

RESEARCH PAPER

Yasuhiro Takemon

Reproductive behavior and morphology of *Paraleptophlebia spinosa* (Ephemeroptera: Leptophlebiidae): implications of variation in copula duration

Received: September 3, 1999 / Accepted: December 9, 1999

Abstract The reproductive behavior and genital morphology of a leptophlebiid mayfly *Paraleptophlebia spinosa* Ueno were studied in a population inhabiting a mountain stream in Kyoto, central Japan. Males formed swarms along gravel shores, caught females in the air, and mated on the ground. The distribution of the oviposition sites coincided with that of the swarming sites along the stream shore. The lobes of the male penis were characterized by a shallow pocket opening on the ventral side. The female vestibule formed a square-shaped chamber into which eggs were released from the oviducts. The number of eggs carried by females caught during copulation in the field showed a greater variation than the number of eggs of virgin females. Copula duration was strongly correlated with the number of eggs carried by the female. Significantly short copula duration with spent females (egg loading less than 20% of the estimated fecundity) may indicate that males could identify spent females. Copula duration with partially loaded females (egg loading between 20% and 80% of fecundity), however, was not shorter than that with fully loaded females. Male mating tactics with respect to copula duration and the possibility of kinematic sperm displacement are discussed with reference to the behavioral and morphological characteristics of the species.

Key words Reproductive behavior · Fecundity · Copula duration · *Paraleptophlebia spinosa*

Introduction

Mating in the Ephemeroptera is characterized by male swarming and copulation initiated in swarms (Brodsky

1973; Savolainen 1978; Sullivan 1981). Some workers have suggested that this mating system does not involve strong male–male competition, because the short lifespan of mayflies tends to preclude multiple copulation (Eberhard 1985; Thornhill and Alcock 1983). On the other hand, Flecker et al. (1988) argued that swarming males of a heptageniid mayfly, *Epeorus longimanus*, are under strong intermale competition. This conclusion was supported by observation of relatively long adult life and multiple copulation by males in another heptageniid, *Epeorus ikanonis* (Takemon 1993).

The occurrence of multiple copulation by males does not mean that females will also mate multiply. If most females lay all their eggs after a single copulation, the overall reproductive success of males will depend on their mating success. In such a case, it is always advantageous for males to copulate with more than one virgin female. In contrast, once multiple copulation spreads among females in the population, sperm competition becomes inevitable (Parker 1970a). The value of virgin females for males may decrease if males can displace the sperm from previous copulations (Arnqvist 1988; Choe and Crepsi 1997; Otronen 1990; Smith 1984; Waage 1979). Given the paucity of information on the reproductive ecology of mayflies in general, elucidating the possibility of female multiple copulation is crucial to understanding their mating systems. Information on the morphology of the genitalia is also needed to assess the possibility of sperm precedence in mayflies. Unfortunately, their swarm mating habits largely preclude artificial copulation under experimental conditions, and therefore the direct measurement of P2 values (sperm precedence values for egg-fertilization derived from the last copulation) through the use of sterilized males (Parker 1970b) cannot be carried out.

In the present paper, the mating and oviposition behavior and the morphology of the genitalia are described in detail for the leptophlebiid mayfly *Paraleptophlebia spinosa* Ueno. The mayfly is common in Japan, inhabiting the upper to middle reaches of mountain streams, and has a univoltine life cycle with adult emergence in early spring (Gose 1970). Variation in copula duration observed in the field was analyzed in relation to egg loading (the number of eggs in

Y. Takemon (✉)

Department of Earth and Life Sciences, College of Integrated Arts and Sciences, Osaka Prefecture University, Gakuen-cho 1-1, Sakai-shi, 599-8531 Japan
Tel. +81-722-54-9742; Fax +81-722-54-9932
e-mail: takemon@el.cias.osakafu-u.ac.jp

the oviducts), relative egg loading (egg loading as a percentage of estimated fecundity), seasonal timing, time of day, air temperature, and male and female body size. A seemingly high incidence of female multiple copulation was noted, and male mating tactics with respect to copula duration are discussed. Further, the possibility of kinematic sperm displacement, which has been found in some damselflies (Waage 1979, 1984; Robinson and Novak 1997), dipterans (Otronen and Siva-Jothy 1991), coleopterans (De Villiers and Hanrahan 1991; Yokoi 1990), orthopterans (Ono et al. 1989), and hymenopterans (Shigemura and Naito 1999), is suggested on the basis of the morphology of the genitalia, the characteristics of the mating behavior, and the oviposition habits of the species.

Materials and methods

Study sites

Field observation and sample collection were conducted at Okunomiya (elevation 340m) in the middle reaches of Kibune Stream (width 2–5 m), a tributary of the Kamo River in Kyoto City (35° 0'N, 130° 0'E). The stream had a series of small dams, and the riparian vegetation was domi-

nated by the plantation of Japanese cedar *Cryptomeria japonica*. A stream section ca. 60 m in length, with a gravel island in the middle, was selected for observations of reproductive behavior (Fig. 1). Subimagines were collected with emergence traps at Yuyaga-dani-deai (elevation 350m), 300m upstream of Okunomiya. The characteristics of Kibune Stream were described in detail in Takemon (1985). The air temperature was recorded at Okunomiya by a thermograph with remote sensors. Data on temperature and humidity at the study site throughout the study period are given in Takemon (1993).

Collection of subimagines

Subimagines were collected with 12 floating emergence traps, each with an enclosed area of 50 × 60 cm. Six traps were set on riffles and the other six on pools. The traps were cleared every day from 20 March to 21 April 1988. Because subimagines of this species emerged mainly between 10:00 and 15:00, the traps were examined after 16:00. The trapped subimagines were kept alive in cages (30 cm × 30 cm × 40 cm) near the stream at Okunomiya to determine the duration of the subimaginal stage under field conditions and to obtain virgin female specimens. The adult specimens were preserved in 75% alcohol for a later analysis of fecundity.

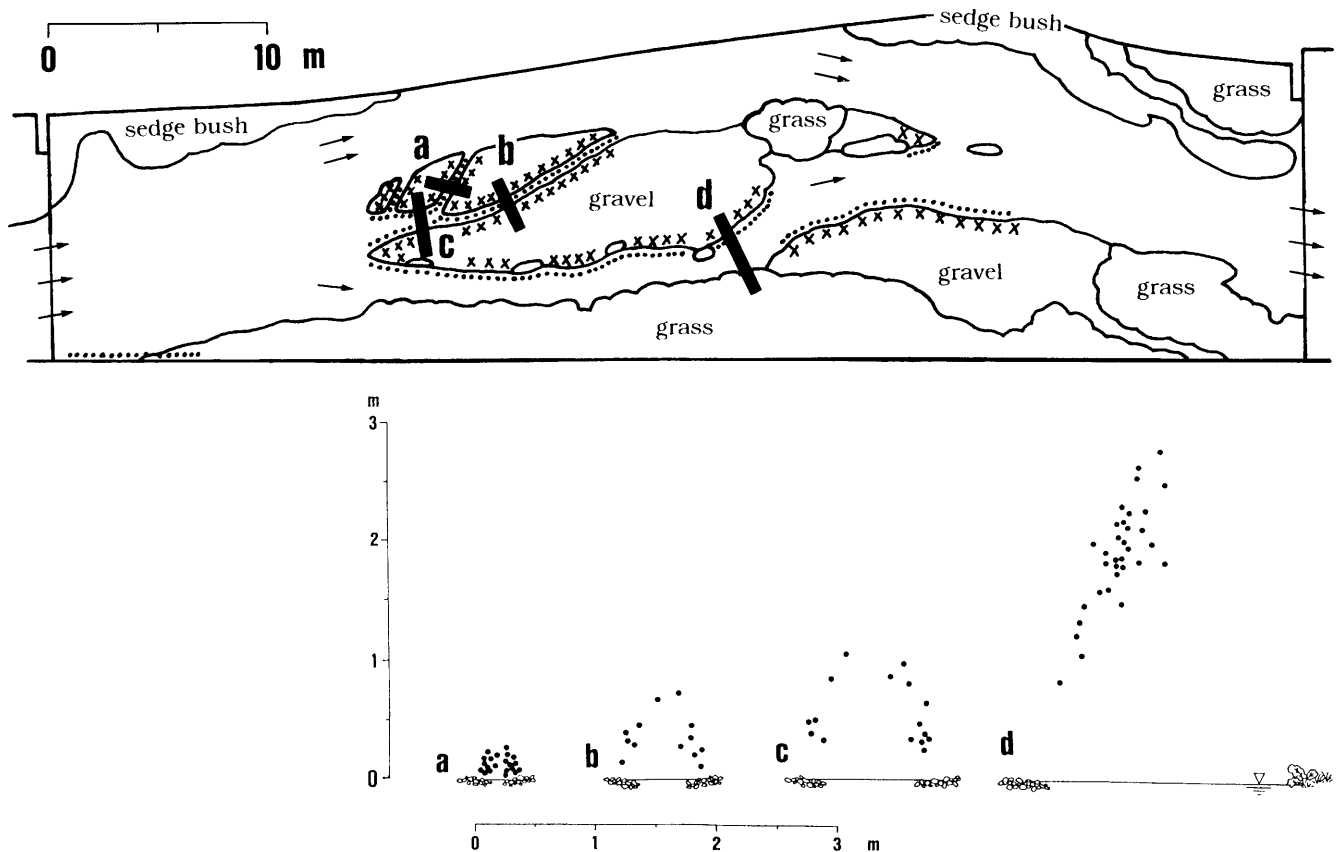


Fig. 1. Map of the study area and the distribution of male swarming sites (dots) and female oviposition sites (crosses) of *Paraleptophlebia spinosa* (top figure). Arrows indicate the direction of water flow. The lower figure shows the distribution of swarming males at different cross

sections a–d in the study area on 3 April 1988. The figure is based on photographs taken at each site, with each dot representing a swarming male

Fecundity–body size relationship

Specimens of adult females obtained from subimagines were dissected on a Petri dish under a binocular microscope. The length of the forewing was measured, and all the eggs in the oviducts were washed out in 10% glycerine–alcohol and counted with a plankton splitter. The fecundity–wing length relationship was used as a standard for estimation of the total fecundity of a field female. The females collected after mating had been observed in the field were kept in individual Petri dishes (12 cm wide and 1.5 cm high) with 50 cc of water for 24 h, during which all the females could perform oviposition. The number of eggs oviposited and those remaining in the oviducts were counted, either directly or after subsampling by a plankton splitter.

Observation of reproductive behavior

Field observations on swarming, copulation, and oviposition behavior were conducted at Okunomiya every day from 20 March to 26 April, except for 12–14 April 1988 and 24–28 March 1991. Male swarms in the observation area were monitored every day, and their locations were recorded on the map of the area. The number of swarming males at each site was counted in the early afternoon (from 12:00 to 14:00) when adult activity reached its peak. Photographs of swarming males were taken at four sites with different channel widths (Fig. 1a–d) on 3 April 1988.

Swarms at sites b and d (Fig. 1) were observed during 11:00–16:00 every day to record copulation behavior, including copula duration and the movement of the penis lobes during copulation. Because males of this species seized females in the air and mating occurred on the ground, I assumed that copulation began when a pair landed on the ground. Copula duration was measured with the use of different stopwatches for monitoring two or three copulating pairs at the same time. Individuals were captured with an insect net after separation on the ground; on return to the laboratory, their body sizes were measured and the numbers of eggs carried by the females were counted.

Analysis of copula duration

Kendall rank and partial rank correlation analyses were carried out between of copula duration and the following seven measures, based on 34 individual observations: egg loading (the number of eggs actually carried by a female), relative egg loading (egg loading as a percentage of the estimated total fecundity for a female of given body size), seasonal timing of copulation, diurnal timing of copulation, air temperature during copulation, and male and female forewing length. Differences in copula duration were also tested by the Kruskal–Wallis test and the Dunn test among three groups of females with different levels of relative egg loading: fully loaded females with over 80% of estimated total fecundity, partially loaded females with

20%–80% of estimated total fecundity, and spent females with less than 20% of estimated total fecundity.

Morphological observations

The morphology of the genitalia of each sex was observed with a binocular microscope and a stereoscopic scanning electron micrograph (JSM-5400LV, JEOL, Japan). The inner morphology of the female genitalia was observed after removing other tissues from the dorsal side of the organ with a pair of entomological pins. The structures of the genitalia were identified by reference to Needham et al. (1935) and Brinck (1957).

Results

Reproductive behavior and morphology

Emergence and swarming

Figure 2 shows the cumulative number of subimagines caught and the variation in the number of swarming males throughout the reproductive period in 1988. The emergence of subimagines of both sexes started on 20 March and ended on 3 April for males and 4 April for females. The mean emergence dates were 28 and 29 March for males and females, respectively. The subimaginal stage lasted 4 to 8 days [mean \pm SD, 5.3 ± 1.0 for males ($n = 65$) and 5.8 ± 0.9 for females ($n = 74$)].

The swarming of males occurred between 27 March and 21 April, with the number of swarming males peaking in the first half of April. No swarming was seen when the air temperature was below 10.0°C (29–31 March and 6–8 April).

Males formed swarms above the water along the gravel shoreline where oviposition also took place (Fig. 1, upper). Males showed different swarming patterns at different sites of the stream. Males located close to the shore kept low, at 5–30 cm, flying with up and down movements of short amplitudes of 5–15 cm. In contrast, males located offshore flew high, at over 100 cm, with movements of amplitudes of 60–100 cm. Swarms occurring above wider channels reached greater heights (Fig. 1, lower). Swarming continued only when the air was calm and ceased once wind started.

Mating and oviposition

Copulation was always initiated by swarming males in the air. They caught females from beneath, using their forelegs, and the pair dropped to the ground. On landing, the male seized the female's abdomen with forceps and inserted his penis into her gonopore.

Males performed a quick, piston-like movement (putting in and taking out the penis in a quick motion) throughout copulation. The entire penis disappeared in the gonopore when it was inserted, and the basal half appeared when

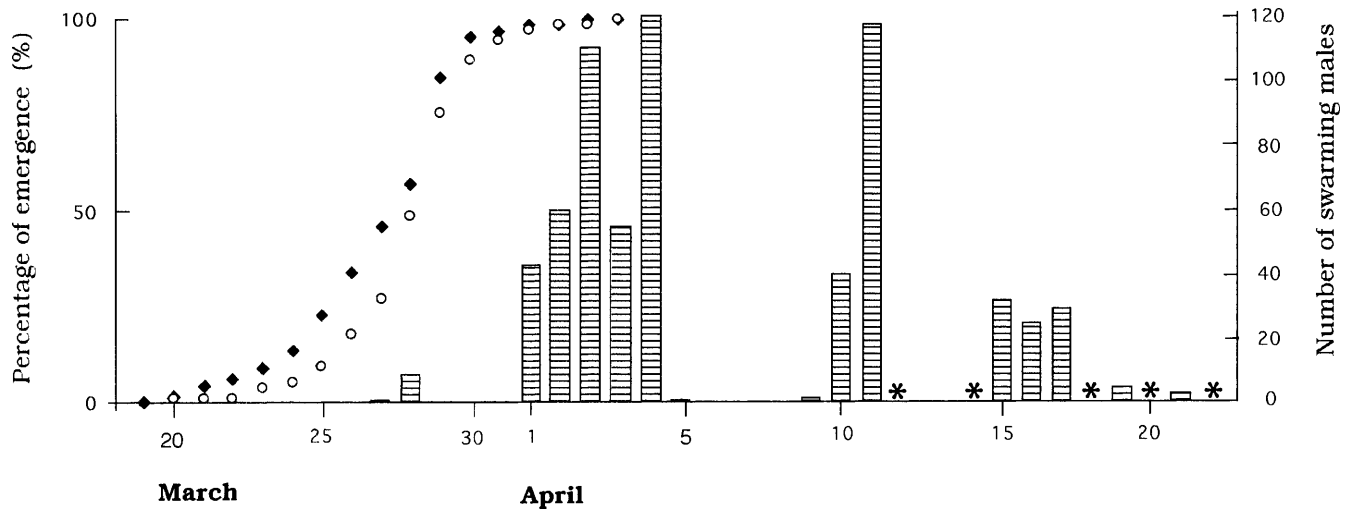


Fig. 2. Cumulative emergence curve of male (◆) and female (○) subimagines and the daily maximum number of swarming males (shaded bars) of *Paraleptophlebia spinosa* at a swarming site (site *b* in

Fig. 1) throughout the reproductive season in 1988. The asterisk and the absence of a bar indicate no data and no swarm occurring on the day; * indicates no data

withdrawn. The frequency of this movement was $80.0 (\pm 5.2, 1\text{SD})$ per minute on average ($n = 12$; range, 74–91). The total number of piston-like movements was highly variable, ranging from 27 to 691, with an average of $292.0 (\pm 200.6, 1\text{SD}; n = 12)$. Copulating pairs separated and took off as soon as the piston-like movement ceased.

Females made intermittent flights along the shoreline before, during, and after oviposition. They flew slowly at a height of 20–60 cm above the ground, dropped suddenly onto the gravel bed, and stepped backwards for a short distance. At the water's edge, they placed the ventral side of the sixth to ninth abdominal segments into the water and started ovipositing. When the landing site happened to be away from the waterlogged area, the females took off again for a short flight. They repeated this behavior until they landed on an appropriate site for oviposition. Ovipositing females were found only at the shore of gravel substrates (Fig. 1).

Oviposition at each site lasted for 11 min 47 s (± 6 min 12 s, 1SD) on average ($n = 88$). Eggs were released into the water one by one or a few at a time at irregular intervals and were scattered into the interstitial space in the gravel bed. Females engaged in repeated bouts of oviposition at different sites (up to five bouts were observed for a female). The total time required for completion of oviposition was unknown, since it was difficult to track a flying individual in the field, but 68 min 29 s was the longest recorded. Of 46 females followed after a bout of oviposition, 3 were observed to remate.

Morphology of genitalia

Male (Figs. 3 and 4). The basal half of the penis is jointed, forming a broad plate, and a ladle-shaped depression ("pocket") exists on the ventral side (Fig. 3b and c). The posterior edge of the pocket is strongly sclerotized, but the

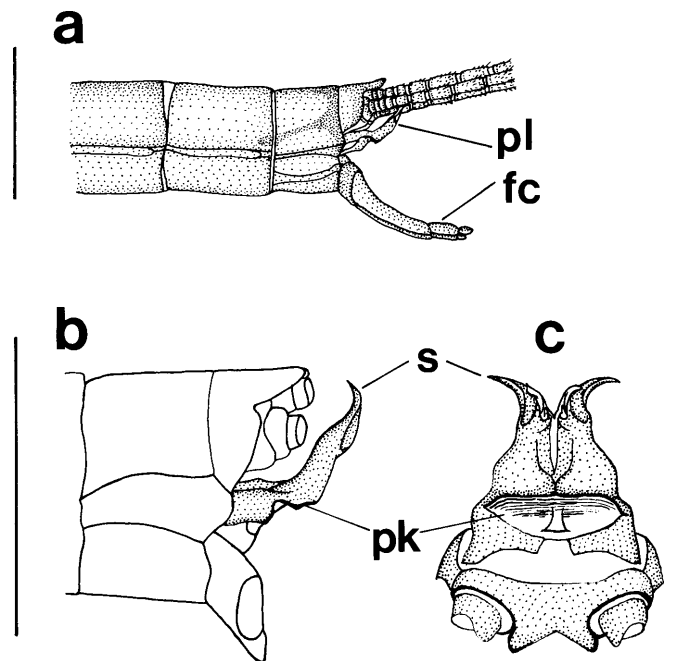


Fig. 3. Male reproductive organ in *Paraleptophlebia spinosa*. **a**, Lateral view of the terminal segments of the abdomen; **b** and **c**, lateral and ventral views of the penis lobes, respectively. *fc*, Forceps; *pk*, pocket; *pl*, penis lobes; *s*, spine. Scales represent 1 mm

inside of the pocket is made of opaque, thin cuticle (Fig. 4a). The whole penis lobe is bent dorsally, so that the apical portion of the penis stands vertically (Fig. 3a and c). The penis is divided into two lobes in the posterior half and ends in a posterolaterally elongated apex (Figs. 3b and c; 4b). The ejaculatory duct opens at the base of this apex (Fig. 4b).

Female (Fig. 5). The subgenital plate is reduced in size and only forms a small hump at the posterior edge of the seventh sternum (Fig. 5a). The gonopore is invisible from

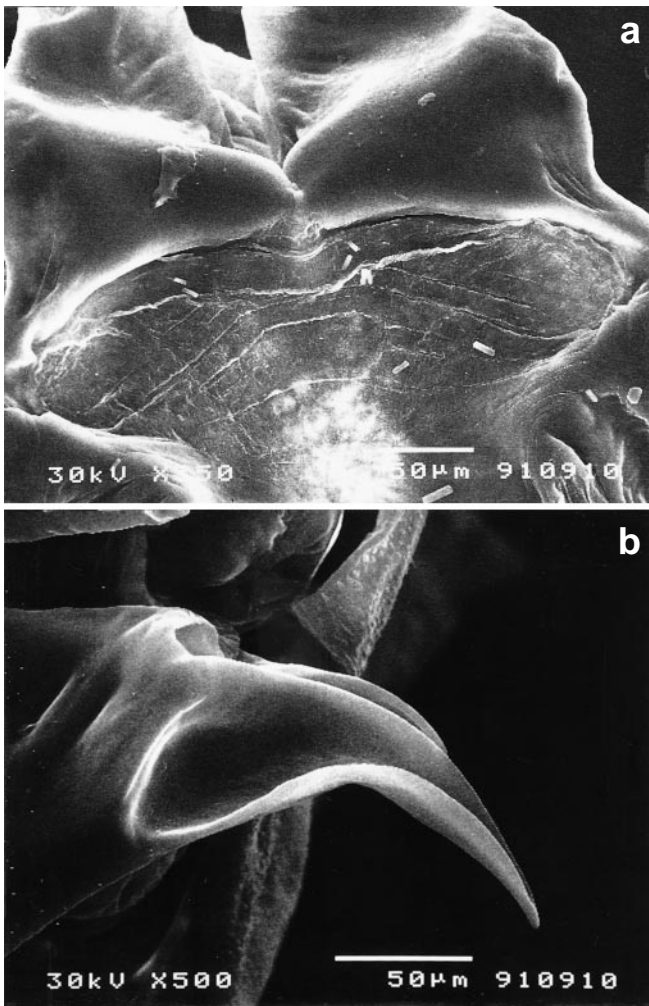


Fig. 4. Stereoscopic scanning electron micrographs of the basal half of the penis lobes showing a pocket with a strongly sclerotized edge (**a**) and the right apex of the penis lobes (**b**) in *Paraleptophlebia spinosa*

outside because it is covered by an opaque membrane. The vestibule forms a square-shaped chamber into which each oviduct opens with a small outlet from the ventral side of the chamber (Fig. 5b and c). The part connecting the chamber to the oviducts is so narrow that eggs must pass through in a single file. The chamber does not have any appendage, such as the seminal receptacle.

Analysis of copulation

Fecundity and copulation

The fecundity of virgin females ranged from 1496 to 3896, with an average of 2571 ($n = 90$). The fecundity (F)–body size (S) relationship of virgin females (Fig. 6) was expressed as

$$\ln F = 0.76 + 2.96 \ln S \quad (r^2 = 0.86, P < 0.001)$$

Relative to virgin females, females collected in the field after copulation ($n = 41$) showed a greater variation in their

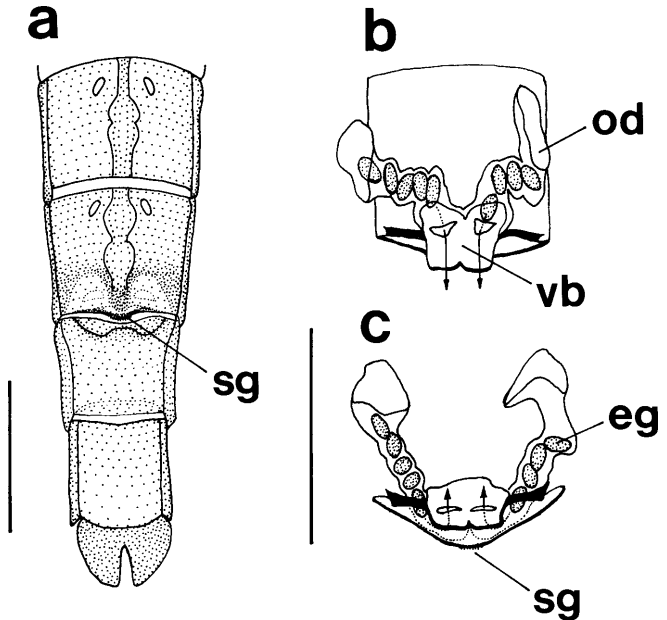


Fig. 5. Female reproductive organ in *Paraleptophlebia spinosa*. **a**, Ventral view of the abdomen; **b**, dorsal view of the outlet of the oviducts (od) and the vestibule (vb) at the posterior end of the 7th sternum after removal of other tissues; **c**, posterior view of the vestibule. eg , Eggs; od , oviduct; sg , subgenital plate; vb , vestibule. Arrows show passage of eggs through the outlet of the oviducts to the vestibule. Scales represent 1 mm

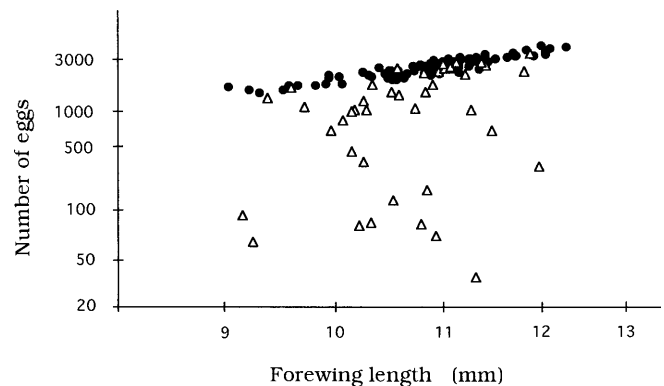


Fig. 6. Relationship between egg loading and body size (forewing length) in *Paraleptophlebia spinosa* plotted on double logarithmic scales. (●) Virgin females ($n = 90$) showing the fecundity–body size relationship; (△) field-collected females ($n = 41$)

egg number than predicted from the above relationship ($P < 0.001$, F -test) (Fig. 6), suggesting that these females copulated again after laying some eggs. Females carrying fewer than about 100 eggs can be considered as having laid all their eggs, because dead females collected at oviposition sites often had similar numbers of eggs remaining in their oviducts.

Copula duration ranged widely from 12s to 8min 32s (mean \pm 1SD, 2min 58s \pm 1min 54s; $n = 65$). The frequency distribution of copula duration indicated a distinctive mode at 120–200s and a secondary mode at less than 40s (Fig. 7). All of the eight females that were engaged in

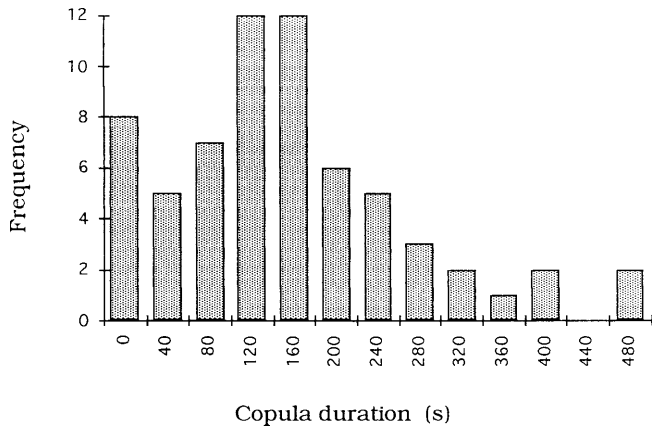


Fig. 7. Frequency distribution of copula duration ($n = 65$) in *Paraleptophlebia spinosa*

Table 1. Results of Kendall rank and partial rank correlation analyses between copula duration of *P. spinosa* and six variables

Variable	n	$\tau(\tau\rho)$	z
× Relative egg loading (RE)	34	0.524	4.355***
× RE – ST	34	0.453	
× RE – TD	34	0.458	
× RE – AT	34	0.465	
× Seasonal timing (ST)	34	-0.344	-2.858**
× ST – RE	34	-0.153	
× Time of day (TD)	34	-0.295	-2.453*
× TD – RE	34	-0.084	
× Air temperature (AT)	34	-0.287	-2.390*
× AT – RE	34	-0.087	
× Female body size	34	-0.020	-0.164 ^{NS}
× Male body size	34	-0.004	-0.030 ^{NS}

*** $P < 0.0001$, ** $P < 0.005$, * $P < 0.01$, ^{NS} not significant

copulation for less than 40s were spent individuals, with a mean of 83.5 eggs ($SD = 74.3$; range, 21–233) remaining. Copula duration showed a significant correlation with both egg loading ($\tau = 0.487$, $z = 4.047$, $P < 0.0001$, Kendall rank correlation) and relative egg loading ($\tau = 0.524$, $z = 4.355$, $P < 0.0001$, Kendall rank correlation) (Fig. 8). Copula duration also correlated significantly with seasonal timing (number of days after the first swarm of the mayfly), time of day, and air temperature, but not with female and male body sizes (Table 1). However, Kendall partial rank correlation analysis revealed that relative egg loading had a stronger effect on the correlation than seasonal timing, time of day, or air temperature. A high level of correlation was retained even after the effects of seasonal timing, time of day, and air temperature were excluded, whereas exclusion of the effect of egg loading resulted in much lower values of partial correlation (Table 1).

Relationship between copula duration and relative egg loading

In order to investigate further the relationship between copula duration and relative egg loading, females were di-

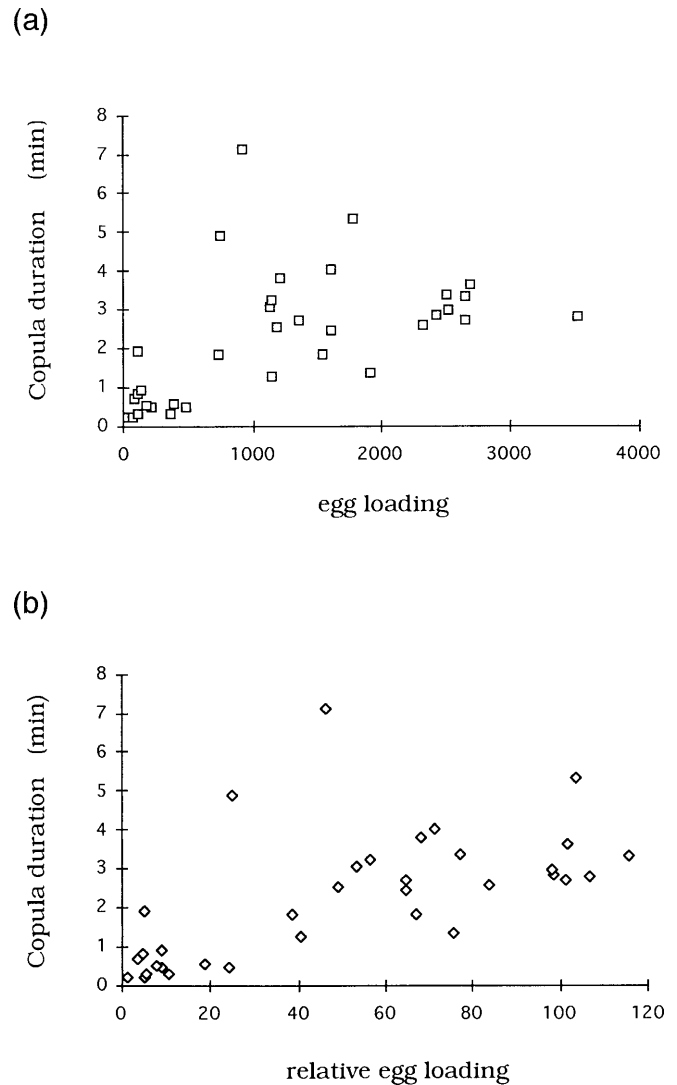


Fig. 8. Relationship of copula duration and (a) egg loading and (b) relative egg loading in *Paraleptophlebia spinosa*. Egg loading and relative egg loading are defined as the number of eggs actually carried by a female and the egg loading as a percentage of the estimated total fecundity for a female of given body size, respectively

vided into three categories on the basis of relative egg loading: fully loaded females with over 80% egg loading, partially loaded females with 20%–80% loading, and spent females with less than 20% loading. Of 41 females observed, there were 14 fully loaded, 16 partially loaded, and 11 spent individuals, indicating that as many as 66% of copulating females had already laid some eggs. The differences in copula duration among the three categories were statistically significant ($H = 18.73$, $P < 0.001$, Kruskal-Wallis test). Multiple comparisons between each two of them by the Dunn test found significant differences between fully loaded and spent females ($Q = 3.50$, $P < 0.01$) and between partially loaded and spent females ($Q = 3.94$, $P < 0.001$), but not between fully loaded and partially loaded females ($Q = 0.14$, ns).

Table 2. Variation in copula duration and copulation sites in mayflies

Species	Copula duration (s)	Copulation site	Reference
<i>Ephoron ladogensis</i>	Several seconds	In flight	Tiensuu (1935)
<i>Ephoron album</i>	Several seconds	In flight	Britt (1962)
<i>Dolania americana</i>	Several seconds	In flight	Peters and Peters (1977)
<i>Paraleptophlebia debilis</i>	Several seconds	In flight	Lehmkuhl and Anderson (1971)
<i>Choroterpes mexicanus</i>	Several seconds	In flight	McClure and Stewart (1976)
<i>Stenonema canadense</i>	Several seconds	In flight	Thew (1958)
<i>Epeorus longimanus</i>	5–30	In flight	Flecker et al. (1988)
<i>Parameletus chelififer</i>	ca. 20	On the ground	Brinck (1957)
<i>Baetis</i> sp.	<30	In flight	Morgan (1913)
<i>Stenonema vicarium</i>	20–40	In flight	Cooke (1940)
<i>Isonychia bicolor</i>	25–60	In flight	Clemens (1917)
<i>Ephemera simulans</i>	<60	In flight	Britt (1962)
<i>E. simulans</i>	Several seconds	In flight	Clemens (1913)
<i>Epeorus assimilis</i>	90	In flight	Degrange (1960)
<i>Paraleptophlebia spinosa</i>	178 ^a	On the ground	Present study
<i>Ecdyonurus venosus</i>	180–240	In flight	Rawlinson (1939)
<i>E. venosus</i>	60–120	In flight	Schoenemund (1930)
<i>Ecdyonurus</i> sp.	360–420	On the ground	Eaton (1883–1888)
<i>Epeorus ikanonis</i>	468 ^b	On the ground	Takemon (1993)

^a Represents an average value (SD = 114s; range, 12–512s; *n* = 65)

^b Represents an average value (SD = 294s; range, 132–978s; *n* = 11)

Discussion

Reproductive habits of *P. spinosa*

Protandrous emergence has been commonly reported for the Ephemeroptera (Britt 1962; Clemens 1917; Harper and Magnin 1971; Rawlinson 1939; Takemon 1990c; Watanabe et al. 1989). For example, the mean emergence date of male *Ephemera strigata* precedes that of females by 2 days within a total emergence period of 2 weeks (Takemon 1990c). In the case of *P. spinosa*, however, males preceded females by only 0.5 day within a total emergence period of 2 weeks (Fig. 2). Protandry is expected to evolve under intermale sexual selection, in which males emerging early have a higher chance of mating than those emerging late (Fagerström and Wiklund 1982; Iwasa et al. 1983; Wiklund and Fagerström 1977). This conclusion, however, depends on the assumption that females mate only once, whereas males are capable of multiple copulation. Zonneveld and Metz (1991) showed that if females are capable of multiple copulation, absence of protandry is the evolutionarily stable strategy for males because of increasing chance of mating success, even for males emerging later in the adult season. This may be the case with *P. spinosa*, since a seemingly high incidence of female multiple copulation is indicated from the present study.

Swarming in mayflies is variable among species with respect to the distance from shoreline, kinds of swarm marker used, and height above the ground or water (Brodsky 1973; Savolainen 1978; Sullivan 1981). The swarming sites of *P. spinosa* were located over the water close to the shoreline, and their locations were very stable throughout the reproductive period. Swarming close to the shore, as observed in

P. spinosa, has also been reported for a congeneric species, *P. debilis*, which swarms 2–4 feet above the water and 2–10 feet from the shoreline (Lehmkuhl and Anderson 1971), whereas another leptophlebiid, *P. memorialis*, is known to swarm up to 200 yards away from a stream (Edmunds et al. 1976). These observations suggest that the selection of swarming sites is not evolutionarily fixed within the genus. Although mayfly swarming sites may correspond to emergence sites, oviposition sites, or neither of these (Allan and Flecker 1989; Savolainen 1978, Sullivan 1981), the swarming sites of male *P. spinosa* clearly coincided with the oviposition sites (Fig. 1), indicating the importance of intercepting females that are close to oviposition.

Previous studies on leptophlebiid mayflies reported very short copulations that were conducted in the air (Edmunds et al. 1976; Lehmkuhl and Anderson 1971; Leonard and Leonard 1962; Morgan 1913; Peters 1971). The mean copulation duration of 178s reported for *P. spinosa* in this study represents a fairly long record among mayflies (Table 2). Records of prolonged copulation (>60s) refer to heptageniid species such as *Epeorus* and *Ecdyonurus* only. Copulation conducted on the ground is another characteristic of *P. spinosa*, which is rather uncommon among mayflies (Table 2). It may be suggested that ground-copulating species tend to perform prolonged copulation. Although *P. debilis* has similar swarming behavior to *P. spinosa*, its copulation is completed in the air within a few seconds (Lehmkuhl and Anderson 1971). Therefore, long copula duration in *P. spinosa* is less of a phylogenetically constrained character and more of an ecologically molded trait through evolutionary time.

The piston-like movement of the penis lobes during copulation is a remarkable feature of *P. spinosa*. Copulatory movement of the penis with a rotation of apical parts of

the penis lobes has been reported in heptageniid mayflies (Harker 1986; Takemon 1990b). The movement of the penis lobes in *P. spinosa*, however, was not a rotation, but a putting in and taking out of the penis itself.

In summary, the following features characterize the reproductive behavior of *P. spinosa*: no or very weak protandry, male swarming located above oviposition sites, prolonged copulation conducted on the ground, occurrence of multiple copulation by females, and the piston-like movement of the penis during copulation.

Multiple copulation by females in *P. spinosa*

The possibility of multiple copulation by females cannot easily be assessed in mayflies because of the difficulty of visual observation and the lack of spermatophores or bunched sperm, which would permit estimation of the number of copulations by a female. A notable exception is a ground-copulating heptageniid mayfly, *Epeorus ikanonis*, for which female multiple copulation was confirmed. The females successively mated with different males in cages (Takemon 1993), and observation in the field confirmed that 12% of all females engaged in multiple copulation within a restricted period of 13 min before oviposition (Takemon 1990a).

In the case of *P. spinosa*, focal animal observation of females was difficult because of their small body size and rapid flight, coupled with the fact that copulation was always initiated in the air by swarming males. In spite of these difficulties, a second copulation by a field female was observed three times in the present study. Apart from this direct evidence, multiple copulation was indicated by the occurrence of copulation by spent and partially loaded females. The high proportion of these females (66% of the total observations, $n = 41$) indicates that more than half of the females may perform multiple copulation. Unfortunately, however, it was impossible to estimate the lifetime frequency of multiple copulation by females.

Multiple copulation in female *P. spinosa* seems to be closely related to their oviposition habits. Since the females change sites after each bout of oviposition, the chance of encounter with swarming males arises with each oviposition flight. Moreover, the long total time required for oviposition may also increase the chance of a second copulation before completion of oviposition. Female *P. spinosa* have narrow oviducts through which eggs must pass one by one, thereby prolonging the total time required for oviposition. This condition contrasts with that in females of mayfly genera such as *Ephemerella*, *Siphonurus*, *Isonychia*, *Centroptilum*, *Baetis*, *Ecdyonurus*, and *Epeorus*, which have oviducts with wider outlets and lay all their eggs in one bout or at one oviposition site in a series of bouts (Brinck 1957; Brittain 1982; Degrange 1960; Needham et al. 1935).

The possible advantages of multiple copulation for females, such as enhanced genetic variability, extra nutrients presented by males, or fertilization success (Alcock et al. 1977; Drummond 1984; Thornhill and Alcock 1983), cannot be assessed from the present data. In the case of males, the

occurrence of female multiple copulation is considered to have a serious impact on their mating strategy, since it tends to result in sperm competition (Parker 1970a) and high variation in reproductive success among males (Fincke 1984; Hafernik and Garrison 1986; Thornhill 1979). Therefore, males of *P. spinosa* may be experiencing relatively strong male–male competition.

Male mating tactics in *P. spinosa*

A significant reduction in copula duration for spent females seems to suggest that males are capable of distinguishing spent from egg-loaded females. Males might be able to detect the presence of water in the oviducts of a spent female that was sucked in when eggs were released into water (Takemon unpublished). Whatever the precise mechanism, the end result is that males can minimize mating investment to spent females, which is likely to enhance the males' reproductive fitness. There is a clear limit to the amount of sperm and eggs available in the adult stage, because mayflies cannot produce extra sperm and eggs after emergence due to their atrophied testes and ovaries (Edmunds and McCafferty 1988; Needham et al. 1935). A good example is the apparent depletion of sperm in males of *Epeorus ikanonis* in the field (Takemon 1990b).

On the other hand, the absence of a significant difference in copula duration between fully loaded and partially loaded females suggests that the latter are still worth a full-length copulation for males. This in turn assumes that a succeeding copulation is effective for fertilizing the eggs remaining in the oviducts. If only the sperm from the earlier copulation is used for fertilization, males should search for virgin females at emergence rather than oviposition sites; this is not the case with *P. spinosa*. It is known that insects that show precedence of sperm from a later copulation tend to establish mating sites at or close to oviposition sites (Fincke 1986; Otronen 1989, 1990; Parker 1978; Waage 1984).

Displacement of the previously stored sperm may be a mechanism leading to the precedence of sperm from a later copulation (Parker 1970a; Choe and Crepsi 1997). In recent years, sperm displacement phenomena have been reported in various orders of insects, such as Odonata (Waage 1979, 1984; Robinson and Novak 1997), Diptera (Otronen and Siva-Jothy 1991), Coleoptera (De Villiers and Hanrahan 1991; Yokoi 1990), Orthoptera (Ono et al. 1989), and Hymenoptera (Shigemura and Naito 1999). Nevertheless, data on the mechanisms of sperm precedence in mayflies are scarce, and only sperm mixing has been suggested in *Epeorus ikanonis* (Takemon 1990b).

The genitalia of *P. spinosa* are characterized by a concave pocket on the ventral side of the penis (Figs. 3b and c; 4a) and a vestibule forming a square-shaped chamber with a very narrow gonopore (Fig. 5b and c). The occurrence of a quick, undulatory movement of the penis during copulation may indicate that the posterior edge of the pocket could be used as a shovel for removing the previously stored sperm during the undulatory movement. The female vesti-

bule, which forms a square-shaped chamber where the sperm is stored until fertilization, might have been a precondition for the evolution of sperm displacement in the male. The fact that the undulatory movement starts soon after insertion of the penis and continues until just before the end of copulation suggests that the total number of undulations is proportional to copula duration. If the undulatory movement functions for kinematic sperm displacement, copula duration may affect the rate of fertilization by the male. In this case, prolonged copula duration would be advantageous for the male's reproductive success.

In summary, male mating tactics in *P. spinosa* consist of distinguishing ovigerous females from spent ones and investing more time and effort in the former; swarming at oviposition sites to intercept females that are close to oviposition; and copulating on the ground for a prolonged period of time, presumably to remove previous males' sperm from the vestibule.

Acknowledgments The author is grateful to Dr. Mutsunori Tokeshi for his helpful advice and critical comments on the manuscript, Ms. Midori Takemon and Mr. Shigeru Kamata for their help with the field work, Mr. Akio Taki for his technical advice on the laboratory work, Mr. Shin-ichi Ishiwata for taking electronmicrographs using his facilities, and Dr. Fumio Hayashi for information on references. Data analysis and manuscript preparation were done while the author was at Queen Mary and Westfield College, University of London, under the JSPS Fellowship for Research at Centers of Excellence Abroad program. This study was supported by Grants-in-Aid of Scientific Research from the Japan Ministry of Education, Science and Culture (Nos. 02954019, 05740466 and 06854046) to the author, partly by a grant (No. 62304003) to Prof. Hiroya Kawanabe and one (No. 05640714) to Dr. Kazumi Tanida, and also by the Foundation for Management of River Environment in Japan.

References

- Alcock J, Eickwort GC, Eickwort KR (1977) Reproductive behavior of *Anthidium maculosum* (Hymenoptera: Megachilidae) and the evolutionary significance of multiple copulations by females. *Behav Ecol Sociobiol* 2:385–396
- Allan JD, Flecker AS (1989) The mating biology of a mass-swarming mayfly. *Anim Behav* 37:361–371
- Arnqvist G (1988) Mate guarding and sperm displacement in the water strider *Gerris lateralis* Schumm. (Heteroptera: Gerridae). *Freshwater Biol* 19:269–274
- Brinck P (1957) Reproductive system and mating in Ephemeroptera. *Opuscula Entomol* 22:1–37
- Britt NW (1962) Biology of two species of Lake Erie mayflies, *Ephoron album* (Say) and *Ephemera simulans* Walker. *Bull Ohio Biol Surv* 1:1–70
- Brittain J (1982) Biology of mayflies. *Ann Rev Entomol* 27:119–147
- Brodsky AK (1973) Swarming behaviour of mayflies (Ephemeroptera). *Entomol Rev USSR* 52:33–39 (in Russian)
- Choe JC, Creps BJ (eds) (1997) The evolution of mating systems in insects and arachnids. Cambridge University Press, London
- Clemens WA (1913) New species and new life histories of Ephemerae or mayflies. *Can Entomol* 45:246–262, 329–341, pls 5–7
- Clemens WA (1917) An ecological study of the mayfly *Chirotenes*. *Univ Toronto Stud Biol Ser* 17:1–43, 5 pls
- Cooke HG (1940) Observations on mating flights of the mayfly *Stenonema vicarium*. *Entomol News* 51:12–14
- Degrange C (1960) Recherches sur la reproduction des Ephemeroptères. *Travaux du Laboratoire d'Hydrobiologie et de Pisciculture de l'Université de Grenoble* 51:7–193
- De Villiers PS, Hanrahan SA (1991) Sperm competition in the namib desert beetle *Onymacris unguicularis*. *J Insect Physiol* 37:1–8
- Drummond BA III (1984) Multiple mating and sperm competition in the Lepidoptera. In: Smith RL (ed) *Sperm competition and evolution of animal mating systems*. Academic Press, Orlando, FL, USA, pp 291–330
- Eaton AE (1883–1888) A revisional monograph of recent Ephemerae or mayflies. *Trans Linn Soc Lond Ser 2 Zool* 3:1–352, 65 pls
- Eberhard WG (1985) *Sexual selection and animal genitalia*. Harvard University Press, Cambridge
- Edmunds GF Jr, Jensen SL, Berner L (1976) *The mayflies of North and Central America*. University of Minnesota Press, Minneapolis
- Edmunds GF Jr, McCafferty WP (1988) The mayfly subimago. *Ann Rev Entomol* 33:509–529
- Fagerström T, Wiklund C (1982) Why do males emerge before females? Protandry as a mating strategy in male and female butterflies. *Oecologia* 52:164–166
- Fincke OM (1984) Sperm competition in the damselfly *Enallagma hageni* Walsh (Odonata: Coenagrionidae): benefits of multiple mating to males and females. *Behav Ecol Sociobiol* 14:235–240
- Fincke OM (1986) Underwater oviposition favors male vigilance and female multiple mating in a damselfly (Odonata: Coenagrionidae). *Behav Ecol Sociobiol* 18:405–412
- Flecker AS, Allan JD, McClintock NL (1988) Male body size and mating success in swarms of the mayfly *Epeorus longimanus*. *Holarctic Ecol* 11:280–285
- Hafernik JE, Garrison RW (1986) Mating success and survival rate in a population of damselflies: results at variance with theory? *Am Nat* 128:353–365
- Harker JE (1986) The effect of the environment and copulatory movements on the taxonomic characters of three species of *Ecdyonurus* (Ephemeroptera). *J Nat Hist* 20:635–647
- Harper F, Magnin E (1971) Emergence saisonnière de quelques épheméroptères d'un ruisseau des Laurentides. *Can J Zool* 49:1209–1221
- Gose K (1970) Life histories of some species of Ephemeroptera and Plecoptera at Ikadaba in the Yoshino River. *Productivity of Yoshino River* 2:8–13 (in Japanese)
- Iwasa Y, Odendaal FJ, Murphy DD, Ehrlich PR, Launer AE (1983) Emergence patterns in male butterflies: a hypothesis and a test. *Theoret Pop Biol* 23:363–379
- Lehmkuhl DM, Anderson NH (1971) Contribution to the biology and taxonomy of the *Paraleptophlebia* of Oregon (Ephemeroptera: Leptophlebiidae). *Pan-Pacific Entomol* 46:268–274
- Leonard JW, Leonard FA (1962) Mayflies of Michigan trout streams. *Cranbrook Inst Sci Bull* 43
- McClure RG, Stewart KW (1976) Life cycle and predation of the mayfly *Choroterpes (Neochoroterpes) mexicans* Allen (Ephemeroptera: Leptophlebiidae). *Ann Entomol Soc Am* 69:134–144
- Morgan AH (1913) A contribution to the biology of mayflies. *Ann Entomol Soc Am* 6:371–413, 13 pls
- Needham JG, Traver JR, Yin-Chi H (1935) *The biology of mayflies*. Comstock Publishers, New York
- Ono T, Siva-Jothy MT, Kato A (1989) Removal and subsequent ingestion of rivals' sperm during copulation in a tree cricket. *Physiol Entomol* 14:195–202
- Otronen M (1989) Female mating behaviour and multiple matings in the fly, *Dryomyza anilis*. *Behaviour* 111:77–97
- Otronen M (1990) Mating behaviour and sperm competition in the fly, *Dryomyza anilis*. *Behav Ecol Sociobiol* 26:349–356
- Otronen M, Siva-Jothy MT (1991) The effect of postcopulatory male behaviour on ejaculate distribution within the female sperm storage organs of the fly, *Dryomyza anilis* (Diptera: Dryomyzidae). *Behav Ecol Sociobiol* 29:33–37
- Parker GA (1970a) Sperm competition and its evolutionary consequences in the insects. *Biol Rev* 45:525–567
- Parker GA (1970b) Sperm competition and its evolutionary effect on copula duration in the fly *Scatophaga stercoraria*. *J Insect Physiol* 16:1301–1328
- Parker GA (1978) Evolution of competitive mate searching. *Ann Rev Entomol* 23:173–196
- Peters WL (1971) A revision of the Leptophlebiidae of the West Indies (Ephemeroptera). *Smithsonian Contrib Zool* 62:1–48

- Peters WL, Peters JG (1977) Adult life and emergence of *Dolania americana* in Northwestern Florida (Ephemeroptera: Behningiidae). *Int Rev Ges Hydrobiol* 62:409–438
- Rawlinson R (1939) Studies on the life-history and breeding of *Ecdyonurus venosus* (Ephemeroptera). *Proc Zool Soc Lond Ser B* 109:377–450
- Robinson JV, Novak KL (1997) The relationship between mating system and penis morphology in ischnuran damselflies (Odonata: Coeagrionidae). *Biol J Linn Soc* 60:187–200
- Savolainen E (1978) Swarming in Ephemeroptera: the mechanism of swarming and the effects of illumination and weather. *Ann Zool Fenn* 15:17–52
- Schoenemund E (1930) Eintagsfliegen oder Ephemeroptera. Die Tierwelt Deutschlands und der Angrenz. Meeresteile 19:1–106, text figs 1–186
- Shigemura T, Naito T (1999) The mechanism of sperm displacement in the turnip sawfly, *Athalia rosae* (Hymenoptera: Tenthredinidae). *Entomol Sci* 2:61–65
- Smith RL (ed) (1984) Sperm competition and evolution of animal mating systems. Academic Press, Orlando, FL, USA
- Sullivan RT (1981) Insect swarming and mating. *Florida Entomol* 64:44–65
- Takemon Y (1985) Emerging behaviour of *Ephemera strigata* and *E. japonica* (Ephemeroptera: Ephemeridae). *Physiol Ecol Jpn* 22:17–36
- Takemon Y (1990a) Reproductive ecology of mayfly *Epeorus ikanonis* (Ephemeroptera: Heptageniidae). PhD thesis, Kyoto University
- Takemon Y (1990b) Functional morphology of the genitalia in *Epeorus ikanonis* (Ephemeroptera: Heptageniidae). *Jpn J Entomol* 58:113–122
- Takemon Y (1990c) Timing and synchronicity of the emergence of *Ephemera strigata*. In: Campbell IC (ed) *Mayflies and stoneflies*. Kluwer Academic Publishers, Dordrecht, pp 61–70
- Takemon Y (1993) Water intake by the adult mayfly *Epeorus ikanonis* (Ephemeroptera: Heptageniidae) and its effect on their longevity. *Ecol Res* 8:185–192
- Thew TB (1958) Studies on the mating flight of the Ephemeroptera I. The mating flight of *Ephoron album* (Say) and *Stenonema canadense* (Walker). *Florida Entomol* 41:9–12
- Thornhill R (1979) Male and female sexual selection and the evolution of mating strategies in insects. In: Blum MS, Blum NA (eds) *Sexual selection and reproductive competition in insects*. Academic Press, New York, pp 81–121
- Thornhill R, Alcock J (1983) *The evolution of insect mating systems*. Harvard University Press, Cambridge
- Tiensuu L (1935) On the Ephemeroptera-fauna of Laatokan Karjala (Karelia Ladogensis). *Ann Zool Fenn* 1:3–23
- Waage JK (1979) Dual function of the damselfly penis: sperm removal and transfer. *Science* 203:916–918
- Waage JK (1984) Sperm competition and the evolution of Odonate mating systems. In: Smith RL (ed) *Sperm competition and evolution of animal mating systems*. Academic Press, Orlando, FL, USA, pp 251–290
- Watanabe NC, Yoshitaka I, Mori I (1989) Timing of emergence of males and females of *Ephoron shigae* (Ephemeroptera: Polymitarcyidae). *Freshwater Biol* 21:473–476
- Wiklund C, Fagerström T (1977) Why do males emerge before females? *Oecologia* 31:153–158
- Yokoi N (1990) The sperm removal behaviour of the yellow spotted longicorn beetle *Psacotheta hilaris* (Coleoptera: Cerambycidae). *Appl Entomol Zool* 25:383–388
- Zonneveld C, Metz LAJ (1991) Models on butterfly protandry: virgin females are at risk to die. *Theoret Pop Biol* 40:308–321