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Spermatophores in the female's bursa copulatrix accumulated through their life span in three species of *Lepidostoma* (Trichoptera, Lepidostomatidae)

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Abstract

In order to estimate mating frequencies of females, spermatophores stored in the bursa copulatrix were examined in three Asian species of the genus *Lepidostoma* Rambur (Lepidostomatidae): *L. complicatum* (Kobayashi 1968), *L. satoi* (Kobayashi 1968), and *L. itoae* (Kumanski & Weaver 1992), during their flight seasons of 2011 to 2016. In all three species, several spermatophores were detected, particularly in late seasons, suggesting that multiple mating occurs in these caddisflies.

Keywords: copulation, polyandry, protogyny, Trichoptera, *Lepidostoma*

Introduction

In most insects, males transfer sperm to the females by enclosing it within a sack, which is called a spermatophore (Khalifa 1949). The number of spermatophores in the bursa copulatrix of females shows their mating frequency, presuming a single spermatophore is transferred at each mating and the outer part of the spermatophore remains after copulation without absorption within the bursa. While Trichoptera are known to produce spermatophores, female mating frequencies have been rarely reported (Khalifa 1949; Matsumoto & Eguchi 1990). In the present study, female mating frequencies were estimated by counting the bursal spermatophores in three Asian species of the genus *Lepidostoma* Rambur (Lepidostomatidae).

Species observed

Lepidostoma complicatum (Kobayashi), *L. satoi* (Kobayashi), and *L. itoae* (Kumanski & Weaver) were used in this study. The first two species are abundant in mountain streams of Japan, but the last one inhabits small brooklets in central to western Japan and Korea (Ito & Yamamoto 2012; Ito 1983, 2016). The life cycles of *L. complicatum* and *L. satoi* in Hokkaido, northern Japan, are univoltine with a summer emergence period and a spring emergence period, respectively (Ito 2016). The emergence patterns of these two species are protogynous; i.e., females emerge earlier than males (Ito 2016). The life cycle of *L. itoae* in Shikoku, central Japan, is also univoltine with an early summer emergence period and with males and females emerging simultaneously (Ito & Yamamoto 2012; Ito 2016).

Examination of spermatophores

Adults of *L. complicatum* were collected for about 2 h after sunset by ultra-violet lamp (20 W) along the middle reach of the small stream, Ichankoppe-zawa, Eniwa, Hokkaido, northern Japan (42º50' N, 141º24' E, 310 m above the sea level). Sampling was conducted weekly from June 22 to August 29 in 2011.

Adult *L. satoi* were collected using sweep nets along the middle reach of the small mountain stream,

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Manosawa-gawa, Bibai, Hokkaido, northern Japan (43º16' N, 141º52' E, 105 m), at weekly intervals in May 2014. Additional adults were collected slightly further upstream (145 m) twice in May 2016.

Adult *L. itoae* were collected using sweep nets at a small brooklet in Matsuyama-joshi Park, Matsuyama, Ehime, Shikoku, central Japan (33º50'43" N, 132º46'06" E, 75 m), on 6 May 2012, 15 May 2013, and 7 May 2014.

Field-captured adults were individually put in small plastic vials (30 mm in diameter, 50 mm deep), cooled (ca. 10˚C), and transferred to the laboratory in Eniwa, Hokkaido, Japan (42º53'51" N, 141º34'05" E, 33 m). Within 24 h, after killing by pressing their thoraces lightly, female specimens were dissected carefully using a needle and internal reproductive organs were transferred to physiological saline for insects (Ephrussi-Beadle solution: 7.5 g NaCl, 0.35 g KCl, 0.21 g $CaCl_2$ in 1000 ml water), and then the spermatophores within the bursa were counted under a stereoscopic microscope (×10 to 20). At the same time, developmental stages of ovaries were recorded according to Novák and Sehnal (1963) and Gower (1967): Stage A (ovaries of immature females) in which ovarioles are very thin and eggs are of similar size; Stage B (ovaries in the middle period of their development) in which the first row of several eggs in each ovariole are markedly larger than the others, but the eggs are not yet mature; Stage C (ovaries just prior to oviposition) in which the first several eggs in each ovariole are completely developed and mature eggs frequently fall out of the ovariole during dissection; and Stage D (ovaries after oviposition) in which only a few or no mature eggs are left in the ovary. Stages A, B, C, and D are referred to as A, B, C, and D in the results below.

Results

In all three species, spermatophores were semitransparent and 0.2 mm in diameter (Fig. 1A) and included in the bursa copulatrix (Fig. 1B).

Adults of *L. complicatum* were collected from 29 June to 17 August with the maximal number on 19 July 2011, but not on 22 June and 29 August (Fig. 2A). The developmental stages of ovaries were B on 29 June, followed by C in and after 6 July, and finally D on 17 August (Fig. 2B). Females with stage A ovaries were not collected. The number of spermatophores increased with development of the ovaries: 0–1 in ovary stage B, 0–3 in C, and 1–4 in D (Fig. 2C).

Adult *L. satoi* were collected from 15 May to 31 May with the maximal number on 20 May 2014 (Fig. 3A). Females of ovary stage B were abundant in the early half of the emergence period and those of C and D increased later (Fig. 3B). Females with A were not collected. The number of spermatophores was always few (0–1 in all ovary stages), but 7 females collected at the upper reach on 26 May 2016 had two spermatophores in their bursa (Fig. 3C).

Female *L. itoae* collected in May of 2012 to 2014 had ovaries in stages C and D (Fig. 4B) and included 0–6 spermatophores in their bursa (Fig. 4C).

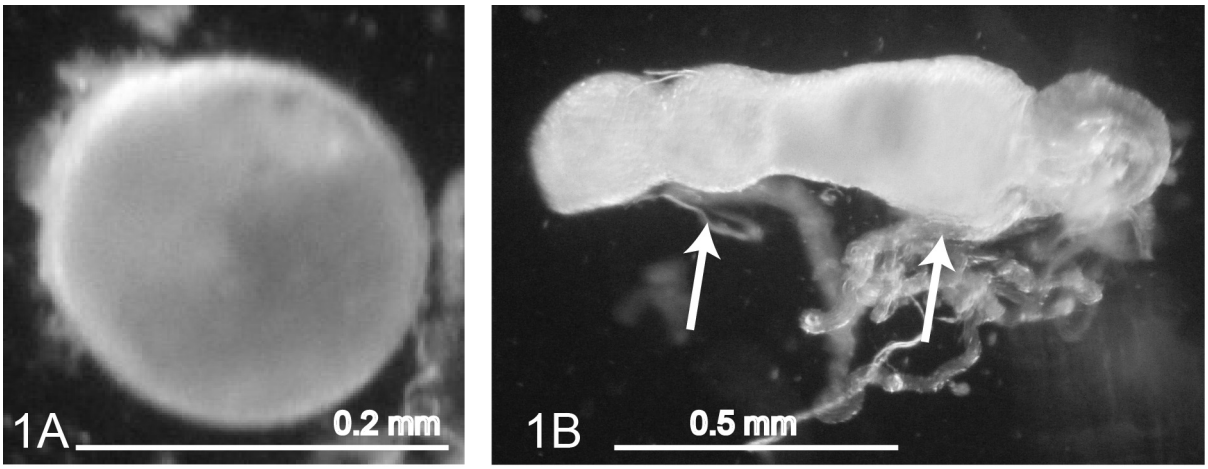

FIGURE 1. Spermatophores of female *Lepidostoma complicatum*. 1A, spermatophore; 1B, two spermatophores in the bursa copulatrix.

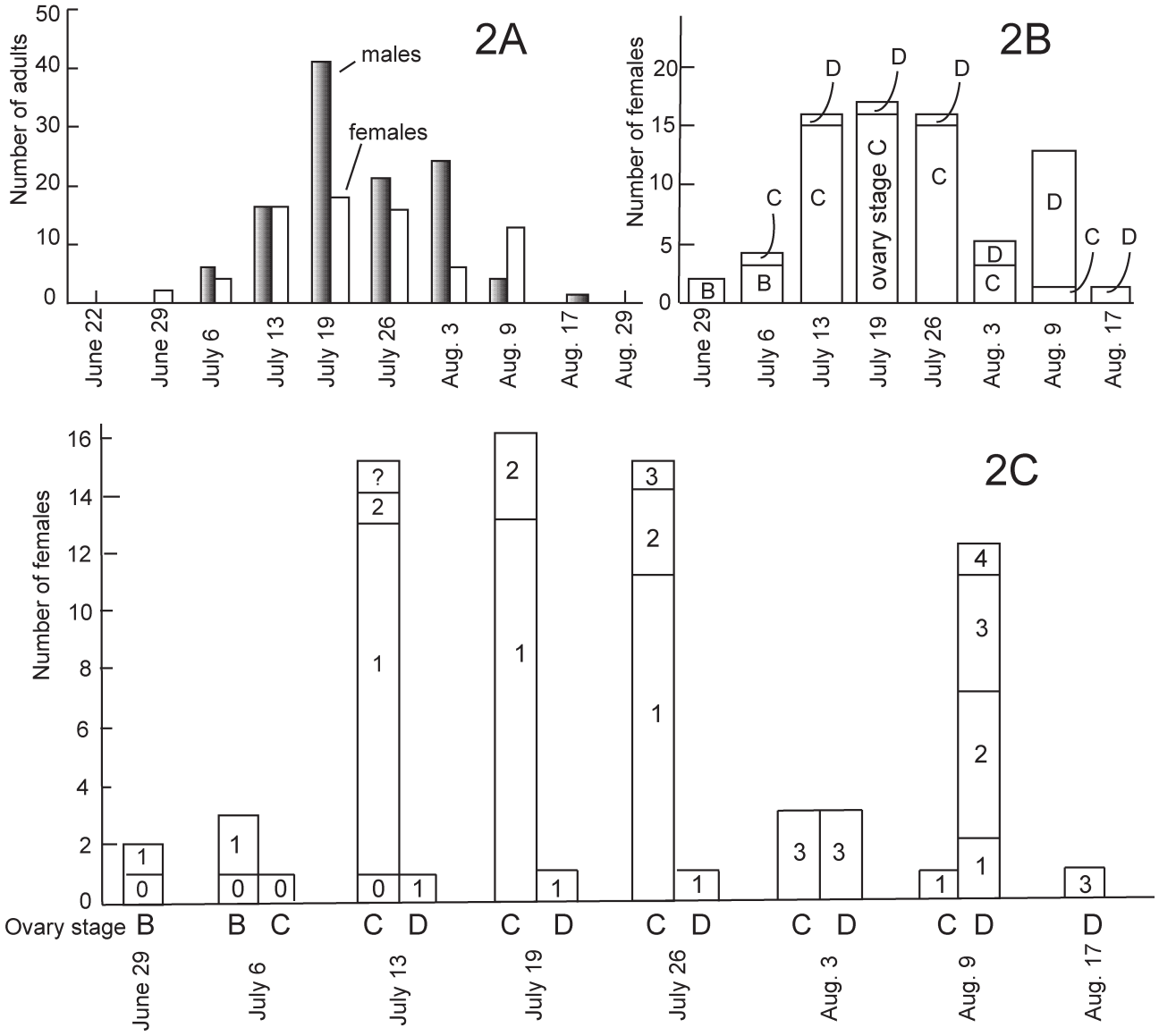

FIGURE 2. *Lepidostoma complicatum* collected from Ichankoppe-zawa from June to August in 2011. 2A, number of males and females collected; 2B, number of females with three developmental stages (B, C, and D) of ovaries; 2C, number of spermatophores in bursa (small figure in column) in each ovary stage and collecting date. "?" = number of spermatophores indistinct due to specimen condition.

Discussion

Females of most insect species mate multiple times, despite the single mating being sufficient to fertilize all eggs laid during their lifetime. Probably because females can increase their life span, egg production rate, and egg fertility by multiple matings (e.g., reviews by Arnqvist & Nilsson 2000; Simmons 2001). In Trichoptera, Petersson (1991) reported the effects of remating on the fecundity and fertilization of female *Mystacides azurea* (L.) of the family Leptoceridae. Females prevented from remating laid fewer eggs with lower fertilization rates than the remated females. In the present study, females of all three species included several spermatophores in their bursa, and therefore, multiple matings (polyandry) occurred in the field. In *Perissoneura paradoxa* (McLachlan) of the family Odontoceridae, one to three spermatophores are simultaneously included in the female bursa (Matsumoto & Eguchi 1990). Multiple mating may be common in Trichoptera, although the evolutionary significance of this behavior remains unclear.

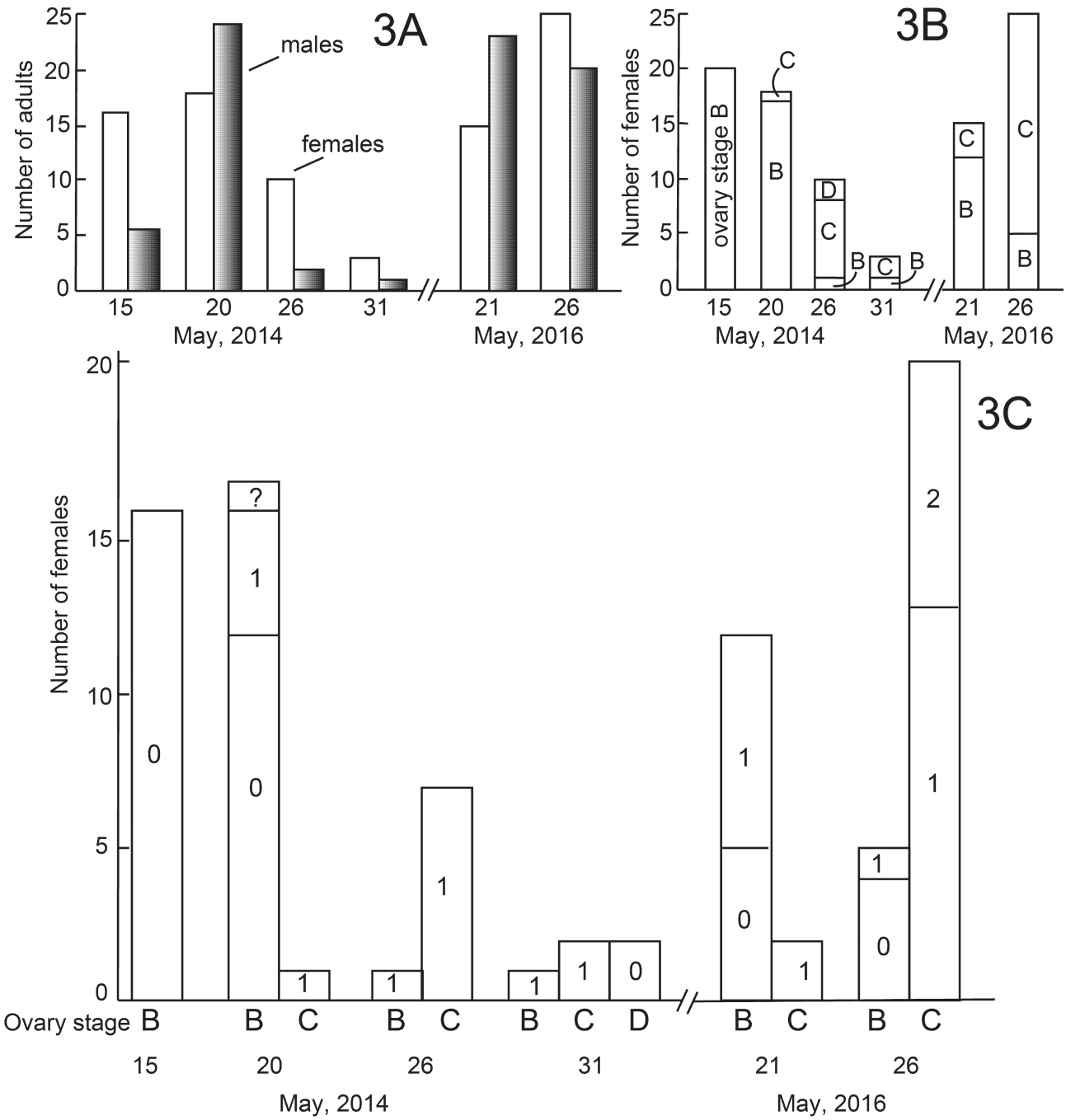

FIGURE 3. *Lepidostoma satoi* collected from Manosawa-gawa in May of 2014 and 2016. 3A, number of males and females collected; 3B, number of females with three developmental stages (B, C, and D) of ovaries; 3C, number of spermatophores in bursa (small figure in column) in each ovary stage and collecting date. "?" = number of spermatophores indistinct due to specimen condition.

From the emergence patterns of males and females in constant rearing condition, *L. complicatum* and *L. satoi* are known to be protogynous, but males and females of *L. itoae* emerge simultaneously (Ito 2016). This offers a good opportunity to compare the mating frequencies among congeneric species with sexually different emergence patterns. Protogyny is usually rare in insects (Simmons 2001). Although several species of Trichoptera have been reported to be protogynous based on daily light trapping data at riversides (Svensson 1972; Nowinszky *et al.* 2016), light trapping is not a useful method to detect protogyny because females are usually attracted to light better than males in some species (i.e., Terra 1978; Usseglio-Polatera 1987; Nozaki & Gyotoku 1990; Kiss 2012).

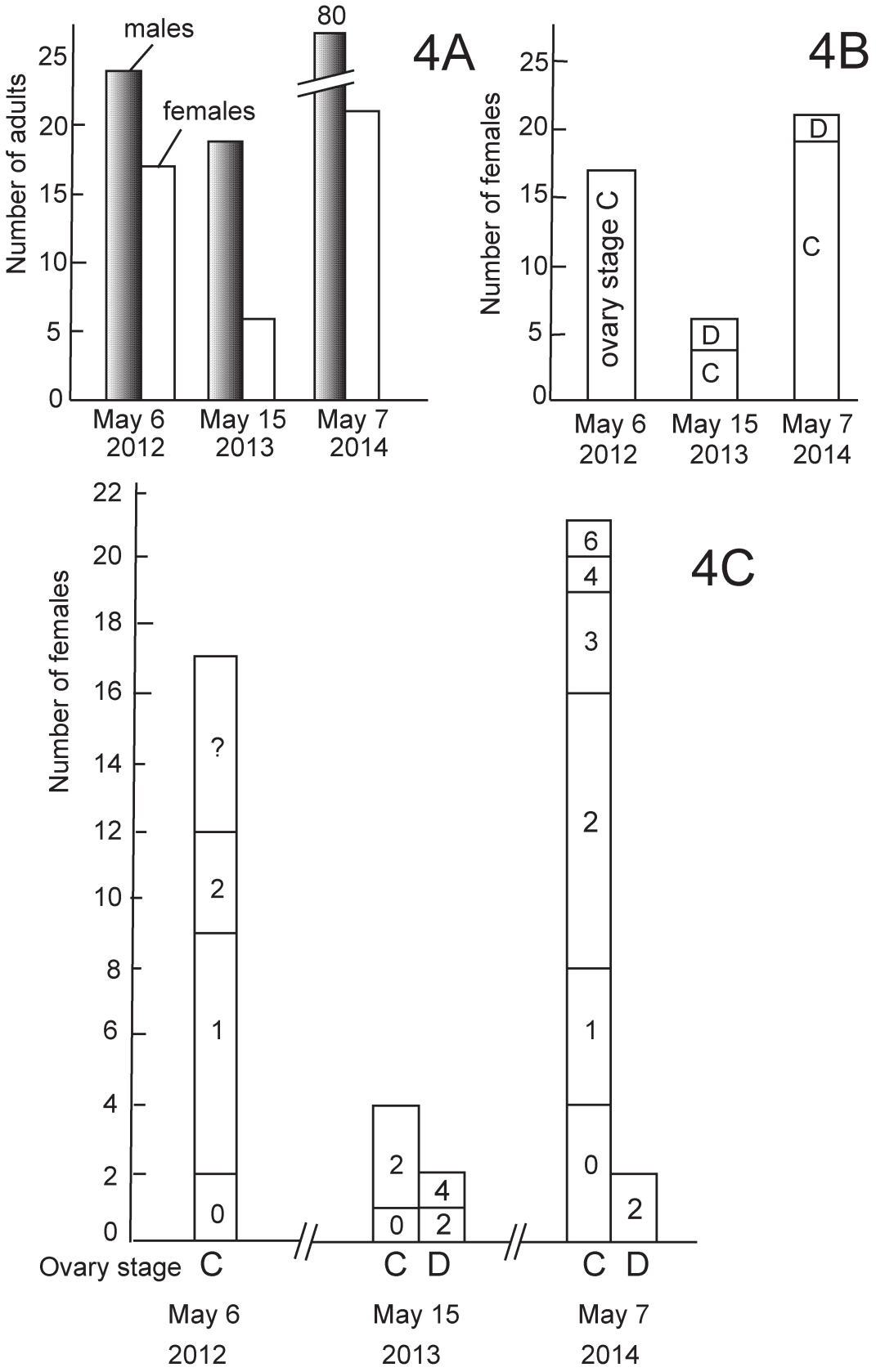

FIGURE 4. *Lepidostoma itoae* collected from a nameless small stream in Matsuyama-joshi Park in May of 2012 to 2014. 4A, number of males and females collected; 4B, number of females with two developmental stages (C and D) of ovaries; 4C, number of spermatophores in bursa (small figure in column) in each ovary stage and collecting date. "?" = number of spermatophores indistinct due to specimen condition.

In the field, females tended to appear earlier than males in protogynous *L. complicatum* (Fig. 2A). Protogyny in *L. complicatum* may be caused by early emergence of females with immature ovaries, which need 3 to 5 days for egg maturation (Ito 2016). In fact, immature females with the ovary stage B were abundant in the beginning of their emergence period in the field (Fig. 2B). Another protogynous species *L. satoi* was also female-biased in the early period (Fig. 3A). The mating frequencies of *L. satoi* were much lower than for the other two species. Since population density, operational sex ratio, and several environmental factors such as temperature and humidity may affect mating frequencies, we must pay attention to these factors in the future studies.

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