

## Potential for Parthenogenesis of Virgin Females in the Mayfly *Ephoron eophilum* (Insecta: Ephemeroptera, Polymitarcyidae)\*

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*Ephoron shigae* is known to be a geographically parthenogenetic species. That is, some populations are bisexual, and others are unisexual (solely females) (Watanabe and Ishiwata, 1997; Tojo *et al.*, 2006; Sekiné and Tojo, 2010a). In the previous study on *E. shigae*, obligatory parthenogenesis (parthenogenesis as the normal mode of reproduction) appeared in the unisexual populations (Tojo *et al.*, 2006). In the populations, the parthenogenetic ability was comparatively high; more than 80% of unfertilized eggs were developed successfully (Tojo *et al.*, 2006). Furthermore, it was revealed that all females reproduced via parthenogenesis were diploid, and the process of parthenogenetic recovery of diploidy was of the automictic type categorized in the typical ‘terminal fusion’ pattern (Sekiné and Tojo, 2010b).

Based on our other haplotype analysis study of mitochondrial DNA (in the 16S rRNA and the COI regions), it has become clear that the unisexual populations scattered throughout Japan are of singular (monophyletic) origin, and its ancestor originates in the western region of Japan (Sekiné *et al.*, in preparation). And, not only in the unisexual populations, but also in the bisexual populations, parthenogenetic ability of the virgin females was also observed (Sekiné and Tojo, 2010a). However, in this case, the average success rate of parthenogenetic development of unfertilized eggs was significantly lower than that of virgin females in the unisexual populations. All individuals reproduced by parthenogenesis in this population were also diploid females (Sekiné and Tojo, 2010a). Accordingly, we suggested that parthenogenetic ability (whether tychoparthenogenesis or facultative parthenogenesis) in bisexual populations of *E. shigae* may facilitate the evolutionary transition to unisexual populations with fully obligatory parthenogenesis.

Parthenogenesis has been reported in some

mayflies (Degrange, 1960; Huff and McCafferty, 1974; Gibbs, 1977; Humpesch, 1980; Sweeney and Vannote, 1987; Glazaczow, 2001; Ball, 2001, 2002). And even in some bisexual mayfly species, the unfertilized eggs of the virgin females often develop parthenogenetically. In most cases, less than 10% of them possess the tychoparthenogenetic ability (Huff and McCafferty, 1974; Templeton, 1982; Ball, 2001). In contrast to these cases, however, the parthenogenetic ability and the presence of its potential in virgin females of bisexual populations of *E. shigae* is considered to be quite high (Sekiné and Tojo, 2010a). For example, in the three bisexually reproducing species (*Ephemera japonica*, *Ephemera strigata*, and *Ephemera orientalis*) the success rates of parthenogenetic development in unfertilized eggs were significantly lower than those of virgin females in bisexual populations of *E. shigae* (Sekiné and Tojo, 2010a). As the parthenogenetic abilities in a facultatively parthenogenetic situation were high in bisexual populations of *E. shigae*, the potential to evolve from a bisexual to a unisexual population may be higher in *E. shigae* than in other mayflies. Is this trend peculiar to *E. shigae* among mayflies, or not?

Here we have studied the parthenogenetic potential in virgin females of a bisexually reproducing species, *Ephoron eophilum*, one of the most closely-related species to *E. shigae*. From the comparison between these two species, we discuss the origin of the obligatory parthenogenesis in *Ephoron* mayflies.

We incubated the unfertilized egg-batches of seven virgin females of the bisexual species, *E. eophilum*, from the Kinu-gawa River population (Jyoso, Ibaraki Prefecture). The average rate of the embryos developed to the final stage was  $16.7 \pm 26.8\%$ . Among these egg batches, however, two particular egg batches showed extremely high rates, 70.2% and 34.7%.

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In the nymphs developed parthenogenetically in the Kinu-gawa River population of *E. eophilum*, only 16 chromosomes were noted ( $n = 18$ ; the number of nymphs examined was 18). However, either 15 or 16 mitotic metaphase chromosomes were counted in nymphs originating from mated females of this species. The sex chromosome(s) in Ephemeroptera are homogametic (XX) in females, and are heterogametic (XY or XO) in males (White, 1973). Accordingly, the number of chromosomes in *E. eophilum* are considered to be  $2n = 15$  (male) and  $2n = 16$  (female). In parthenogenetic females of *E. eophilum*, 14 diploid autosomes and the homogametic sex chromosomes were observed ( $2n = 16$ ). Therefore, the parthenogenetic mode of this mayfly may be a diploid thelytokous parthenogenesis as in *E. shigae*.

From these results, it is considered that the high parthenogenetic development rate, more than 10%, of *E. eophilum* unfertilized eggs, which in fact exceeds that of *E. shigae*, is not species specific in *E. shigae*.

As the life spans of adult *E. shigae* and *E. eophilum* are extremely short (less than two hours), some females may die before the copulation. Furthermore, females of these species often discharge eggs with minimal external stimuli (e. g., changes of moisture or a touch on the body) even before mating. In the *E. eophilum* specimens individually reared, all of the examined virgin female subimagos actually oviposited as a result of the stimulus of their landing on water. Therefore, the parthenogenetic ability may have made females produce some measure of reproductive product without mating.

There are several remaining issues related to the viability of offspring (e. g., the hatching rate of parthenogenetically developed embryos and the survival rate of hatched nymphs). If these rates are remarkably high and the high rates descend to the following generations, the proportion of eggs developing via parthenogenetic reproduction may have gradually increased through generations and the female ratio of the population gradually become dominant. A transition from the tychopterogenesis to the full obligatory partheno-

genesis through the facultative parthenogenesis is hypothesized in some populations of *E. eophilum*, as in those of *E. shigae*.

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