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Effects of temperature and light on the embryogenesis of genus *Ephoron* mayflies (Insecta: Ephemeroptera, Polymitarcidae)

Kazuo NAKAMURA, Shuichiro TSURUMARU and Kumiko KAWAMURA

Faculty of Agriculture, Utsunomiya University, 350 Mine-machi, Utsunomiya, Tochigi 321–8505, Japan

Introduction

Three species of mayflies belonging to the genus *Ephoron* occur in Japan. All of them emerge in temperate seasons and pass the winter during egg stage (Ishiwata, 1996). It has been known that the chilling treatment is neccesary for *E. shigae* eggs to hatch (Nakamura, 1985), thus it is understood that a kind of diapause operates in these eggs (Watanabe and Ishiwata, 1997).

Through the incubation of eggs of three *Ephoron* species under various temperatures, we found that the thermal treatment exerted unique effects upon their embryogenesis. Besides the temperature, light conditions given to eggs also affected hatching patterns of the embryos in these species. The present report gives an outline of our experiments and results obtained, to provide some new basis for elucidation of the mechanism regulating the embryogenesis and diapause in these species.

Materials and Methods

Subimagos of *Ephoron shigae* (Takahashi), *E. limnobium* Ishiwata, and *E. eophilum* Ishiwata were collected respectively from the Abukuma River in Fukushima City, Fukushima Prefecture, Lake Biwa in Biwa and Shin-asahi Towns, Shiga Prefecture, and the Kinu River in Ishige Town, Ibaraki Prefecture. Egg clusters extruded from subimagos attracted by a mercury lamp were directly dipped into water.

Eggs obtained from at least 20 subimagos were mixed and several hundred eggs were fixed in each petri dish, with aid of sticky polar cap threads of eggs themselves. For examining the hatching rate of individual eggs in a single egg batch, eggs obtained from a subimago were incubated separately in a well of polystylene microplates (12-Well Polystylene Microplate, Iwaki, Japan).

Eggs were incubated in chambers of a gradient temperature incubator controlled at a constant temperature from 10℃ to 35℃ at intervals of 2.5℃. Light conditions in these cases, were left in nature coming through two glass walls. When light conditions were controlled, the eggs were placed in chambers of an incubator (Bio Multi Incubator LH–30–8CT, Nippon Medical & Chemical Instruments, Japan), in which temperature and illumination by fluorecent lamp (8 W from 23 cm height) were regulated periodically.

Embryonic stages after late anatrepsis were determined by the microscopic pictures taken at intervals. To distinguish earlier embryonic stages, eggs fixed with Petrunkewitsch solution (Romeis, 1968) were stained with thionin. Developmental stages of the embryo were expressed corresponding to those described by Tojo and Machida (1997) for an ephemerid *Ephemera japonica*; namely, stages 5 and 6: early and late anatrepsis, stage 7: longest embryo, stage 8: segmentaion of embryo, stage 9: proctodaeum formation, stage 10: katatrepsis. On the division from stages 11 to 13 some modifications were made. That is, eye spots were invisible in stage 11 and evident in stage 12. With the progressive growth of embryo, the midgut, which is clearly defined with contained yolk, elongates. The posterior extremity of midgut does not attain the level of a half of egg long axis in stage 12 but gets beyond it in stage 13.

Results

1. *Embryonic development at constant temperature*

The results on *E. shigae* are shown in Figure 1, and the critical points are summarized in Figure 2.

The embryogenesis went on slowly but continuously until hatching in the eggs incubated at 10 and 12.5℃. At temperatures ranging from 15 to 25℃, the embryo developed up to the specific stages depending on temperature (designated as 'initial phase'), and then rested for a period. After that, embryos reached

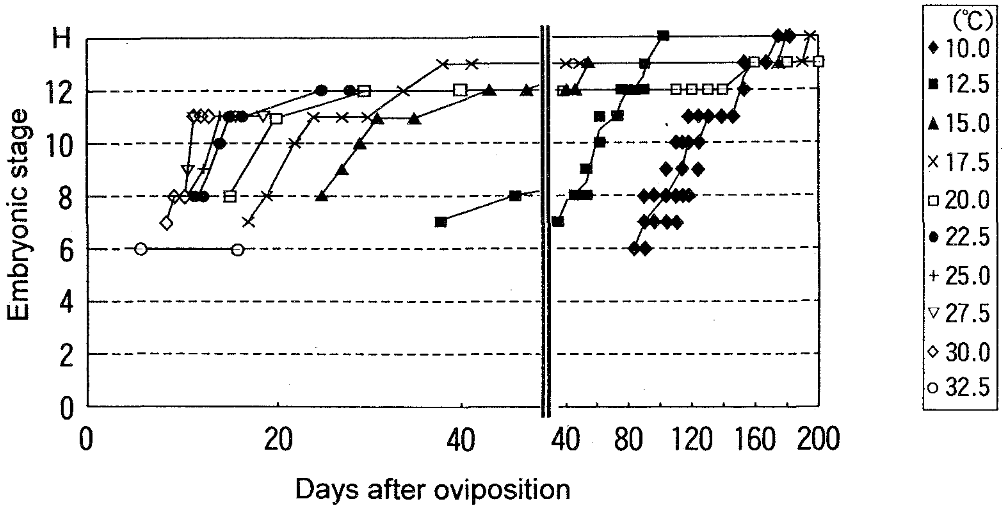

Fig. 1 Time course of embryonic development in *Ephoron shigae* under constant temperatures. The earlier and later periods were differently shown in time scales, to show details. The embryonic stages were analyzed by inspecting the photomicrograph pictures taken at certain intervals (30 to 60 eggs per each time), and predominant stages at each time are plotted. In the experiment at 32.5℃, stages were determined by staining. Stage numbers are according to the standard by Tojo and Machida (1997). H: hatch.

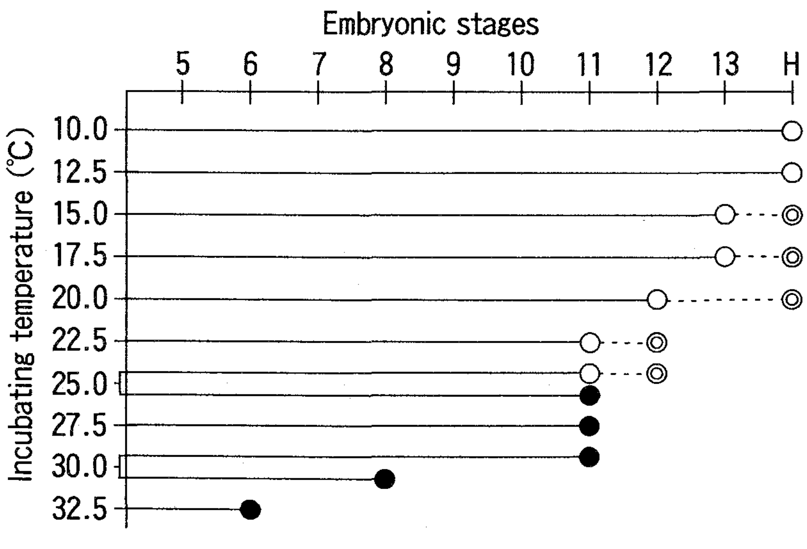

Fig. 2 Effect of incubation temperature on the developmental process of *Ephoron shigae*. Open circles connected with straight lines indicate the duration of the initial phase (see text for definition) of embryogenesis. Double circles with dotted lines mean a period of delayed development after the initial phase. Solid circles denote that no progress was detected after the initial phase at the temperature. Stage numbers according to the standard by Tojo and Machida (1997). H: hatch.

the final stages peculiar to thermal condition, including hatching. In eggs incubated at temperature higher than 27.5℃ and in some eggs at 25℃, embryos showed the growth of initial phase alone and made no more progress. At 35℃, eggs showed an abnormal appearance and embryogenesis did not proceed. Growth rates during the initial phase were proportional to temperature.

As the results of these processes, hatching occurred first at 12.5℃, then at 10 and 15℃ and after around one year at 20℃ (Fig. 3). No nymphs were obtained at 22.5℃ or higher temperatures. The total percentage of eggs hatching at 10℃ was considerably low (12%).

The resting eggs after initial developmental phase could resume further embryogenesis, when transferred to an accceptable lower temperature at a suitable period. It might be suggested that the embryogenesis in these eggs of *Ephoron* was temporarily suppressed at higher temperatures. In a preliminary experiment, eggs were developed to stage 13 without retardation in 30 days after oviposition by gradually lowering the temperature.

The responses to temperature mentioned above were essentially similar to the embryos of the other two species of *Ephoron* as far as examined, except that developmental synchronisms among embryos were not clearly recognizable in *E. eophilum* and *E. limnobium*, and the hatching percentage was low in *E. limnobium* (about 35%).

2. *Effect of light on hatching*

Figure 4 indicates that hatching patterns of *Ephoron* eggs are substantially influenced by photoperiods and that the effect of light is specific among the species. In *E. eophilum*, a longer light period accelerated hatching, but in contrast, in *E. shigae* the light seemed to suppress hatching. In *E. linobium*, the light might stimulate hatching, although hatching percentages were considerably low in this species.

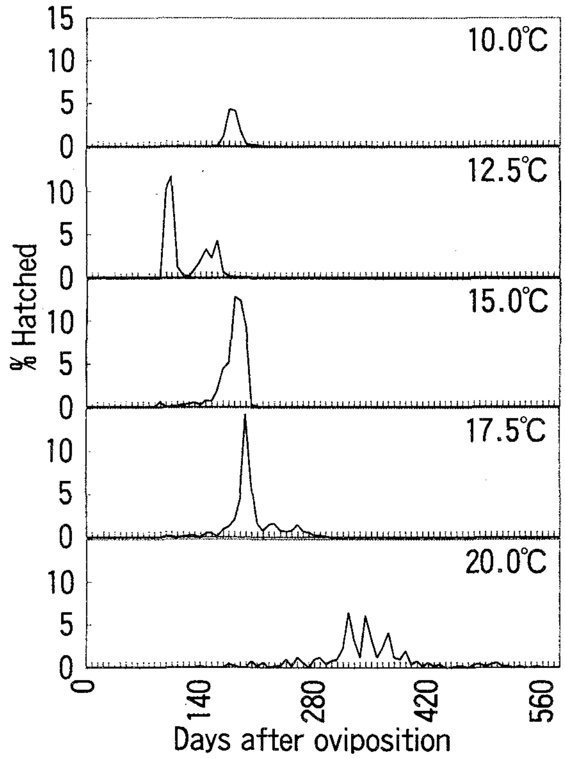

Fig. 3 Hatching of *Ephoron shigae* larvae incubated under constant temperatures. Larvae hatched from the incubations in Fig. 1 were counted and removed from the dish daily, or at an interval of every two days in the earlier period and once per week in the later period.

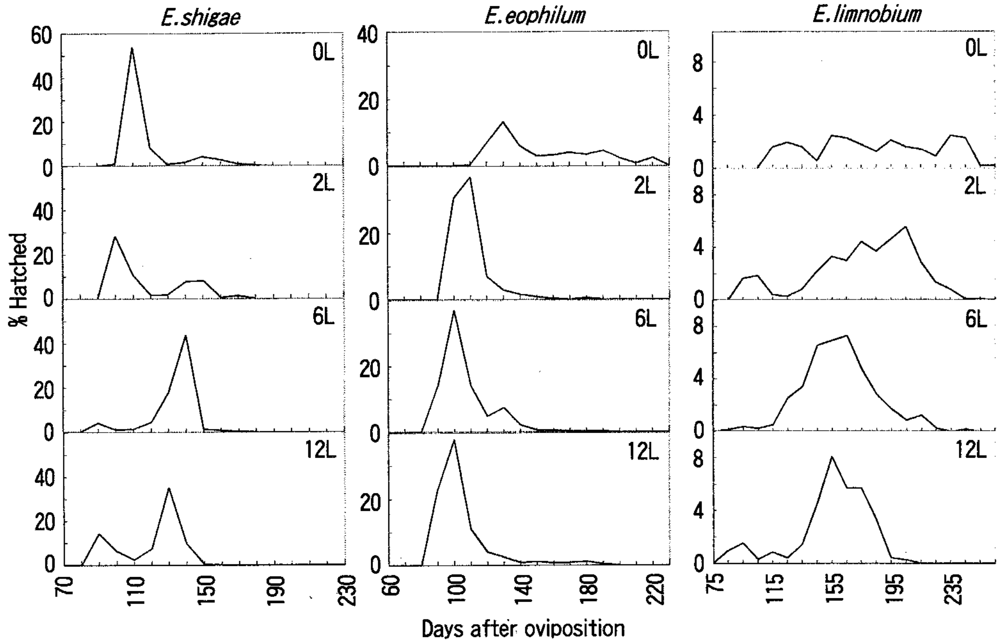

Fig. 4 Hatching of three *Ephoron* species incubated under different photoperiod. Eggs of three mayfly species were incubated at 12.5℃ with different photoperiods. Treatment of hatched larvae was the same as in Fig. 3. 0L: continuous dark, 2L: 2 h light per day, 6L: 6 h light per day, 12L: 12 h light per day.

3. *Hatching of larvae from individual egg batches*

Eggs obtained from individual batches were separately incubated until hatching. Four examples for each species are shown in Figure 5. Time courses of hatching were variable among batches in *E. eophilum*, and almost coincided with each other in *E. shigae*. In *E. limnobium*, the results were ambiguous, due to low percentages of eggs hatching.

Discussion

The general response of *Ephoron* eggs to thermal conditions may seemingly resemble the diapause, namely embryos stop their development in some condition and require a chilling treatment to develop further.

Among the expressions used for dormant state of organisms, Shelford (1929) suggested the term 'quiescence' for the simple case in which development is temporarily inhibited by an unfavorable environment and is resumed as soon as the hindrance is removed (cf. Andrewartha, 1952). The term 'diapause' is restricted to cases where development is autonomously arrested and does not respond immediately to the cessation of adverse conditions.

Examinations of *Ephoron* eggs at different temperatures revealed that the embryogenesis was arrested temporarily and that the embryos began to develop when transferred to acceptable lower temperatures. Thus the dormant nature of *Ephoron* should be classified as a kind of quiescence caused by high temperatures. Similar modes of embryonic development in resonse to temperature have been reported fragmentally in the mayfly (Bohle, 1969) and in the locust (Cherrill and Begon, 1989).

In ordinary quiescence, the development of organisms is stopped by low temperatures, and the organisms are released from suppression by exposure to high temperature. But the relationship is reversed in *Ephoron*, in which the embryogenesis is inhibited by exposure to high temperature and resumed by a chilling. With respect to the response to thermal conditions, the nature is analogous to aestivation to avoid the hot season, but *Ephoron* adopts this nature for hibernation.

The light circumstance to female silkworm moths determines the diapause attitude of her progeny *via*

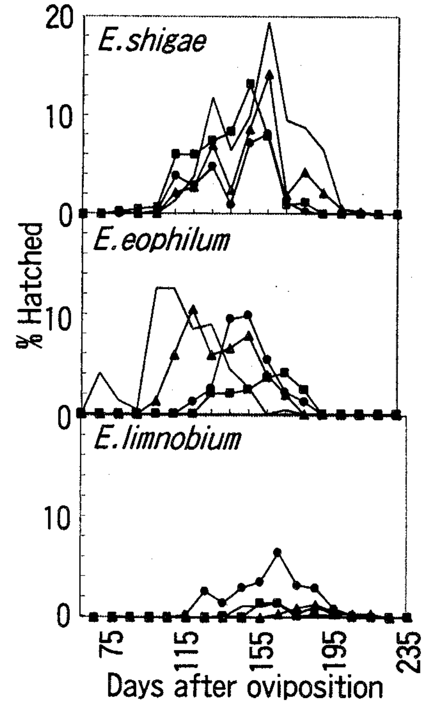

Fig. 5 Hatching of larvae from individual egg batches in three *Ephoron* species. Eggs obtained from individual subimagos were incubated under 12 h light (22.5℃) and 12 h dark (12.5℃) daily cycle. The hatching processes of four batches were expressed by different symbols.

amounts of diapause hormone transferred to the egg (Yamashita and Hasegawa, 1985). In *Ephoron*, the illumination regimes given directly to egg seem to affect the hatching process. Along with temperature conditions, light circumstances might exert complex influences upon embryogensis in these mayflies.

Adults of *E. shigae* are known to emerge synchronously in September (Watanabe *et al.*, 1998), and their larvae hatch within a short period in spring (Watanabe *et al.*, 1993). In contrast to this, adults of *E. eophilum* hatch and emerge over four months (Aoyagi *et al.*, 1998). Less variable hatching patterns among *E. shigae* egg batches compared to those in *E. eophilum* under experimental conditions, might possibly explain the difference of hatching and emerging patterns between them.

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