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Geographical variation in Japan in egg development of the mayfly, *Ephoron shigae* (Ephemeroptera: Polymitarcyidae)

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SUMMARY

1. The effect of temperature on embryonic development was compared in four populations, two bisexual and two unisexual, of *Ephoron shigae*, including one each near the northern and southern periphery of the species range in Japan.
2. Eggs from every population were chilled at 4, 8 or 12 °C for diapause development after 50 days at 20 °C for pre-diapause development (experiment I). Some eggs hatched during chilling at 8 °C or 12 °C, whereas no eggs hatched at 4 °C. The rate of hatching in a given condition of chilling was higher for the eggs from warmer winter environments.
3. Chilling at 4 or 8 °C effectively facilitated diapause development. Chilling at 12 °C was, in general, not so effective, but relatively effective for the eggs from warmer winter environments.
4. Eggs were incubated at 8, 12, 15 or 20 °C after chilling at 4 °C to examine the effect of temperature on post-diapause development (experiment II). The eggs incubated at higher temperature after chilling hatched quicker and more synchronously and had higher hatching success.
5. The relationship between temperature and the days required for hatching after chilling was well described by the power function. There was no significant difference in the slope of the regression lines (i.e. temperature dependency) among local populations. However, a longer time was required for hatching at a given temperature for the population from the colder winter environment.
6. There was no detectable difference in the observed intraspecific variations between unisexual and bisexual populations.

Introduction

Intraspecific variation in embryonic development, including egg diapause, has been considered an important adaptation synchronizing life cycle with seasonal conditions for many insect species (Masaki, 1965; Danks, 1987). For aquatic insects, however, the studies on intraspecific variation of embryonic development are limited. A stonefly, *Diura bicaudata,* has been known to have diapause eggs in populations in high-altitude streams but usually has non-diapause eggs in lake populations (Khoo, 1968; Elliott, 1992). Humpesch (1980) also found that the eggs of a mayfly, *Ecdyonurus dispar,* from a river needed a much longer incubation period than those from lakes. Geographical intraspecific variations in hatching success at various temperatures or in the threshold temperature for hatching have also been reported for some European stoneflies (Lillehammer, 1987; Brittain, 1991; Elliott, 1995; Frutiger, 1996; Zwick, 1996). On the other hand, many mayflies and stoneflies show little or no intraspecific variation in embryonic development (Humpesch & Elliott, 1980; Brittain, Lillehammer & Saltveit, 1984; Elliott, 1988a, 1988b, 1989, 1991a, 1991b).

The burrowing mayfly, *Ephoron shigae* (Takahashi)

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(Polymitarcyidae) is distributed in Japan, Korea and the Russian Far East (Ishiwata, 1996). This species has been reported from Japanese rivers ranging from about 33–39 °N in latitude, and no, or few, males have been found in some populations (unisexual populations), whereas many males have been collected in other populations (bisexual populations) (Watanabe & Ishiwata, 1997). Over its entire range, *E. shigae* has a univoltine life cycle, emerging mainly in September (Watanabe *et al*., 1998). In this paper, the response of embryonic development to temperature is compared in four populations of *Ephoron shigae*, two bisexual and two unisexual, including one each near the northern and southern periphery of the species range in Japan.

Materials and methods

Egg collection

Females of the genus *Ephoron* lay eggs and die as subimagos. Eggs of *E. shigae* were obtained from female subimagos collected in the four rivers: the Abukuma-gawa (37°45′N, 140°29′E), the Shonai-gawa (35°15′N, 137°02′E), the Asahi-gawa (34°41′N, 133°56′E) and the Oita-gawa (33°12′N, 131°35′E). The Abukuma-gawa and the Oita-gawa are located near the northern and southern peripheries, respectively, of the species' range. The populations in the Shonai-gawa and the Oita-gawa are unisexual, whereas those in the Abukuma-gawa and the Asahi-gawa are bisexual (Watanabe & Ishiwata, 1997). The collections of mayflies were made using a light trap in 1995 on 10 September in the Oita-gawa, on 13 September in the Asahi-gawa, on 15 September in the Shonai-gawa and on 16 September in the Abukuma-gawa. The light trap was set at around 20.00 h when almost all females had mated in the bisexual populations (Watanabe, Yoshitaka & Mori, 1989). The presence of sperm in their egg masses was, however, checked under a microscope for some females collected with the light trap in two rivers. All the female subimagos examined (*c.* twenty individuals) in the Asahi-gawa were fertilized, whereas all of those in the Shonai-gawa were unfertilized. Females in the other two rivers were not examined. As soon as the abdomen of a female subimago was dipped into water, egg masses were expelled. About twenty females in each river were forced in this way to lay eggs in a beaker filled with river water. After the eggs had been mixed in the beaker, they were distributed as evenly as possible with a syringe into forty Petri dishes (40 mm in diameter) half-filled with river water. When the eggs eventually became attached to the bottom of the dish, a folded piece of cotton gauze was put in the dish to soak up the water for carrying convenience, and then the dish was covered. The dishes, packed in a plastic box with stuffing, were put in a cooler without ice or coolant, and transported to the laboratory within 2 days.

Experimental procedure

The eggs of *Ephoron* require a period of cold for diapause development (Edmunds, Nielsen & Larsen, 1956; Britt, 1962; Watanabe & Takao, 1991; Giberson & Galloway, 1985; Kureck & Fontes, 1996). Two series of experiments were therefore designed to compare the eggs from the four rivers in terms of the effects of temperature on diapause development (experiment I) and on post-diapause development (experiment II). The experimental procedures are summarized in Fig. 1, and explained in detail below.

After the gauze was removed, all the dishes were checked and some that contained too few eggs were used for measurement of egg sizes. Major and minor axes of fifty eggs were measured from each river. The other dishes were half-filled with dechlorinated city water and were kept at 20.9 ± 0.9 °C (average ± SD) for 50 days (first incubation), which provided a period long enough for pre-diapause development (Nakamura *et al*., 1987; Matsumura, 1989). No eggs hatched during this period. The dishes were then checked again and some were excluded from the experiments because eggs detached easily from the bottom, probably as a result of unclean glass surfaces. For each population, twenty-four dishes were used in experiment I and eight dishes in experiment II.

Three series of eight dishes each for experiment I were chilled at 3.9 ± 0.3 °C, 8.1 ± 0.2 °C and 12.0 ± 0.1 °C, respectively, using a temperature-gradient chamber (type TG-100-AD; Nippon Medical & Chemical Instruments). Four, two-dish sets of each series were kept there for 30, 45, 60 and 75 days, respectively. After chilling for the respective periods, the dishes were brought back to 20.1 ± 0.4 °C (second incubation). When egg hatching commenced, newly

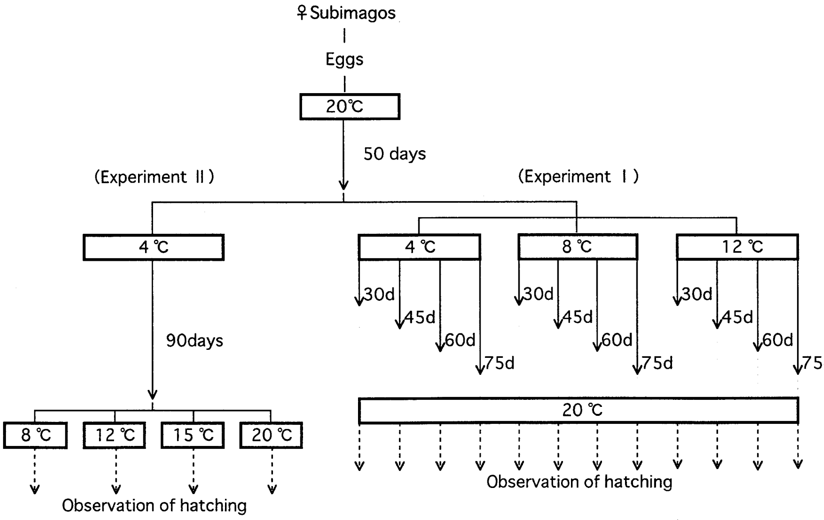

Fig. 1 Experimental procedures for the embryonic development of *Ephoron shigae*. The temperatures are approximate and accurate values are given in the text.

hatched larvae were removed daily and counted under a binocular microscope.

Eight dishes for experiment II were kept at 4.0 ± 0.3 °C for 90 days. After that, four series of two dishes each were transferred to 8.1 ± 0.3 °C, 12.0 ± 0.2 °C, 15.4 ± 0.5 °C and 20.3 ±0.4 °C, respectively, for the second incubation, and egg hatching was observed daily. For convenience, integers closest to actual mean temperatures, i.e. 4, 8, 12, 15 and 20 °C, are quoted subsequently in the text and in the tables and figures. However, in calculations, precise mean temperatures during the respective experimental periods were used.

The photoperiod in the chamber was set at light : dark 12 : 12 h throughout the experiments, using artificial lights. There was no forced aeration. The water was replenished almost every week during the first incubation and the period of chilling. During the second incubation, the water was changed every day after the observation of hatching. The water used for replenishment or change had been kept at the same temperatures as the respective dishes.

When hatching was terminated in both the experiments, the number of eggs attached to the bottom of dish, including hatched and unhatched eggs, was counted with a pen-type colony counter.

Results

Thermal conditions in the four rivers

Seasonal changes in water temperature in the four rivers where the eggs were obtained are shown in Fig. 2. The temperature from autumn to spring was lowest in the Abukuma-gawa, where average temperatures below 5 °C lasted for about 3 months from mid-December to mid-March. In contrast, the average temperature did not fall below 5 °C in the Oita-gawa. Winter temperature decreased in the order, the Oita-gawa, the Shonai-gawa, the Asahi-gawa and the Abukuma-gawa.

Egg sizes

Egg sizes of *E. shigae* from the four rivers were compared (Table 1). The major axis of eggs did not differ significantly in the four populations. The minor axis of eggs in the Oita-gawa was shortest and significantly different from those in the Asahi-gawa ($t_{98} = 3.098$, $P < 0.01$) and in the Abukuma-gawa ($t_{98} = 2.163$, $P < 0.05$). The volume of egg, assumed to be right circular cylinders (see scanning electron microscope view by Ishiwata, 1996), was significantly smaller in the Oita-gawa than in the Asahi-gawa ($t_{98} = 2.283$, $P < 0.05$).

Final hatching success and hatching rates within 10 days after different conditions of chilling

Results of egg hatching after different conditions of chilling in experiment I are summarized in Table 2. Number of eggs per dish varied considerably (311–2856) because of difficulty in distributing eggs evenly to the dishes. Although final hatching success also varied from 29 to 96%, the number of eggs did not

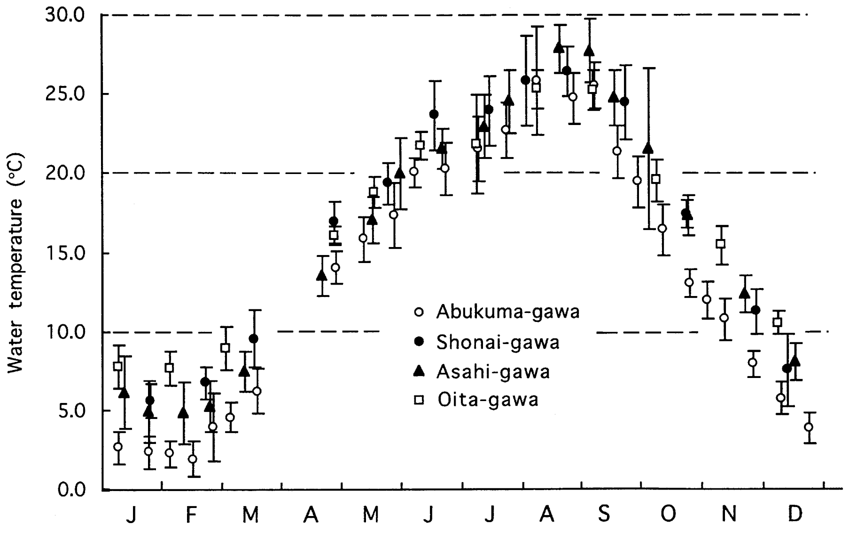

Fig. 2 Seasonal changes in water temperature in the four Japanese rivers where eggs were obtained. Temperature measurements at the point nearest to the site where females of *Ephoron shigae* had been collected in each river were selected from the Annual Report of Water Quality (River Bureau, 1984–93), and averaged for the 10 years from 1984 to 1993. Error bars represent $CL_{0.95}$.

Table 1 Egg sizes of *Ephoron shigae* in four Japanese rivers

	Rivers			
	Abukuma	Shonai	Asahi	Oita
Major axis (μm) $\bar{x}$ ± SD	260.7 ± 11.7	258.2 ± 12.1	261.5 ± 12.5	264.3 ± 17.1
Minor axis (μm) $\bar{x}$ ± SD	193.8 ± 13.9	192.2 ± 13.0	197.7 ± 14.6	185.3 ± 16.4
		*	**	
Egg volume (μm^3) $\bar{x}$ ± SD	7.7 ± 1.1×10^6	7.5 ± 1.1×10^6	8.1 ± 1.4×10^6	7.2 ± 1.6×10^6
			*	

t-test: $^{**}P < 0.01$; $^{*}P < 0.05$.

affect hatching success. The eggs that hatched without delay when returned to 20 °C can be regarded as having completed their diapause development during chilling. Therefore, the rates of eggs hatched within 10 days compared to the total of hatched eggs, including the eggs already hatched during chilling as described below, are shown in the table. After chilling at 4 and 8 °C, the majority of eggs hatched within 10 days regardless of the length of cold period. However, the eggs from the Abukuma-gawa and the Asahi-gawa had somewhat lower hatching rates after 30 days at 4 or 8 °C. The hatching rate within 10 days decreased after shorter periods at 12 °C in every river. After a given period at 12 °C, hatching rate became higher in the order, the Abukuma-gawa, the Asahi-gawa, the Shonai-gawa and the Oita-gawa. In other words, even relatively high temperatures facilitated more effective diapause development of eggs from the river with warmer winter temperatures. On the other hand, only a small percentage of the eggs from the Abukuma-gawa hatched within 10 days after 30 or 45 days at 12 °C; most of the eggs had not completed their diapause development.

The time required for hatching after chilling and hatching period

The number of days required for 50% of eggs to hatch after chilling and the length of hatching period (days for 5–95% hatch) are shown in Table 3. Some eggs had already hatched when the cold periods were finished (column A). The percentage of such eggs was generally higher after chilling at higher temperatures for longer periods, with no egg hatching at 4 °C. The percentage of eggs hatching during a given chilling temperature was highest in eggs from the Oita-gawa followed by the Shonai-gawa, the Asahi-gawa and the Abukuma-gawa, respectively. This was the order of warmer winter temperature.

After chilling at 4 and 8 °C, eggs hatched quickly

Table 2 Final hatching success and cumulative hatching rate on the tenth day after chilling (compared to the eggs that eventually hatched) for eggs of *Ephoron shigae* from four Japanese rivers (experiment I). The data from two dishes at the same temperature treatment were combined.The temperatures are shown approximately; see the text for the exact values

River	Chilling temperature (°C)	Days	Eggs (*n*)	Hatching success (%)	Hatching within 10 days (%)
Abukuma-gawa	4	30	500	66.0	92.1
	4	45	513	90.6	98.1
	4	60	1050	88.4	99.7
	4	75	666	86.2	99.8
	8	30	865	72.3	88.5
	8	45	852	93.3	99.9
	8	60	432	93.5	100.0
	8	75	502	89.2	100.0
	12	30	1163	64.8	0.8
	12	45	746	83.0	2.4
	12	60	468	94.0	80.7
	12	75	526	96.0	99.4
Shonai-gawa	4	30	1227	41.7	99.2
	4	45	2300	58.0	99.0
	4	60	1732	53.6	99.1
	4	75	1591	50.3	96.4
	8	30	420	71.7	99.7
	8	45	2339	61.1	99.9
	8	60	2218	55.6	99.9
	8	75	2529	54.1	100.0
	12	30	1288	44.4	28.0
	12	45	2085	64.3	63.8
	12	60	2172	57.1	98.8
	12	75	750	66.8	100.0
Asahi-gawa	4	30	1101	62.2	93.4
	4	45	794	69.0	99.3
	4	60	1381	73.3	100.0
	4	75	400	63.5	100.0
	8	30	903	45.3	99.3
	8	45	2269	91.7	100.0
	8	60	642	39.9	100.0
	8	75	1317	77.4	99.9
	12	30	854	56.1	18.8
	12	45	731	77.8	49.4
	12	60	1040	86.6	93.6
	12	75	1510	43.0	100.0
Oita-gawa	4	30	1703	44.2	99.9
	4	45	2856	67.7	99.9
	4	60	1845	50.6	99.9
	4	75	587	29.0	100.0
	8	30	1331	76.2	99.5
	8	45	311	77.8	99.2
	8	60	773	43.3	100.0
	8	75	1177	82.0	100.0
	12	30	1198	59.6	77.2
	12	45	1034	54.5	86.5
	12	60	1133	67.5	99.9
	12	75	1951	85.2	100.0

Table 3 The number of days required for 50% hatch after chilling and the length of hatching period (days for 5–95% hatch) in eggs of *Ephoron shigae* from four Japanese rivers (experiment I). Column A gives the percentage of eggs hatched during chilling periods

		Rivers											
		Abukuma-gawa			Shonai-gawa			Asahi-gawa			Oita-gawa		
Chilling temperature (°C)	Days	A	50%	5–95%	A	50%	5–95%	A	50%	5–95%	A	50%	5–95%
4	30	0.0	7	5	0.0	6	2	0.0	7	6	0.0	6	2
	45	0.0	7	3	0.0	6	3	0.0	7	2	0.0	6	2
	60	0.0	6	2	0.0	6	3	0.0	7	2	0.0	6	1
	75	0.0	6	2	0.0	6	5	0.0	7	2	0.0	6	3
8	30	0.0	6	12	0.0	5	2	0.0	6	3	0.0	5	1
	45	0.0	4	3	0.0	4	3	0.0	5	4	0.0	4	3
	60	0.0	4	3	0.2	3	2	0.0	5	5	0.3	2	3
	75	1.1	3	3	19.2	1	> 5	4.3	4	6	48.1	1	> 4
12	30	0.0	58	117	0.0	21	31	0.2	19	56	0.0	7	9
	45	0.0	20	69	7.5	9	> 22	0.2	11	16	1.2	4	11
	60	0.0	7	20	13.3	5	> 8	0.2	6	9	34.2	1	> 5
	75	1.8	4	6	62.9	–	> 4	41.5	2	> 5	76.5	–	> 4

and synchronously except for a somewhat prolonged hatching period after 30 days at 8 °C for the eggs from the Abukuma-gawa. The time required for hatching was slightly shorter after chilling at 8 °C than at 4 °C, and also became shorter after longer periods at 8 °C. After the relatively short periods at 12 °C, hatching was delayed and occurred over a long period, except in the Oita-gawa. Even chilling at 12 °C was effective in facilitating diapause in the Oita-gawa where winter temperatures were highest. The number of days required for hatching and the hatching period after chilling at 12 °C were longest in the Abukuma-gawa and shortest in the Oita-gawa.

Effect of temperature on post-diapause development

Table 4 shows the effect of temperature for second incubation on egg hatching after chilling at 4 °C for 90 days in experiment II. No eggs had hatched during the chilling at 4 °C in this experiment either. The time required for hatching of 50% of the eggs eventually hatched was shorter at the higher temperatures in the range of 8–20 °C. The hatching period (5–95% hatching) was also shorter at the higher temperatures. In addition, the rate of hatching success was generally higher at the higher temperatures, with the exception of a higher value at 15 °C than 20 °C in the Abukuma-gawa. Therefore, higher temperature facilitated the break of diapause and the ensuing post-diapause development more effectively. The rate of hatching success in the Oita-gawa, where winter temperature was highest, was very low at 15 °C and at lower temperatures, whereas hatching success in the other rivers were 40–50%, even at 12 °C. These low rates of hatching success in the Oita-gawa were not always a result of mortality during chilling at 4 °C, because 56% of the eggs hatched successfully when returned to 20 °C. The eggs from the Oita-gawa may enter an intense diapause during chilling at an unusually low temperature.

Figure 3 shows the relationship between temperature and the days required for 50% of eggs to hatch after chilling, using data from Table 4. The relationship was clearly linear on logarithmic scales for the Abukuma-gawa, Shonai-gawa and Asahi-gawa. The number of days required for hatching in eggs from the Oita-gawa at 8 °C was the same as at 12 °C. However, the value at 8 °C is probably erroneous because the rate of hatching success was extremely low (0.6%). When the value at 8 °C in the Oita-gawa is excluded, the relationship for all populations is well described by the regression equation:

$$\log Y = \log a - b \log T$$

or the power function:

$$Y = aT^{-b}$$

where a and b are constants, the values of which are

Table 4 Post-diapause development in *Ephoron shigae* eggs from four Japanese rivers (experiment II). Number of days required for hatching of 50% of the eggs that eventually hatched and hatching period (5–95% hatching) at different incubation temperatures after chilling at 4 °C for about 90 days, with rates of hatching success. The data of two dishes at the same temperature treatment were combined

		Rivers			
Temperature (°C)		Abukuma	Shonai	Asahi	Oita
8	50% hatch (days)	57	48	53	12
	Hatching period (days)	28	28	12	43
	Hatching success (%)	12.2	13.0	3.0	0.6
12	50% hatch (days)	24	19	21	12
	Hatching period (days)	10	13	6	10
	Hatching success (%)	44.6	49.3	41.5	5.6
15	50% hatch (days)	14	11	15	9
	Hatching period (days)	3	6	4	8
	Hatching success (%)	93.2	53.8	50.4	8.0
20	50% hatch (days)	9	6	6	4
	Hatching period (days)	3	2	1	1
	Hatching success (%)	81.1	58.0	84.0	56.2

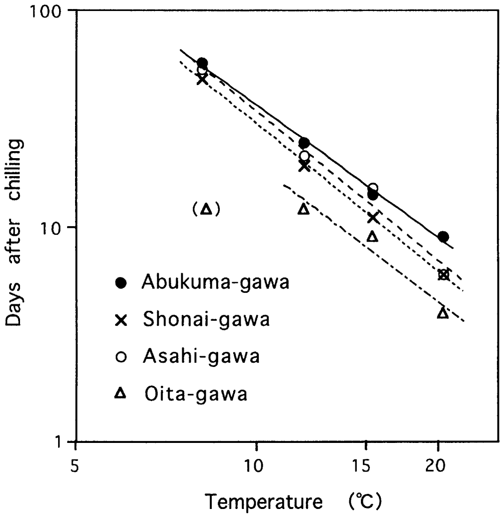

Fig. 3 Relationship for *Ephoron shigae* between temperature and days required for 50% of eggs hatched after chilling for the four Japanese rivers. The regression equation constants for each river are given in Table 5.

given for each river in Table 5. There was no significant difference in constant *b* ('degree of temperature dependency', Brittain, 1990) among the local populations. On the other hand, significant differences in constant *a* (number of degree days required for hatching) were apparent between the Oita-gawa and each of the other rivers, and between the Abukuma-gawa and the Shonai-gawa (Table 5). The time required for hatching at a given temperature in the range of 8–20 °C increased in the following order: the Oita-gawa, the Shonai-gawa, the Asahi-gawa and the Abukuma-gawa (Fig. 3).

Table 5 Comparison of the regression equations ($Y = aX^b$) for post-diapause development in *Ephoron shigae* between pairs of Japanese rivers (experiment II). Solid and broken lines indicate significant differences at '0.5% level' and '5% level', respectively, between two rivers (ANCOVA). There were no significant differences between the Asahi-gawa and the Abukuma-gawa or the Shonai-gawa

River	R^2	*b*	log *a*	*a*
Abukuma-gawa	0.997**	–2.024	8.243	3801
Shonai-gawa	0.999**	–2.252	8.570	5271
Asahi-gawa	0.976**	–2.269	8.741	6254
Oita-gawa	0.930*	–2.090	7.766	2359

t-test of correlation coefficient: $^{**}P < 0.01$; $^{*}P < 0.05$.

Discussion

Effect of temperature on diapause and post-diapause developments

No eggs hatched during chilling at 4 °C, but after being brought back to 20 °C eggs hatched quickly and synchronously. Therefore, chilling at 4 °C seems to facilitate diapause development effectively, but not to break diapause. Some eggs hatched during longer periods at 8 °C. In addition, the time required for hatching was slightly shorter after chilling at 8 °C than at 4 °C, and also became shorter after longer periods at 8 °C. Therefore, the temperature of 8 °C not only facilitates diapause development effectively, but also permits post-diapause development. Chilling at 12 °C was generally not so effective for facilitating diapause development because hatching was delayed after short periods of chilling. After diapause was broken, however, post-diapause development seemed to have taken place quickly because a large percentage of eggs hatched over extended periods at 12 °C.

Even after a shorter period of chilling at 12 °C, a large percentage of eggs eventually hatched at 20 °C. Watanabe & Takao (1991) reported that eggs kept continuously at 20 °C after oviposition began to hatch sporadically after about 120 days. Therefore, when the diapause development was not completed during chilling in the present experiment, the eggs might have finished the remaining process of diapause development at 20 °C as well as post-diapause development and might have hatched after a long time.

The time required for hatching after 90 days at 4 °C was shorter at the higher temperatures in the range of 8–20 °C. Similar relationships were reported for post-diapause embryonic developments of a mayfly, *Ephemerella ignita* (Bohle, 1972) and a stonefly, *Diura bicaudata* (Elliott, 1992). A longer hatching period at lower temperatures shown in experiment II has also been known for post-diapause development of *D. bicaudata* (Elliott, 1992) and for non-diapause developments of many mayflies and stoneflies (e.g. Elliott, 1972, 1991b; Friesen, Flannagan & Lawrence, 1979).

Geographical variations in thermal effect on diapause and post-diapause development

Geographical variations in the embryonic response of *E. shigae* to temperature were clearly shown by the experiments. Diapause development was facilitated more effectively even by the relatively high temperature (i.e. 12 °C) in the eggs from the river with warmer winter temperatures. These characteristics are clearly adaptive in the rivers where water temperature does remain relatively high even in winter.

Only a small percentage of the eggs from the coldest river, the Abukuma-gawa, broke their diapause even during the longest period (75 days at 8 or 12 °C), whereas the eggs from the warmer rivers broke diapause more easily. This may be explained as a mechanism to keep the populations in colder rivers from hatching at an unfavourable time before the onset of spring as suggested for a stonefly, *Dinocras cephalotes* by Zwick (1996) and Frutiger (1996).

According to the relationship between temperature and days required for hatching after chilling, the eggs from the population in the colder environment required a longer time for hatching at a given temperature in the range of 8–20 °C. Slower embryonic development of eggs from colder environments has also been reported for two species of the mayfly *Ecdyonurus* (Humpesch, 1980). The temperature dependency of post-diapause development did not, however, differ among the four populations. Few differences in temperature dependency of embryonic development and differences in incubation period or hatching success at given temperatures have been reported for local populations of stoneflies (Lillehammer, 1987; Brittain, 1991; Elliott, 1995; Frutiger, 1996). It may possibly be the general strategy in embryogenesis for aquatic insects to adapt to a wide range of thermal conditions.

Differences between unisexual and bisexual populations in embryonic development

Two unisexual populations studied were from warmer winter environments and two bisexual populations were from colder winter environments. However, it is unlikely that the occurrence of unisexual and bisexual populations is related to ambient water temperature, because geographical distributions of unisexual and bisexual populations broadly overlap in Japan (Watanabe & Ishiwata, 1997).

None of the observed intraspecific variations in diapause and post-diapause developments differed recognizably between unisexual and bisexual populations. Instead they showed gradients related to field winter temperatures as shown above.

Effect of increase in temperature on egg hatching

Only a small percentage of the eggs hatched during chilling at 8 and 12 °C in the Abukuma-gawa in experiment I. Nevertheless, a much higher percentage of eggs hatched at similar temperatures after chilling at 4 °C in experiment II. This suggests that the change from lower to higher temperature may facilitate the break of diapause and/or post-diapause development, and thus low temperature may be important as a contrast to vernal increase in temperature as discussed by Hodek & Hodkovà (1988).

Effect of egg size on the post-diapause development

Some authors have suggested that smaller eggs develop more quickly than larger ones (Munro & White, 1975; Humpesch, 1980). In *E. shigae*, the smallest eggs from the Oita-gawa also developed the most quickly after chilling. However, the eggs from the Abukuma-gawa, smaller than those from the Asahi-gawa, developed more slowly, although the difference was not statistically significant. A clear conclusion cannot be drawn from the small samples in this experiment for the relationship between egg size and the rate of post-diapause development.

Water temperature as a limiting factor of geographical distribution

Watanabe & Ishiwata (1997) hypothesized that the species range of *E. shigae* was determined by the thermal regime from autumn to spring, on the basis of Watanabe & Takao's (1991) experiments using the eggs from the Asahi-gawa. They considered that the distribution of this species was limited to the north of the Banjo-gawa (32°57′N) in Japan because the winter temperature is too high for diapause development in the more southern areas. According to the present experiments, however, the limit could extend further southward because even relatively high temperatures effectively facilitated diapause development of the eggs from warmer winter environments. Actually, mass emergence of *E. shigae* was reported in 1996 from the Hitotsuse-gawa (32°07′N) in Saito City, about 100 km south of the Banjo-gawa, although data for temperature regimes are not yet available.

On the other hand, Watanabe & Ishiwata (1997) considered that at the northern periphery of the species' range, a slower vernal increase of water temperature owing to melting snow may retard egg hatching and diminish the potential period of nymphal development, and that this may determine the northern limit of their distribution. As shown in the present experiments, the eggs from colder winter environments required a longer time for hatching after diapause at a given temperature. This characteristic may amplify the delay in egg hatching under low vernal temperatures.

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