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Origin of Parthenogenesis in the Geographically Parthenogenetic Mayfly *Ephoron shigae* (Ephemeroptera: Polymitarcyidae) - Potential for Parthenogenesis of Females in a Bisexual Population -

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Abstract

The burrowing polymitarcyid mayfly *Ephoron shigae* is widely distributed across Japan. Some populations are bisexual, and others are unisexual, *i.e.*, geographically parthenogenetic. Moreover, the distribution of the bisexual and unisexual populations overlap broadly in their respective geographic ranges. *Ephoron shigae* provides a good model to study the differentiation of unisexual and bisexual populations, the establishment of parthenogenesis, and the dispersal of parthenogenetic individuals. In the previous study for this mayfly, obligatorily diploid thelytoky appears in unisexual populations. However, the potential for parthenogenesis or parthenogenetic ability of females in a bisexual population is still not clearly understood. From this background, we examined the parthenogenetic ability of virgin females in the bisexual populations. As a result, it is revealed that females of this population potentially have parthenogenetic ability. The parthenogenetic individuals of bisexual population were also indicated to be of the same diploid thelytokous type as found in the other examined unisexual populations.

Key words: *Ephoron shigae*, geographic parthenogenesis, mayfly, parthenogenesis, Polymitarcyidae, thelytoky

Introduction

The burrowing polymitarcyid mayfly *Ephoron shigae* (Takahashi) is distributed widely in Japan. Some populations are bisexual, and others are unisexual (solely females), so that *E. shigae* is a geographically parthenogenetic mayfly (Watanabe and Ishiwata, 1997; Tojo *et al.*, 2006; Sekiné and Tojo, 2007; Fig. 1). In general, parthenogenetic populations are often found under that extreme environments such as at high latitudes and altitudes, in xeric as opposed to mesic conditions (e.g., Suomalainen *et al.*, 1987), in isolated habitats such as islands and island-like areas, and at the peripheral regions of the taxon's range (Cuellar, 1977). In *E. shigae*, however, the distribution of the bisexual and unisexual populations overlap broadly in their respective geographic ranges. Therefore, *E. shigae* provides a uniquely well-suited model to study the differentiation of unisexual and bisexual popu-

lations, the establishment of parthenogenesis, and the dispersal of parthenogenetic individuals.

In the previous study on *E. shigae*, obligatory parthenogenesis (the normal mode of reproduction) appears in unisexual populations (Tojo *et al.*, 2006). Furthermore, it is revealed that all individuals reproduced by parthenogenesis are diploid females ($2n=12$), indicating the occurrence of thelytokous parthenogenesis (Sekiné and Tojo, 2007). However, the parthenogenetic ability of females in the bisexual populations has not been thoroughly studied. Although it was reported that twenty of the females examined in the Asahi-gawa River population (a bisexual population) showed no parthenogenetic ability (Tojo *et al.*, 2006), it is still unclear whether this parthenogenetic potential is peculiar to the unisexual populations or not. From this background, we examined the parthenogenetic potential of virgin females in the bisexual populations.

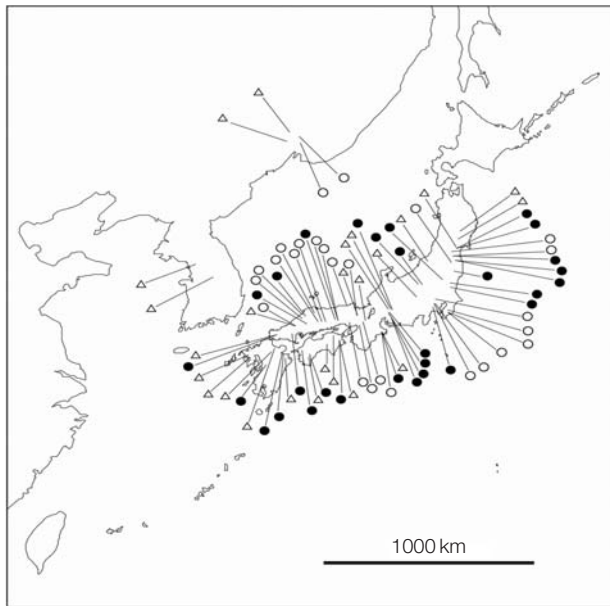


Fig. 1. Distribution of *Ephoron shigae*. Data from the present study added to that from Sekiné *et al.* (2006) and Tojo *et al.* (2006). Open and solid circles indicate bisexual and unisexual populations, respectively. Open triangles indicate the populations in which the sex ratio was not determined.

Materials and Methods

Development Rate of Fertilized and Unfertilized Eggs of *Ephoron shigae*

Three bisexual populations of *Ephoron shigae* were examined; from Hino-yosui flume (irrigation canal; Hino, Tokyo Prefecture: 36° 24'N, 138° 15'E), Ara-kawa River (Konosu, Saitama Prefecture: 36° 05'N, 139° 26'E), and Asahi-gawa River (Okayama, Okayama Prefecture: 34° 41'N, 133° 56'E). In the Hino-yosui, Ara-kawa, and Asahi-gawa populations, many males as well as females were observed: the ratio of males to females almost one to one. On the other hand, a unisexual Chikuma-gawa population (Chikuma, Nagano Prefecture: 36° 32'N, 138° 6'E) was examined. Wherein no males were found indicating a fully unisexual population (Tojo *et al.*, 2006).

We collected ten final instar female nymphs from the Hino-yosui population (Sep. 1, 2006) and reared them individually in the laboratory until the subimaginal stage to ensure all were virgin females. Thereafter unfertilized eggs were collected from these ten virgin females. In the Ara-kawa and the Asahi-gawa populations, we dissected final instar female nymphs and obtained their unfertilized eggs (five female nymphs from the Ara-kawa River population: Sep. 1, 2006, and also five female nymphs from the Asahi-gawa population: Sep. 19, 2006). As a control, fertilized eggs were obtained from six mated females in the Hino-yosui popula-

tion (bisexual) and unfertilized eggs were obtained from twenty four virgin females in the Chikuma-gawa population (unisexual).

These fertilized and unfertilized eggs were incubated separately in batches at $20 \pm 0.5^\circ\text{C}$. At about three months of incubation, successful development rates were analyzed for each female's egg batch of fertilized or unfertilized eggs collected from the four different populations. During embryogenesis of this mayfly, diapause commences during the last embryonic stage (Watanabe *et al.*, 1993; Nakamura *et al.*, 1999; Nakamura and Endo, 2001): equivalent to "stage 13" in embryos of a closely related burrowing mayfly *Ephemera japonica* (cf. Tojo and Machida, 1997a, b), and release of diapause (*i.e.*, hatching) requires a change in environmental conditions like water temperature and light conditions (under the incubated conditions in the laboratory, a long incubation time was required for completion of hatching). Differences were observed in developmental and successful hatching rates during short-term experiments over a range of several months to a half-year. Under natural conditions, several years may be required for hatching, in rare cases (Sekiné *et al.*, 2007). Consequently, the development rate of eggs was focused upon in this study rather than the hatching rate. Judging as to whether an egg was developing or not was relatively simple because the developing and undeveloped eggs are clearly differing in color (Tojo *et al.*, 2006).

Chromosome Number Analysis

Upon hatching of the nymphs, chromosome preparations were made and chromosomes were counted for each of the respective offspring. The nymphs were prepared on slides wounded with a needle and forceps and incubated for 30 minutes in ion-exchange water containing 0.005% colcemide. The nymphs were then fixed with fixative solution I (acetic acid : ethanol : ion-exchange water = 1 : 1 : 2). A drop of fixative solution II (acetic acid : ethanol = 1 : 1) was placed on each nymph before it was macerated with a needle and forceps while fixative solution I was spreading over the slide. After one minute, glacial acetic acid was dropped onto the slides which were then air-dried. These air-dried chromosome preparations were stained with 3% Giemsa solution (Nacalai Tesque) in phosphate buffer (Sigma-Aldrich Japan; pH 6.4) for 30 minutes. The slides were then dipped in the water for one second, and air-dried. Chromosome spreads on the slides were mounted in a mounting agent (Entellan New, Merck) and photographed under a microscope at $1000\times$ magnification.

Results

Table 1 summarizes the developmental rate of fertilized and unfertilized egg batches of *Ephoron shigae* collected from

Table 1. Developmental rate of fertilized or unfertilized eggs of *Ephoron shigae*

	No. of females examined	No. of eggs examined	Percentage of embryos developing to the final embryonic stage	
			Mean \pm SD	Minimum-Maximum
Hino-yosui Irrigation Canal ^a				
fertilized eggs	6	32289	98.1 \pm 1.9	94.8-99.9
unfertilized eggs	10	23956	14.1 \pm 13.5	0.3-34.7
Chikuma-gawa River ^b				
unfertilized eggs	23	66698	89.2 \pm 4.6	76.2-96.9
Ara-kawa River ^a				
unfertilized eggs	5	7425	21.5 \pm 27.5	0-53.3
Asahi-gawa River ^a				
unfertilized eggs	5	5881	3.0 \pm 4.0	0-8.0

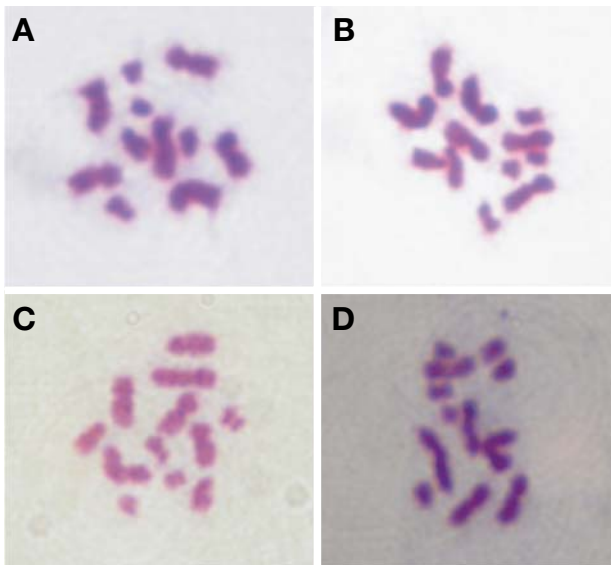
^aBisexual population^bUnisexual population: no males were found

Fig. 2. The mitotic metaphase chromosomes prepared from nymphs of the mayflies, *Ephoron shigae*. A, Chromosomes of 2n=11, obtained from a fertilized nymph in the bisexual population (*Ephoron shigae*; Hino-yosui), B, Chromosomes of 2n=12, obtained from a fertilized nymph in the bisexual population (*Ephoron shigae*; Hino-yosui), C, Chromosomes of 2n=12, obtained from a parthenogenetic nymph in the bisexual population (*Ephoron shigae*; Hino-yosui), D, Chromosomes of 2n=12, obtained from a parthenogenetic nymph in the unisexual population (*Ephoron shigae*; Chikuma-gawa).

four different populations. In the bisexual population (Hino-yosui flume) of *E. shigae*, the fertilized eggs from six mated females developed almost to the final embryonic stage in three months. The percentage of embryos developing to the final stage was $98.1 \pm 1.9\%$ (mean of three egg batches \pm SD). Whereas the development rate of embryos to the final stage in the unfertilized egg batches was $14.1 \pm 13.5\%$ (ten batches; there were no batches with a developmental rate of zero). The developmental rates of unfertilized egg batches in Ara-

Table 2. Chromosome numbers of *Ephoron shigae*

Population	Mode of reproduction	Number of specimens examined		
		Total	2n=11 (♂)	2n=12 (♀)
Hino-yosui	Sexual	6	2	4
Hino-yosui	Parthenogenetic	33	0	33
Chikuma-gawa	Parthenogenetic	8	0	8

kawa River and Asahi-gawa River populations were $21.5 \pm 27.5\%$ (five batches) and $3.0 \pm 4.0\%$ (five batches), respectively. In the unisexual Chikuma-gawa River's population, the developmental rate of unfertilized egg batches was $87.8 \pm 7.0\%$ (twenty four batches; this data included prior data collected in Tojo *et al.* [2006], $89.0 \pm 4.6\%$ for twenty batches).

For nymphs hatched from fertilized eggs in the Hino-yosui *E. shigae* population, either eleven or twelve mitotic metaphase chromosomes were counted. However, amongst nymphs parthenogenetically hatched from unfertilized eggs in the Hino-yosui *Ephoron shigae* population, only 12 chromosomes were observed (Fig. 2, Table 2).

Discussion

In the present study, the parthenogenetic ability of virgin females in bisexual populations of *E. shigae* is investigated for the first time, and the parthenogenetic development rates of unfertilized eggs were considerably lower than that of virgin females in the unisexual populations (Table 1). In Hino-yosui population, although the parthenogenetic development rates varied widely, parthenogenetic ability was recognized in all of the individuals examined (n=10). In Ara-kawa population, the parthenogenetic development rates of two egg batches were relatively high (53.3% and 49.8%), while two other egg batches failed to develop at all. For these egg batches, we obtained eggs from final instar nymphs (female), ra-

ther than obtaining and dissecting matured and unfertilized eggs from winged (subadult) virgin females. Therefore, the parthenogenetic abilities of the Ara-kawa and the Asahi-gawa populations may be higher than indicated in the above results.

In *Ephoron shigae*, Sekiné and Tojo (2007) indicated that all parthenogenetic offspring from the unisexual populations were female ($2n=12$), and that the reproductive mode of the unisexual populations is a diploid thelytokous parthenogenesis. The present result also indicates that all of the parthenogenetic offspring from bisexual population are females ($2n=12$; Fig. 2, Table 2), similar to parthenogenetic mode of the unisexual populations. Consequently, the parthenogenetic ability of bisexual populations would be relevant in the evolutionary path to parthenogenetic evolution of unisexual populations.

Thelytokous parthenogenesis is divided into two main types from a cytogenetic standpoint as follows (White, 1973); (1) automixis or meiotic thelytoky, in which completely meiosis occurs in oogenesis, but is compensated for by a doubling of the chromosome number at some stage in the life cycle, and (2) apomixis or ameiotic thelytoky, in which meiosis has been entirely suppressed, where the maturation division or divisions in the oocyte is mitotic in character (*i.e.*, without any formation of bivalents). However, the process and mechanisms of egg maturation and parthenogenetic recovery of diploidy have not been studied, as to whether this mayfly's parthenogenesis is of the automictic or apomictic type. Hereafter, it is need that we will observe and describe the oogenesis and early embryogenesis of this mayfly.

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References

- Cuellar O (1977) Animal parthenogenesis. *Science* **197**: 837-843.
- Nakamura K, Endo T (2001) Development of mayfly eggs in the river laid at different period. *Proceedings of the Arthropodan Embryological Society Japan* **36**: 17-19. (in Japanese).
- Nakamura N, Tsurumaru S, Kawamura K (1999) Effects of temperature and light on the embryogenesis of genus *Ephoron* mayflies (Insecta: Ephemeroptera, Polymitarcyidae). *Proceedings of the Arthropodan Embryological Society Japan* **34**: 11-16.
- Sekiné K, Tojo K (2007) A comparative study of the number of chromosomes between bisexual reproductive populations and parthenogenetic populations of the burrowing mayfly *Ephoron shigae* (Insecta: Ephemeroptera, Polymitarcyidae). *Proceedings of the Arthropodan Embryological Society Japan* **42**: 7-8.
- Sekiné K, Tsuruda D, Tojo K (2007) Life cycle of the burrowing mayfly *Ephoron shigae* (Ephemeroptera, Polymitarcyidae) in Hino-yosui population of the Tama-gawa River basin. *Japanese Journal of Limnology* **68**: 253-260. (in Japanese with English abstract).
- Suomalainen E, Saura A, Lokki J (1987) Cytology and Evolution in Parthenogenesis. CRC Press, Boca Raton.
- Tojo K, Machida R (1997a) Embryogenesis of *Ephemera japonica* McLachlan (Insecta: Ephemeroptera). *Proceedings of the Arthropodan Embryological Society Japan* **32**: 25-28.
- Tojo K, Machida R (1997b) Embryogenesis of the mayfly *Ephemera japonica* McLachlan (Insecta: Ephemeroptera, Ephemeridae), with special reference to abdominal formation. *Journal of Morphology* **234**: 97-107.
- Tojo K, Sekiné K, Matsumoto A (2006) Reproductive mode of the geographic parthenogenetic mayfly *Ephoron shigae*, with findings from some new localities (Insecta: Ephemeroptera, Polymitarcyidae). *Limnology* **7**: 31-39.
- Watanabe NC, Ishiwata S (1997) Geographic distribution of mayfly, *Ephoron shigae* in Japan, with evidence of geographic parthenogenesis (Insecta: Ephemeroptera: Polymitarcyidae). *Japanese Journal of Limnology* **58**: 15-25.
- Watanabe NC, Nakamura K, Hatta K, Hisada K, Ishiwata S, Hoshi K (1993) Studies on the mechanism of mass emergence of mayflies. Research Projects in Review 1993. *Nissan Science Foundation* **16**: 151-162. (in Japanese with English abstract).
- White M JD (1973) Animal cytology and evolution 3rd ed. Cam. Univ. Press, London.