The Origin and Distribution Expansion of Unisexual Populations in the Geographically Parthenogenetic Mayfly, *Ephoron shigae* (Insecta: Ephemeroptera: Polymitarcyidae)*

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The burrowing polymitarcyid mayfly Ephoron shigae (Takahashi) is distributed widely in Japan, and it is also reported to be present in Korea and Far East Russia. Some populations are bisexual, while others are unisexual (solely females). As such E. shigae is a geographically parthenogenetic mayfly (Watanabe and Ishiwata, 1997; Tojo et al., 2006). In general, parthenogenetic populations are found in harsher environments such as at high latitudes and altitudes, in xeric as opposed to mesic conditions (Suomalainen et al., 1987), in isolated habitats such as islands and island-like areas, and in the peripheral regions of the taxon's range (Cuellar, 1977). In E. shigae, however, the distributions of bisexual and unisexual populations overlap broadly in their respective geographic ranges through Japan (Honshu, Shikoku, and Kyushu). E. shigae therefore, provides a uniquely well-suited model to study the differentiation of unisexual and bisexual populations, the establishment of parthenogenesis, and the dispersal of parthenogenetic individuals.

In a previous study on *E. shigae*, obligatory parthenogenesis (*i. e.*, parthenogenesis is the normal mode of reproduction) appeared in unisexual populations (Tojo *et al.*, 2006). Furthermore, all the individuals that reproduced by parthenogenesis were diploid females (2n=12; sex chromosome type was XX), indicating thelytokous parthenogenesis (Sekiné and Tojo, 2007, 2010). The restoration mode into diploidy in parthenogenetic eggs is automictic in that the female pronucleus fuses into the second polar body following the completion of meiosis.

In this study, we have examined whether the unisexual populations are of polyphyletic or monophyletic origin, as inferred from partially sequenced mitochondrial 16S rRNA (373 bp) and COI (658 bp) regions (Fig. 1). Although various haplotypes [16S rRNA: 29 haplotypes, COI: 58 haplotypes] were observed among the bisexual populations, only one haplotype each (16S rRNA: one haplotype, named "16S_s1"; COI: one haplotype, named

"COI_s1") was found among the unisexual populations through Japan widely. The "16S_s1" and "COI_s1" haplotypes were allocated to each of the western Japanese clades (group I; Fig. 1). Therefore, the unisexual populations were of monophyletic origin and recently differentiated somewhere within western Japan. Furthermore, the distribution range of parthenogenetic strains has expanded rapidly across Japan, especially eastern Japan.

Parthenogenetic individuals that enter a bisexual population or a new locality are fully able to reproduce without a mate. Conversely, the efforts of males that mate with a parthenogenetic female are wasted. That is, the parthenogenetic individuals that enter a bisexual population possess the superior reproductive strategy. Furthermore, in order for the range of a bisexual population to expand, at least one pair of male and female must disperse simultaneously. In the case of a parthenogenetic strain, however, even if only one individual relocates, she is able to reproduce, and so establish a population. Therefore, parthenogenetic strains naturally have greater dispersal and establishment potential than bisexual strains.

The results of our genetic analysis indicated that the parthenogenetic strains are monophyletic in origin, and originated in western Japan, from where they must have expanded into eastern Japan.

In the Abukuma-gawa River (Fukushima Prefecture) and the Ara-kawa River (Saitama Prefecture) bisexual populations of eastern Japan, female individuals having the "s1-haplotype", which is common to parthenogenetic strains, were observed. Within these populations, the sex ratio in the Ara-kawa population was significantly biased toward females ($\mathcal{P}: \mathcal{J} = 102:58$, binomial test p < 0.05) as of 2006. The Ara-kawa River has a mixed unisexual/bisexual population that is considered to have resulted from the invasion of the originally bisexual population by a parthenogenetic strain. In the Abukuma-gawa population (locality: Fukushima City), Watanabe *et al.* (1998) reported

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Fig. 2 Sex ratios and composition of haplotypes of the mayfly *Ephoron shigae* at four study sites along the Abukuma-gawa River. Although research into sex ratios was conducted in both 2009 and 2010, similar results were obtained. Only female individuals were used in the haplotype analysis based on the COI mitochondrial DNA region. The haplotype categorized as "COI_s1" and other haplotypes are displayed separately as distinct groups. The "COI_s1" haplotype is common to all of the specimens from the parthenogenetic populations, and the other haplotypes were referred to collectively, as they together represent the haplotypes of the bisexual populations of eastern Japan.

that the sex ratio was not significantly biased toward females and that many male individuals were observed in 1991 and 1992. However, in our recent experience at the same site, almost all individuals were female: $\hat{\gamma} : \hat{\sigma} = 100:0$ in 2009 (p < 0.01), and $\hat{\gamma} : \hat{\sigma} = 105:1$ (p < 0.01) as per our 2010 observations (Fig. 2). Also, of great interest, the ratio of males tended to increase upstream, even within the same river system [Fig. 2; *e. g.*, in the uppermost population located in Nakajima village (ca. 60 km upstream from Fukushima City), $\hat{\gamma} : \hat{\sigma} = 50:74$ in 2009, and 61:64 in the 2010 observations].

Furthermore, an analysis based on the mitochondrial region of COI revealed the "COI_s1" haplotype to be significantly more frequent than non "COI_s1" females in the downstream populations (Fig. 2). Whereas in the upstream populations, the opposite pattern was observed. Therefore, in the Abukuma-gawa River, parthenogenetic individuals must have entered and established themselves first within the downstream population in or around Fukushima City, which has since come to be almost completely composed of parthenogenetic females in less than 20 years. The increasing parthenogenetic bias in the sex ratio of the local populations within this river system is increasing progressively starting downstream and rapidly moving upstream. This situation represents a very important example of the replacement phase of the transition from a bisexual population to a fully parthenogenetic female-only population. This process, which may have occurred or presently be happening in other parts of Japan, would explain the formation of other parthenogenetic populations across Japan.

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