Oviposition sites and the number of larval instars of two mountain blackfly species, *Simulium japonicum* and *S. rufibasis* (Diptera: Simuliidae)*

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Key words: blackfly, *Simulium japonicum*, *Simulium rufibasis*, oviposition site, larval instar.

Abstract: Oviposition sites and the number of larval instars of two mountain blackfly species, *Simulium japonicum* Matsumura and *S. rufibasis* Brunetti, were investigated. The eggs of both species, which were indistinguishable from each other by egg size, coexisted on bryophytes growing on a water-splashed boulder. They were laid individually without making egg masses, and most of them were found lower than 5 cm above water level. Two eggs of *S. japonicum* were also found on a partially submerged fallen leaf. Nine larval instars were identified in both species based on the measurements of head capsules of laboratory-reared individuals. The growth rates calculated from the size of head capsules between successive two instars were almost constant, i.e., a mean of 1.27 ± 0.082 (S.D.) and 1.25 ± 0.045 for *S. japonicum* and *S. rufibasis*, respectively. The oviposition habit of these species was discussed in relation to the habitat conditions.

INTRODUCTION

It is well recognized that the preimaginal stages of blackflies tend to utilize the particular habitat types of the fluvial system (Ogata and Fukui, 1957; Orii *et al.*, 1964). The habitat segregation between species will partly be attributed to the specific differences of larval preference for hydro-chemical and physical characters, as reviewed by Colbo and Wotton (1981) and Grunewald (1981). The different egg-laying habits of blackflies

also undoubtedly have close relation to their distribution and seasonal prevalence since preimaginal simuliids begin to develop at or near the sites of oviposition (Colbo and Wotton, 1981).

Little study has been made on the oviposition habit of Japanese blackflies except *Simulium aokii* (Takahasi) which is one of the common and multivoltine species inhabiting a wide range of habitat conditions from lowland to mountain streams (Ogata, 1954). It usually is difficult to observe the oviposition behavior in the field, particularly of the species inhabiting mountain regions where trailing vegetation, the commonest oviposition substrate, is scarce. In this paper, we report for the first time the oviposition sites of two mountain species, *S. japonicum* Matsumura and *S. rufibasis* Brunetti, together with the number of larval instars and larval growth

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pattern.

MATERIALS AND METHODS

Study site. The field study was conducted at a small stream, Kinun-kyo (360 m above sea level), a tributary of the Yamaguni River, near the town of Kusu, Oita Prefecture. The stream (~5 m in width and ~0.5 m in depth) consisted of a mosaic construction of swift shallows and small pools. The stream bed was rocks or pebbles. The dominant preimaginal simuliid species in this stream were: *Prosimulium kiotoense* Shiraki in winter, and *S. japonicum*, *S. rufibasis*, *S. bidentatum* (Shiraki) and *S. aokii* in other seasons (Baba *et al.*, unpublished data).

Oviposition in the laboratory. Two gravid females (1 *S. japonicum* and 1 *S. rufibasis*) were captured with a sweep net on April 10, 1985. Each female was allowed to oviposit at $14\pm1^\circ\text{C}$ in a polystyrene tube (1.5×0.7 cm) with a strip of paper and *ca.* 2 ml of distilled water. After the completion of oviposition, their residual eggs were checked. The laid eggs were allowed to develop at a constant temperature of $10\pm1^\circ\text{C}$ and embryonic development was observed daily. The first instar larvae and remaining unhatched eggs were preserved and measured with the aid of an ocular calibrator (OSM-D2, Olympus) or a caliper.

Egg collection in the field. Partially submerged fallen leaves and trailing vegetation were collected weekly from the stream between November 1986 and December 1987. In April and May 1988, bryophytes growing on the rock surface wetted by spray from the rapids, were also collected. Two-by-two cm areas of bryophytes were scraped at 0, 5 and 10 cm above water level. These substrates were brought to the laboratory and the eggs were searched carefully under binoculars (×10–40). The eggs were submerged in distilled water in Petri dishes (6 cm in diameter) and kept at $14\pm1^\circ\text{C}$. Since eggs and earlier stages of larvae of most Japanese blackflies were not identifiable, some of the hatched larvae were reared in the laboratory, and identified based on the morphology of grown-up larvae and pupae. Remaining larvae were preserved for later measurements.

Rearing procedure. The larvae were

reared using the stir-bar system at a constant temperature of $14\pm1^\circ\text{C}$. One to 98 first-instar larvae were placed in circular dishes (9×6 cm) with 250 ml of food suspending water. TetraMin (Tetra Werke), which was ground and sieved through No. 325 mesh (44 μm), was used for the food. The food concentration was adjusted to 40 mg/liter. Rearing water was replaced at 2–3 day intervals in the following manner. About 200 ml of the water was removed with a syringe and replaced with water suspending the same concentration of food. Larval carcasses and head capsules (exuviae) were recovered from the removed water under binoculars (×10–40). The measurements of head capsules were made on postgenal length, head length, maximum width of cephalic apotome and distance between corner teeth of hypostomium (Fig. 1).

RESULTS

1. Laboratory oviposition

Each female of *Simulium japonicum* and *S. rufibasis* laid eggs successfully by the next day of the capture. The eggs were deposited individually on the strip of paper or inside wall of the oviposition tube.

Daily examination of the eggs incubated at $10\pm1^\circ\text{C}$ showed similar embryonic development in both species. Eye spots were recognized from the 6th day, egg burster was visible from the 8th day and hatching occurred from the 11th day after oviposition.

Table 1 shows the number of laid eggs, the hatching rate and the sizes of eggs and larvae. No residual eggs were found in the females' ovarioles. Both females laid about 200 eggs. However, the actual fecundity may be higher than these figures, since both females were able to have laid a part of their eggs before capture.

The eggs of both species were semitriangular in shape. The eggs of *S. japonicum* were significantly larger than the eggs of *S. rufibasis* ($t=4.926$, $p<0.001$ in length, and $t=6.872$, $p<0.001$ in width). Consequently, the size of first-instar larvae of the former species was larger than the latter.

2. Oviposition sites and egg characters

Seventy egg batches deposited on fallen

Fig. 1 Measurements direction of head capsule.

A: dorsal view; B: lateral view; C: hypostomial teeth. 1, postgenal length; 2, head length; 3, maximum width of cephalic apotome; 4, distance between corner teeth of hypostomium.

Table 1 Laboratory oviposition of *Simulium japonicum* and *S. rufibasis*.

		<i>S. japonicum</i>	<i>S. rufibasis</i>
Eggs	No. of oviposited	204	224
	No. of hatched larvae	144	203
	(%)	(70.6)	(90.6)
	Length $\pm$ S.D.* (μ m)	290.4 $\pm$ 20.31	267.1 $\pm$ 5.89
	Width $\pm$ S.D.* (μ m)	170.1 $\pm$ 14.10	147.1 $\pm$ 5.02
First instar	Head width $\pm$ S.D.** (μ m)	125.4 $\pm$ 3.86	110.0 $\pm$ 2.71
	Total length $\pm$ S.D.** (mm)	0.709 $\pm$ 0.0223	0.610 $\pm$ 0.0163

* $n=20$; ** $n=10$.

leaves or trailing vegetation were collected between November 1986 and December 1987. Among them, one of two eggs on a fallen leaf collected in November 1987 was identified as *S. japonicum* as stated below. These two eggs measured 272×144 and 280×139 μ m. The remaining egg batches were: 11 *S. aokii*, 4 *S. bidentatum*, 3 *S. quinquestriatum* (Shiraki) and 51 unidentified. There were no eggs of *S. rufibasis* on these substrates.

Many eggs were found on bryophytes growing on a water-splashed boulder in April–May 1988 (Figs. 2 and 3). The eggs were laid individually on the stems or leaves of bryophytes and most of the eggs were distributed lower than 5 cm above water level (Table 2). They were identified as *S. japonicum* and *S. rufibasis* by the laboratory-reared larvae and a pupa. The size of eggs was measured for 91 specimens (Fig. 4). Though the eggs included two species, there were no distinct size clusters among them.

Fig. 2 Oviposition site of a water-splashed boulder by *Simulium japonicum* and *S. rufibasis* (arrow).

3. Larval development and the number of instars

Results of laboratory rearing are given in Table 3. Lot 1 is the result of rearing of two

Fig. 3 Simuliid egg (arrow) deposited on a bryophyte.

Fig. 4 Size distribution of simuliid eggs collected from bryophytes.

Table 2 Vertical distribution of simuliid eggs deposited on bryophytes growing on a water-splashed boulder.

	Height above water level (cm)		
	0	5	10
No. of samples	2	6	3
No. of eggs			
Developing egg	18.5	20.5	0
(range)	(9, 28)	(6-47)	(0)
Egg shell	22.5	14.8	0.7
(range)	(1, 44)	(0-52)	(0-2)
Total	41.0	35.3	0.7
(range)	(10, 72)	(7-63)	(0-2)

Figures indicate the number of eggs found in 2×2 cm areas.

eggs collected from a fallen leaf. One of two larvae of lot 1 died after the first moulting on the 9th day of rearing, and the other pupated on the 35th day. A male *S. japonicum* emerged 5-6 days after pupation. Lots 2 to 5 are the successful examples of the rearing of eggs on bryophytes. Four and three individuals among 32 first-instar larvae were finally identified as *S. japonicum* and *S. rufibasis*, respectively.

The number of larval instars was estimated from the size distribution of exuviae. The measurements of *S. japonicum* were made on a set of larval exuviae of lot 1 in Table 3. The first-instar larvae were easily distinguished from others by the presence of egg-burster sclerite on the cephalic apotome. As

Table 3 The laboratory rearing of eggs of *Simulium japonicum* and *S. rufibasis* collected from different oviposition substrate.

	Lot No.				
	1	2	3	4	5
Oviposition substrate	Fallen leaf	Bryophytes	Bryophytes	Bryophytes	Bryophytes
No. of first instar	2	7	4	19	2
Minimum larval period (days)	35	42	—	39*	—
Final development stage	Adult	Pupa	Immature larva	Mature larva	Immature larva
No. of larvae identified as					
<i>S. japonicum</i>	1	0	0	3	1
<i>S. rufibasis</i>	0	1	1	1	0

Eggs were incubated at 14±1°C. * Died just before pupation.

Fig. 5 Relationship between instar number and larval size of laboratory-reared *Simulium japonicum* (A) and *S. rufibasis* (B).

HL, head length; PGL, postgenal length; WCA, maximum width of cephalic apotome; DCT, distance between corner teeth of hypostomium. Figures indicate the number of specimens measured.

Table 4 Regression equation between instar number (X) and logarithmic value of larval size (Y) of *Simulium japonicum* and *S. rufibasis*.

Y	<i>S. japonicum</i>		<i>S. rufibasis</i>	
	Regression equation	r	Regression equation	r
Postgenal length	$Y=0.104X+1.801$	0.997	$Y=0.096X+1.793$	0.996
Head length	$Y=0.102X+2.020$	0.994	$Y=0.101X+1.985$	0.997
Maximum width of cephalic apotome	$Y=0.114X+1.660$	0.994	$Y=0.098X+1.724$	0.999
Distance between corner teeth of hypostomium	$Y=0.098X+0.901$	0.993	$Y=0.099X+0.908$	0.998

seen in Fig. 5A, nine instars were recognized in this species.

The number of instars of *S. rufibasis* was estimated from the exuviae of lot 2 in Table 3, where only 1 of 7 larvae survived through 12 days of rearing and it pupated on the 42nd day. In a strict sense, *S. japonicum* larvae could have been included in the early

instars which could not be identified, but all instars were assumed to be *S. rufibasis* here. The effect of this assumption was negligible since the size variation among larvae was small. Eight instars were confirmed from recovered larval exuviae (Fig. 5B). However, growth rates in linear size between the 3rd and the 4th exuviae were markedly

high, *i.e.*, 1.50–1.75, compared with the other values, *i.e.*, 1.11–1.42. The results suggested that the head capsule of the 4th instar could not be recovered. Thus, this species is thought to pass through 9 instars.

The growth rates calculated from the size of exuviae between successive instars were nearly constant, a mean of 1.27 ± 0.082 (S.D.) for *S. japonicum* and 1.25 ± 0.045 for *S. rufibasis*. Thus, there was linear relationship between instar number and logarithmic value of larval size (Fig. 5), and the regression equations are summarized in Table 4.

DISCUSSION

Golini and Davies (1987) reviewed the oviposition of blackflies and recognized three types of oviposition behavior, *i.e.*, (1) oviposit freely into the water while flying, (2) on substrates while landed and (3) on submerged substrates while submerged in water. The oviposition behavior of Japanese blackflies has been studied only for *S. aokii* which engages type (2) oviposition. It oviposits as egg mass on substrates, mainly vegetation trailing on running water (Ogata, 1954). The present study confirmed that the two mountain blackfly species, *S. japonicum* and *S. rufibasis*, also adopt type (2) oviposition behavior. However, it is noteworthy that both *S. japonicum* and *S. rufibasis* dispersed their eggs while most of the other species categorized in type (2) oviposition lay eggs in mass. Further, no blackfly species has been known so far to use bryophytes as an oviposition substrate except *Prosimulium kiotoense* (Baba and Takaoka, in preparation).

There may be some advantages to lay eggs individually on bryophytes and not in mass, especially for the species breeding in mountain streams. Firstly, bryophytes available for oviposition substrate are commonly distributed while other substrates such as trailing grasses are scarce in mountain streams. Second, bryophytes may protect the eggs from desiccation because waterdrops can be retained between the leaves. The risk of egg desiccation will be lowered further when bryophytes in the water-splashed area are selected by ovipositing females, as observed in our study. Finally, dispersed egg laying

will be favored in relatively unstable habitats to avoid entire loss of an egg batch. The water level fluctuates greatly with precipitation in mountain regions, hence the risk of egg desiccation might be higher than in lower streams.

The egg size variation is usually small within the blackfly species (Davies and Peterson, 1956). It was, however, very difficult to separate the field-collected eggs of *S. japonicum* from those of *S. rufibasis* by size, in spite of the clear difference observed in laboratory-oviposited eggs between these species. Laboratory oviposition could be observed for only one female each in this study, and the egg size variation among individuals of both species might be great enough to obscure the difference between species.

Many studies had been conducted on the number of larval instars based on the measurements of field-collected or laboratory-reared specimens. However, there are some difficulties in discriminating the instars, because of size variation among individuals within a population or sexual dimorphism (Ross and Merritt, 1978; Brenner *et al.*, 1981; Post, 1983). In the present study, measurements of exuviae of a small number of laboratory-reared larvae enabled us to determine the instars of two species. The difficulty of laboratory rearing of these blackflies living in clear water rapids did not allow collection of many sets of exuviae, hence hampering efforts to assess the effect of temperature and/or food supply on the number of instars, as seen in *P. mixtum* Syme and Davies, *P. fuscum* Syme and Davies (Ross and Merritt, 1978) and *S. erythrocephalum* De Geer (Post, 1983).

The growth pattern of larvae more or less coincided with Dyar's law (*cf.* Gillott, 1980). On the other hand, Brenner *et al.* (1981) pointed out that application of Dyar's law to larval blackflies is sometimes inadequate. Though slightly lower growth can be noticed between the 8th and 9th instars than between the others in the present result, more data are required for further discussion on the growth of larval blackflies.

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摘 要

溪流に発生するブユ2種
(*Simulium japonicum*, *S. rufibasis*)
の産卵場所と幼虫期の齢数

山間部に発生する2種のブユ *Simulium japonicum*, *S. rufibasis* の産卵場所および幼虫期の齢数を調べた。両種とも卵は飛沫のあたる岩上に成育した蘚類に個別に産卵されていた。大部分の卵は水面上5 cm までにみられた。また、前者は部分的に冠水した落葉上にも産卵していた。野外で得られた卵のサイズから2種を区別することは困難であった。飼育個体の脱皮殻の計測値から、両種とも幼虫期は9齢を経ることが推定された。各齢期ごとの計測値の比は、おのの平均 1.27 ± 0.082 (S.D.) および 1.25 ± 0.045 で、ほぼ Dyar の法則に合致した。ブユの産卵方法と生息場所との関連について考察した。