

[SHORT COMMUNICATION]

# A Comparative Study of the Number of Chromosomes Between Bisexual Reproductive Populations and Parthenogenetic Populations of the Burrowing Mayfly *Ephoron shigae* (Insecta: Ephemeroptera, Polymitarcyidae)

Kazuki SEKINÉ<sup>1)</sup> and Koji TOJO<sup>2,3)</sup>

<sup>1)</sup> Department of Environmental System Science, Graduate School of Science and Technology, Shinshu University, Asahi 3–1–1, Matsumoto, Nagano 390–8621, Japan

<sup>2)</sup> Department of Biology, Faculty of Science, Shinshu University, Asahi 3–1–1, Matsumoto, Nagano 390–8621, Japan

<sup>3)</sup> Institute of Mountain Science, Shinshu University, Asahi 3–1–1, Matsumoto, Nagano 390–8621, Japan

E-mail: ktojo@shinshu-u.ac.jp (KT)

## Introduction

The burrowing polymitarcyid mayfly *Ephoron shigae* (Takahashi) is distributed widely in Japan. Some populations are bisexual, and others are unisexual (solely females). Our previous studies presented experimental evidence for the geographic parthenogenesis in this mayfly (Tojo *et al.*, 2002, 2006). We considered that the parthenogenesis in this mayfly species most probably began in originally bisexually reproducing populations, because each egg from females in unisexual populations has well-developed micropyles.

In general, parthenogenetic populations are often found within harsh environments such as at higher latitudes and altitudes, in xeric as opposed to mesic ecosystems (Suomalainen, 1950, 1962; Glesener and Tilman, 1978; Suomalainen *et al.*, 1987), some isolated conditions such as on islands and island-like habitats, and the periphery of a species distribution area (Cuellar, 1977). In *Ephoron shigae*, however, the distributions of the bisexual and unisexual populations overlap broadly in their geographic ranges. Previous studies (*e. g.*, Watanabe and Ishiwata, 1997) stated no difference in topographical or hydrological characteristics in any environmental data between the habitats (rivers) of unisexual and bisexual populations.

From the above mentioned background, *Ephoron shigae* may provide a good means of studying a differentiation process between the unisexual and bisexual populations, the establishment of parthenogenesis, and the dispersal of parthenogenetic individuals. In this paper, we compared the reproduction modes between the bisexual and unisexual populations, with special reference to the number of chromosomes.

## Materials and Methods

The Abukuma-gawa river (Fukushima City, Fukushima Prefecture) and Hino-yosui (artificial waterways of the Tama-gawa river basin: Hino City, Tokyo Prefecture) populations of *Ephoron shigae* were studied as representatives of bisexual populations. The Natorigawa river and Hirose-gawa river populations (Sendai City, Miyagi Prefecture) and Chikuma-gawa river population (Chikuma City, Nagano prefecture) were studied as representatives of unisexual populations.

Eggs were obtained from virgin or mated females in the respective populations, and incubated separately in batches (immersed in distilled water [DW], at 20±0.5°C in a thermostatic room) until the early katarptysis-stage equivalent to stage 10 in the embryogenesis of a closely related burrowing mayfly *Ephemera japonica* (Tojo and Machida, 1997).

The embryos were dissected from the chorion and incubated for 40–60 min in a hypotonic solution (10% Ephrussi-Beadle solution [0.75% NaCl + 0.035% KCl + 0.021% CaCl<sub>2</sub>; cf. Tojo and Machida, 2003] which is diluted with DW) containing 0.005% colchicin or colcemide. The embryos were then individually placed on slides and macerated with a needle in a drop of fixative solution I (acetic acid : ethanol : DW = 3 : 3 : 4). Several minutes later, fixative solution II (acetic acid : ethanol = 1 : 1) was dropped onto the slides. After 1 min, 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 10 min. Then, the slides were dipped in the DW for 1 sec, and air-dried. Chromosome spreads on the slides were mounted in a mounting agent (Entellan New, Merck) and photographed under a microscope at 1000x magnification.

## Results and Discussion

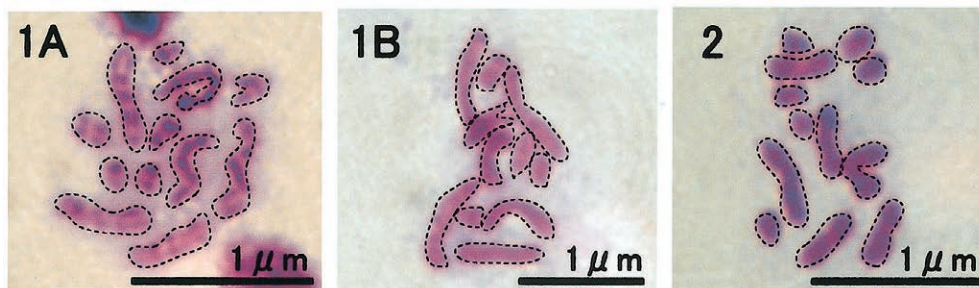
In the two bisexual populations of *Ephoron shigae*, either eleven or twelve mitotic metaphase chromosomes were counted amongst the embryos originated from 5 mated females in the Abukuma-gawa population, and from 6 mated females in the Hino-yosui population (Fig. 1A, B, Table 1). In the three unisexual populations (Natori-gawa, Hirose-gawa and Chikuma-gawa), however, only one chromosomal type (twelve chromosomes) was observed amongst the embryos originated from 20 virgin females in the Natori-gawa population, 14 virgin females in the Hirose-gawa population, and 8 virgin females in the Chikuma-gawa population (Fig. 2, Table 1).

Consequently the numbers of chromosomes of *Ephoron shigae* are considered to be  $2n=11$  (male) and  $2n=12$  (female). The sex chromosome types thus may be XX (female) and XO (male). This interpretation is in agreement with earlier observations of *Ephoron shigae* and *Ephoron eophilum* (Aoyagi, 1994), and of some species in the closely related family Ephemeridae (Soldán and Putz, 2000). The present results also indicate that the embryos from the three unisexual populations are all females ( $2n=12$ ), and that the reproductive mode of the unisexual populations is a diploid thelytokous parthenogenesis.

It remains to be determined whether this parthenogenesis is of the automictic or apomictic type. We have started histological studies on the oogenesis of *Ephoron shigae* in the unisexual populations, with special reference to meiosis; whether it occurs or not, and if it does, how it occurs.

Table 1 Chromosome numbers of *Ephoron shigae*

Population	Number of specimens examined		
	Total	$2n = 11$ (♂)	$2n = 12$ (♀)
<b>Bisexual population</b>			
Abukuma-gawa	5	3	2
Hino-yosui	6	2	4
<b>Unisexual population</b>			
Natori-gawa	20	0	20
Hirose-gawa	14	0	14
Chikuma-gawa	8	0	8



Figs. 1–2 The mitotic metaphase chromosomes prepared from embryos of *Ephoron shigae*.

Fig. 1 Chromosomes of  $2n=12$  (A) and  $2n=11$  (B), obtained from an embryo in the bisexual population (Hino-yosui).

Fig. 2 Chromosomes of  $2n=12$ , obtained from an embryo in the unisexual population (Chikuma-gawa).

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