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Geographical variation in egg diapause in *Sympetrum frequens*

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The effects of photoperiod and temperature on the termination of egg diapause were examined in *Sympetrum frequens*. Eggs were obtained from adult females collected from three locations in Japan and incubated under short- or long-day photoperiods at a constant temperature of 25, 20, or 15°C. Egg diapause was eventually terminated in all treatments. Because differences in the average developmental time were small among the temperature treatments, it was concluded that the rate of diapause development is more rapid at lower temperatures, as in other Odonata species. A clear geographic trend was not found in the egg period, which may reflect the life cycle of *S. frequens*: the timing of reproduction is the same or even earlier in higher than in lower latitudes. Relatively large variations in embryonic period were found among populations and even within a population at 15°C, suggesting that the rate of diapause development is also variable. The results also suggested that a short photoperiod might prevent *S. frequens* eggs from hatching before the onset of winter.

Keywords: Odonata; dragonfly; *Sympetrum frequens*; egg diapause; photoperiod; temperature; seasonal adaptation; diapause intensity; diapause development; geographical variation

1. Introduction

Insects in temperate regions typically exhibit diapause in their life cycles (Danilevsky, 1961; Danks, 1987; Košt'ál, 2006; Tauber, Tauber, & Masaki, 1986). The intensity of diapause is important in determining its duration because the resumption of post-diapause morphogenesis before winter is usually fatal (see Masaki, 1999, 2002). It has been shown that diapause intensity often exhibits a clear latitudinal cline within a single species (Masaki, 1999, 2002); in univoltine species, an intense diapause is favored in low latitudes to prevent its termination before winter.

Diapause induction is followed by another important physiological process, diapause development, which paves the way toward the end of diapause (Andrewartha, 1952). The effects of temperature on the rate of diapause development have been studied extensively (Danks, 1987; Hodek, 1996, 2002; Hodek & Hodková, 1988; Košt'ál, 2006). Because the optimum temperatures for diapause development are often lower than those for non-diapause development, insects terminate diapause physiologically during winter, and resume growth or reproduction as soon as the temperature increases in spring (Danks, 1987; Hodek, 2002; Hodek & Hodková, 1988; Košt'ál, 2006; Lees, 1955).

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Corbet (2004) classified the life cycle of Odonata into several categories. Species with obligatory diapause in the egg stage are predominant in Lestidae and in the genus *Sympetrum* of Libellulidae; larvae hatched from diapause eggs in spring develop quickly to emerge as adults in summer (Corbet, 2004). The rate of diapause development in eggs of Odonata species is often rapid at low temperatures: temperatures around 10°C are the most effective for the termination of diapause (Boehms, 1971 [as cited in Corbet, 2004]; Corbet, 1956; Sawchyn & Church, 1973; Schaller, 1968). It was also shown that diapause development is affected not only by temperature but also by photoperiod in *Lestes disjunctus* and *L. unguiculatus*: egg periods were prolonged by short-day photoperiods (Sawchyn & Church, 1973).

Sympetrum frequens (Selys) is one of the most common dragonfly species in Japan. It is widely distributed from Kyushu to Hokkaido (Sugimura, Ishida, Kojima, Ishida & Aoki, 1999). Eggs are often laid in paddy fields after rice is harvested in autumn. Hatching occurs the next spring when irrigation water is introduced to the field, and adults emerge in early summer (Sugimura et al., 1999). Adults in southern lowland populations do not initiate reproduction but enter estival diapause, migrate to high altitudes, and then return to the lowlands in autumn for reproduction (Asahina, 1984). Because of the diapause, the onset of oviposition in northern regions is the same or earlier than that in southern regions, although the timing of adult emergence is earlier in southern Japan, reflecting temperature gradients (Uéda, 1988, 1993). These findings may indicate that females lay eggs only when the temperature decreases, regardless of latitude. If so, it may not be necessary for insects to adapt to the local climate by changing the intensity of egg diapause, because the period from oviposition to winter would be similar among geographical populations. In the present study, we tested the hypothesis that *S. frequens* displays small or insignificant geographical variation in the intensity of diapause. Eggs laid by females collected in northern and southern localities in Japan were incubated under controlled laboratory conditions. The effects of temperature and photoperiod on diapause development were also examined.

2. Materials and methods

Adult *S. frequens* females were collected around ponds in lowland of Sapporo, Hokkaido (43°00′N, 141°12′E), Goshogawara, Aomori (40°48′N, 140°24′E) and Mihara, Hiroshima (34°24′N, 132°54′E) on 1 September, 9 October and 30 October, respectively (Figure 1). Nine, eight and seven females from Sapporo, Aomori and Hiroshima, respectively, were used for experiments. Their abdomens were immersed in water to encourage the release of eggs, which were then transferred to the laboratory within a few days of oviposition, and incubated under controlled experimental conditions. During the transfer from the collection site to the laboratory, eggs were kept at room temperature in a cooler bag. Each egg batch was divided into six groups and stored in tap water in plastic cups (80 ml) under two photoperiods at 25 ± 1, 20 ± 1 or 15 ± 1°C to examine the interaction between photoperiod and temperature. We used a long-day photoperiod of L16:D8, which is equivalent to the day length around summer solstice in Sapporo and Aomori if twilight periods are taken into consideration. A short-day photoperiod of L12:D12 was used as well to simulate the natural day length slightly after the autumnal equinox. The water was replaced by tap water once a week. Moldy eggs were removed periodically, and hatchings were recorded twice a week. Experiments were continued until all the eggs were hatched or dead. These experiments were not replicated.

In this study, we define the egg development time as the time between the transfer of eggs to experimental conditions and the hatching. The hatching data from all egg batches within each experimental treatment were combined together. To detect the difference among experimental treatments, the results for the mean values were assessed using analysis of variance (ANOVA)

Figure 1. *Sympetrum frequens* collection sites.

with embryonic period as the dependent variable, and photoperiod, temperature, and collection site, as independent variables. The Tukey test at $p = 0.05$ (Zar, 2010) was used for the multiple comparison among the groups.

3. Results

Frequency distributions of egg development times under the three temperature regimes are shown in Figure 2. At 25°C, hatching started at approximately day 30 in the Sapporo population. The number of larvae began increasing at approximately day 60, and the peak hatching extended over more than 1 month. After day 200, only one additional egg of the Sapporo population hatched at 25°C. In the Aomori population, the peak hatching period was slightly earlier (by about 10 days) than in the Sapporo population. In the Hiroshima population, the peak hatching was slightly later than in the Sapporo and Aomori populations. Hatching characteristics at 20°C were similar to those at 25°C in the Sapporo population. In the Aomori population, hatching at 20°C was later than that at 25°C, and was concentrated over days 130–150. A similar concentrated peak in hatching was found in the Hiroshima population over days 130–140 under L16:D8. At 15°C, many eggs hatched at approximately 90 days in the Sapporo population. The peak hatching period arrived later in the Aomori and Hiroshima populations. A bimodal distribution was recorded in the Hiroshima population. The egg period could vary from batch to batch and even within a batch. In four of the seven batches, a first (day 80–110) and second (after day 150) peak of the hatching was recorded. For example, 74.6% of the eggs hatched between days 79 and 111 in an egg batch under L12:D12 at 15°C, whereas the others hatched between days 163 and 224. In the other two batches, the first peak was not clear, and only the second one was observed.

The average (with SD) egg development times at 25°C in the Sapporo population were 81 ± 19 and 82 ± 22 days under L16:D8 and L12:D12, respectively (Figure 3). They were significantly longer than those in the Aomori population, but shorter than those in the Hiroshima populations ($p < 0.05$). There was no significant difference in the average egg development times between the two photoperiods in either population ($p > 0.05$). At 20°C, egg development times were slightly longer than at 25°C. Differences between the tested photoperiods were recorded in all three populations ($p < 0.05$): in the two northern populations, egg development times under

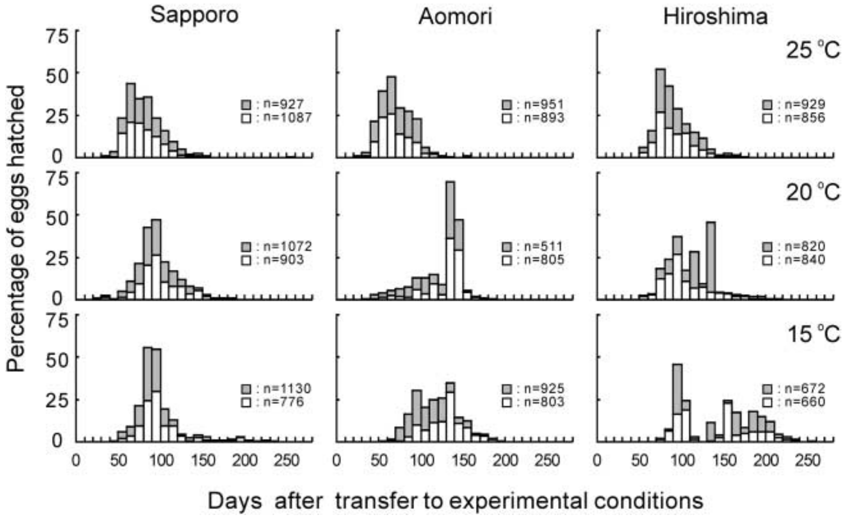

Figure 2. Frequency distribution of the egg development time in *Sympetrum frequens*. The vertical axis indicates the percentage of eggs hatched. Open columns: L12:D12; shaded columns: L16:D8.

L12:D12 were longer than those under L16:D8, whereas the egg period under the short-day photoperiod was shorter than its long-day counterpart in the Hiroshima population. At 15°C, the length of the egg development time was negatively correlated to the latitude of the collection site. Average egg development times under L12:D12 were significantly longer than those under L16:D8 in the Sapporo and Aomori populations ($p < 0.05$). In the Hiroshima population, there was no significant difference between the photoperiods ($p > 0.05$).

4. Discussion

It is known that several insects can complete diapause without being exposed to low temperatures (Hodek, 2002; Hodek & Hodková, 1988). Sawchyn and Church (1973) found that egg diapause in *Lestes disjunctus* and *L. unguiculatus* was terminated even at a constant temperature of 21°C, while most eggs of *L. congener* remained in diapause at that temperature and eventually died. In the present study, eggs of *S. frequens* hatched at all temperatures tested (Figure 2), which is similar to the results obtained with *L. disjunctus* and *L. unguiculatus*. Hatching started as early as 1 month after oviposition at 25°C, suggesting that diapause in *S. frequens* can be terminated even at high temperatures. We collected eggs of the Hiroshima population in the end of October when daily average temperatures were at or below 15°C. Since oviposition is arrested during summer in central or southwestern Japan, it is unlikely that eggs in *S. frequens* are exposed to high temperatures for several months in a natural environment. Thus, it would be unnecessary for this species to adopt a mechanism to prevent eggs from hatching at high temperatures.

Diapause development progresses within a certain range of temperatures for the completion of diapause, with the highest rate dependent on a certain optimal temperature (Danks, 1987). The temperature demand for egg diapause has been studied in several Odonata: Corbet (1956) found that diapause in *L. sponsa* terminates quickly when eggs were maintained at about 10°C, and similar results were obtained in *Aeschna mixta* (Schaller, 1968), *S. vicinum* (Boehms, 1971 [as cited in Corbet, 2004]), *L. disjunctus* and *L. unguiculatus* (Sawchyn & Church, 1973). In the present study, mean egg development time in *S. frequens* was shorter at 25°C than at 20 or

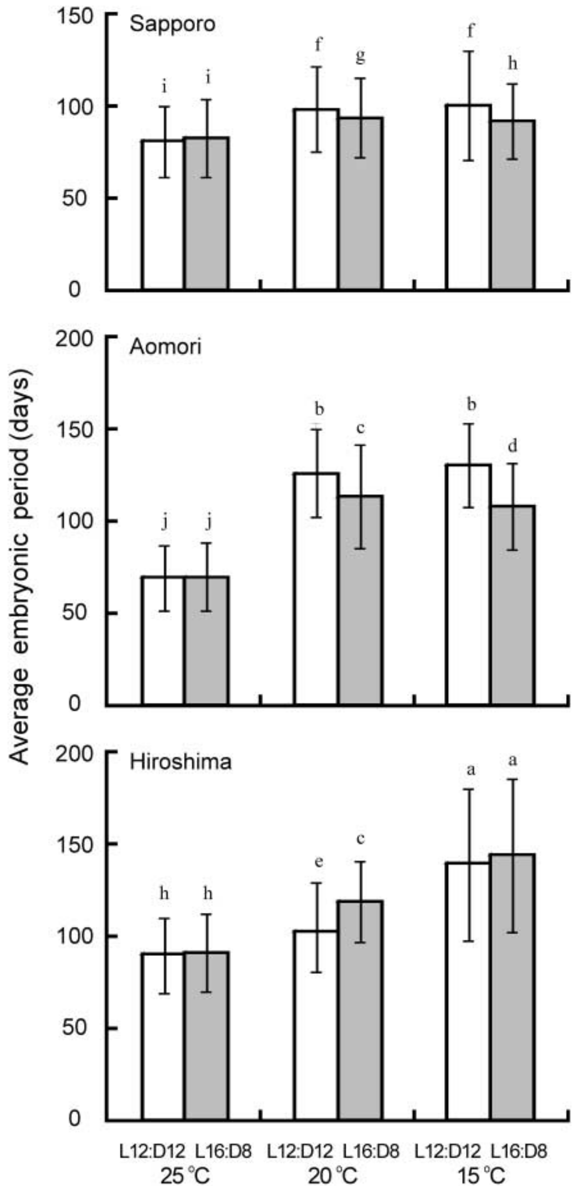

Figure 3. Effects of temperature and photoperiod on the average egg development time in *Sympetrum frequens*. Open rectangles: L12:D12; shaded rectangles: L16:D8; error bars: standard deviations. Data with same letter indicates no significant difference by Tukey test at $p = 0.05$ after ANOVA (Zar, 2010); $n = 511$–1130.

15°C (Figures 2, 3). However, differences among temperatures were small. Because pre- and post-diapause morphogenesis is slow at low temperatures, the small differences confirm that low temperatures are effective at completing diapause development in *S. frequens* as well.

In several insects, geographical variation in the duration of diapause has been demonstrated (see Danks, 1987; Masaki, 1999, 2002). In univoltine insects with obligatory winter diapause, the diapause is more intense in lower latitudes (Masaki, 1999, 2002). For example, the egg development time in the univoltine cricket, *Teleogryllus emma* (Orthoptera: Gryllidae), displays a negative linear correlation to the latitude of habitat (Masaki, 1962). This geographical variation is regarded as an adaptation to avoid untimely hatching before autumn: diapause is more intense at lower latitudes because insects must experience a longer warm period until cold weather in winter arrests embryonic development (Masaki, 1999; 2002). In *S. frequens*, however, such a clear geographical variation in diapause intensity was not indicated at 25 and 20°C (Figures 2, 3). These results may indicate that the period between diapause induction and the arrival of winter is not largely different among populations. Uéda (1988, 1993) revealed that the timing of reproductive activity in *S. frequens* is the same or even earlier in higher than in lower latitudes. The present results appear to confirm those findings.

Asahina (1984) suggested that *S. frequens* is a dragonfly of northern origin: insects in Odonata and other orders expanded their distribution range southward from the Asian continent to the Japanese archipelago during the last glacial period. This idea was supported by a recent study that analyzed mitochondrial 16S rRNA sequences and revealed that Japanese populations of *S. frequens* and Korean populations of *S. depressiusculum* comprise a single clade (Sawabe, Uéda, Higashi, & Lee, 2004). Uéda (1988) speculated that *S. frequens* evolved an adult estival diapause mechanism to adapt to increasingly longer summers as they extended their habitat southward. The current results suggesting that diapause is not intense even at low latitudes support this hypothesis. Because the eggs of *S. frequens* are often laid in very shallow water bodies or on wet ground (Sugimura et al., 1999) where they may be subjected to drought or other unpredictable environmental disturbances during summer, the development of an adult estival diapause in populations in central and southwestern Japan is an adaptation that is evolutionarily superior to the more intense egg diapause.

At 15°C, the mean length of the embryonic period was negatively correlated to the latitude of the collection site (Figure 3). Such a geographic trend in the egg development time is common in univoltine insects such as *T. emma* (Masaki, 1962). As discussed above, such a clear geographical trend was not found at higher temperatures. Therefore, the observed differences in egg development times might indicate that the rate of diapause development is slower in the eggs of populations from low latitudes. Furthermore, the embryonic period in the Hiroshima population displayed a bimodal distribution (Figure 3). This suggests that not only the diapause intensity but also the rate of diapause development may differ among populations, within a population, and even within an egg batch.

Photoperiod is the most important environmental factor that regulates diapause (Danilevsky, 1961; Danks, 1987; Košt'ál, 2006; Tauber et al., 1986). Among dragonflies, it has significant roles not only in larval diapause (see Corbet, 2004; Norling, 1984) but also in embryonic diapause (Sawchyn & Church, 1973). The first phase of diapause development in eggs of *L. disjunctus* and *L. unguiculatus* is typically complete by late October; however, it was revealed that the second phase of diapause persists if their eggs are incubated at a photoperiod shorter than 12 to 14 h (Sawchyn & Church, 1973). These results clearly reveal that short day length in winter helps maintain diapause (Sawchyn & Church, 1973). In the present study, significant differences in the average egg period between short- and long-day photoperiods were detected in all three populations at 20°C, and in the Sapporo and Aomori populations at 15°C although the differences were not large (Figure 3). Except for the eggs of the Hiroshima population at 20°C, egg development times under L12:D12 were longer than those under L16:D8. Therefore, paralleling what has been observed in *L. disjunctus* and *L. unguiculatus*, *S. frequens* may possess a mechanism to prevent its eggs from hatching under short day lengths. In the Hiroshima population, however, the egg development times under L12:D12 was shorter than under L16:D8 at 20°C. The adaptive significance of the photoperiodic response is still unclear. Further study with additional geographical populations and/or larger numbers of specimens within a population might be necessary to clarify the mechanisms involved.

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