.7. This measurement was the maximum value during the prediapause period. After reaching this value, the rate of consumption decreased. This decrease continued until the fifteenth day. Thus, during the embryonic development of *Teleogryllus emma*, oxygen consumption increased and continued to increase until the seventh day, when it began to decrease. This result indicates that the embryos entered diapause on the seventh day. This phenomenon was also reported by Tomeba et al. (1988), who analyzed the changes in amino acids in diapausing *Teleogryllus emma* eggs, and in a study of nucleotide pools by Izumiyama and Suzuki (1986).

The absorption of water in relation to the egg stage after oviposition was examined to determine the time at which
physiological changes occur in the diapausing *T. emma* eggs (Fig. 4). At oviposition the eggs primarily contained water (45%). The water content of the eggs increased gradually over time but no major change occurred. Between the seventh and eighth day of oviposition, however, the water content abruptly increased to over 65%. When cricket eggs are deposited, they contain all the necessary materials for embryogenesis except water. As is the case for other insect eggs that are deposited in moist substrates (such as soil or plant tissue), cricket eggs absorb water through their chorion when the embryo has reached a particular stage of development. In *Teleogryllus commodus*, this event occurs just before the embryo reaches the stage at which diapause occurs (Browning, 1953, 1965). In the eggs of *Acheta configuratus*, *Gryllodes supplicans*, and two sibling species of *Acheta domesticus* (Canadian and Pakistani), water absorption generally occurs at an earlier stage. Moreover, embryonic development is more rapid. These findings indicate that the embryonic stage during which water is absorbed is similar among these species (McFarlane *et al*., 1959). However, water can be absorbed in the absence of an actual embryo. The serosa and yolk cleavage are responsible for water absorption in *Scapsipedus marginatus* (Grellet, 1971). In any case, the eggs must absorb water to develop beyond a certain stage. The amount of water absorbed varies from approximately 60 to 120% of the weight of newly laid eggs, depending on the species or strain (Browning, 1965; McFarlane *et al*., 1959).

This water requirement seems to be universal among crickets. When an egg undergoes diapause, water uptake occurs either just before or after diapause, but the timing of this event is fixed for each species. Thus, *Teleogryllus yezoemna*, *Velarifictorus micado* and *Gryllus pennisylvanlcus* absorb water before entering diapause, whereas *Loxoblemnum aomoriensis*, *Dianemobius nigrofasciatus*, *D. mikado*, and *Pteronemobius ohmachii* tend to absorb water only after the completion of diapause (Masaki, 1960). In the latter case, swelling is an unmistakable sign of an egg’s termination of diapause. The find that water absorption can take place either before or after diapause indicates that water supply is not generally involved in the induction of diapause. Furthermore, Hunter-Jones (1964) reported that during the development of *Schistocerca gregaria* eggs, the weight of the eggs suddenly changed within six to eight hours as moisture was absorbed. This observation indicates that the female oviposits in moist soil to obtain sufficient moisture for the developing eggs. The cold treatment from the eighth day following oviposition obtained, a satisfactory result, with a hatching capacity of approximately 80% (Fig. 5).
In *T. emma*, diapause is intensified by exposure to high temperature at the early egg stage (Fig. 6). This response is probably an adaptation to maintain diapause before winter. If eggs of *T. emma* are held at constant temperatures, the mean duration of development is 7 days at 30°C and 8 days at 20°C and 25°C. The maintenance of diapause at high temperatures is ecologically important because, eggs laid early in adult life should persist in diapause under warm conditions before the winter. The observed intensity of diapause in the morphogenetic range of temperature seems to meet this requirement.

**Conditions for the cold treatment of eggs**

The hatching capacity test results for the cold treatment conditions are shown in Table 1. The eggs of the Emma field cricket generally had a hatching capacity of 56.0-65.1% after oviposition if kept at 28°C for 14 days and a hatching capacity of 11.1-54.8% if kept for 7 days. This result shows a superior hatching capacity for the 14-day treatment period. However, the hatching capacities found for the 40-day, 60-day, and 90-day storage periods at 7.5°C and 10°C after keeping the eggs for 14 days at 28°C did not differ appreciably. A temperature of approximately 10°C produced a higher hatching capacity than a temperature of 7.5°C. However the hatching capacity was generally highest on the 60th day at 10°C after storage for 7 days (66.7%). This study considered the shortening of generations during indoor breeding throughout the entire year. The group that received 40 days of cold treatment at 10°C on the 14th day after oviposition was found to be the most successful in the short term, with a hatching capacity of 62.1%. Furthermore, the hatching capacity was 65.1% if the cold treatment was performed for 90 days at 7.5°C on the 14th day following oviposition. This procedure would be appropriate as a long term approach. Moreover, the results of the statistical analysis of the cold treatment of eggs in relation to temperature were highly significant (*P* < 0.0001).

**The effects of temperature on diapause termination in eggs**

To examine the appropriate cold treatment period in relation to the shortening of generations and the long-term storage conditions for the indoor rearing of Emma field crickets, the hatching capacity after cold treatment at 10°C was investigated from the 30th to the 365th day (Fig. 7).
The results a cold treatment period of 40 days varied with the duration of storage. The hatching capacity was 61-63%, for 150-180 days of storage and was 77-90% for 50-120 days of storage. The eggs did not hatch for a storage period greater than 300 days. These results indicated that the appropriate cold treatment period for the eggs was 40-180 days. After the cold treatment, however, the hatching capacity was greater than 70% at an incubation temperature of 17.5-30°C. However, the hatching capacity decreased if the temperature exceeded 30°C and was lower than 30% at 15°C. The time required for hatching after the cold treatment was approximately 11-12 at all incubation temperatures other than 15°C (Fig. 8). Like the eggs of many other hibernating insects, T. emma eggs can be complete diapause at temperatures well below the developmental threshold. If diapausing eggs of T. emma were incubated at 25°C after exposure to 10°C for 40-180 days, they resumed development without an appreciable delay. Moreover, hatching clearly attained a maximum in 2-3 weeks. These eggs completed all or nearly all of their diapause development during cold exposure. The proportion of eggs hatching in 2-3 weeks increased in a sigmoid fashion as a function of the duration of cold exposure. The median effective duration of cold can be determined from a curve of this type (Browning, 1952a, b).

The termination of diapause by artificial or natural exposure to cold has also been observed in Loxoblemmus aomoriensis (Masaki, 1960), Teleogryllus yezoemma (Masaki, 1961), T. commodus (Browning, 1952a, b; Hogan, 1960; Masaki et al., 1979), Oecanthus nigricornis (Bell, 1979), Gryllus pennsylvanicus (Rakshpal, 1962), Nemobius sylvestris (Brown, 1978), N. allardi (Rakshpal, 1964), Pteronemobitus nigrovus (McIntyre, 1978), and P. ohmachii, Dianemobius nigrofasciatus, and D. mikado (Masaki, 1960). T. commodus is the most extensively studied of these species. Browning (1952b) determined that 12.7°C is the optimal temperature for promoting diapause development by treating eggs before the morphological stage of diapause and that a lower temperature, e.g. 8.5°C, is less effective. Hogan (1960) used eggs that were already in diapause and found that 10°C was near the optimum in the nonfreezing temperature range. He also observed that the time of cold exposure required for terminating diapause was shorter if the subsequent incubation temperature was higher. Similar tendencies have been found in Loxoblemmus aomoriensis and Dianemobius nigrofasciatus (Masaki, 1960). Masaki et al. (1979) analyzed such interactions of temperatures in more detail with T. commodus and found that in the late stage of diapause, a high temperature tended to terminate diapause within a short time even if the eggs had not been previously exposed to cold. In particular, if eggs of this cricket that have been in diapause for approximately 60 days at 20°C are exposed to 30°C for only 3 days and are then returned to 20°C, many of the eggs resume development and a clear maximum of hatching occurs. An increase in temperature seems to serve as a trigger for the resumption of development. Therefore, the effect of cold exposure on diapause termination should be interpreted carefully because the results may vary with the subsequent incubation temperature.

Fig. 9 summarizes the results of observations of the duration of the incubation period at 20°C, 22.5°C, 25°C, 27.5°C, 30°C, 32.5°C, and 35°C. Many nymphs hatched from the eggs kept continuously at these temperatures. The egg stage was shortened by increasing the temperature. However, no eggs hatched before the 25th day of

**Fig. 8.** Hatching capacity and hatching time after cold treatment of T. emma eggs at various temperatures after storage at 10°C for 50 days with an 8-day pre-period at 25°C after oviposition.

**Fig. 9.** Comparison of hatching distributions of diapause in T. emma eggs as a function of the duration of exposure (from deposition) to high temperatures.
incubation, even at the highest temperature. This finding indicates that all of the eggs showed longer or shorter periods of diapause irrespective of the incubation temperature. However, the eggs were affected by temperature in the longer periods of treatment at a high temperature. At 25°C, the eggs began to hatch on approximately the 45th day. Hatching then increased continuously to a maximum on approximately the 80th day. At 27.5°C, hatching first occurred on the 34th day and was most intense from the 60th day to the 100th day. At 30°C, hatching first occurred on the 28th day and was most intense from the 55th day to the 85th day. At the higher temperatures of 32.5°C and 35°C, hatching began on approximately the 40th day and the hatching capacity was low.

Overall, these results indicate that the Emma field cricket eggs should set in within eight days following oviposition. Moreover, the duration of the cold treatment period should be 40 to 120 days at 17.5-30.5°C. These findings suggest that breeding Emma field crickets to ensure a steady supply throughout the year will become possible if the timing of the cold treatment during artificial hibernation of the diapausing eggs, the length of the cold treatment, and the diapause termination technology used for long-term high temperature treatment are all harmonized to establish a year-round _T. emma_ supply system.

**Acknowledgement**

This study was carried out with the support of the “Research Program for Agricultural Science & Technology Development (PJ907019), National Academy of Agricultural Science, Rural Development Administration, Republic of Korea.

**References**

Bae YJ (1998) In Insects’ life in Korea I. The Korean Entomological Institute, pp.154-155, Korea University.


McIntyre ME (1978) Some aspects of diapause in the field cricket _Pteronemobius nigrovus_ and _P. bigelowi_ (Orthoptera: Nemobiinae), with notes on their ecology. Mauri Ora 6, 3-10.


