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Observations on the mating, blood feeding and oviposition of *Simulium takahasii* (Rubtsov) (Simuliidae, Diptera) in the laboratory

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Abstract: The mating, blood feeding and oviposition of *Simulium takahasii* (Rubtsov), an anautogenous, multivoltine Japanese blackfly, were observed in the laboratory. The results showed that this species readily mates in captivity, and when a male and a female were put together in a small polystyrene tube, the insemination rate was 100% (of 43 pairs examined). In addition, blood feeding was successfully induced on a man's hand and a rabbit's ear on the day of emergence. Fifteen (14.7%) of 102 females fed on a hand, while 6 (37.5%) of 16 females fed on a rabbit. Three or 4 days after the blood feeding, oviposition was observed with 11 inseminated females. However, 2 females which had not been inseminated failed to oviposit. A mean of 158 eggs was laid by a female which had fed on a man, whereas 195 eggs were deposited by a female which had fed on a rabbit. Five to 12 days after oviposition, 80.9–97.9% of eggs laid by 7 females hatched, although the rate was only 38.3% with an egg batch laid by 1 female. Thus, it was found that *S. takahasii* is a candidate species suitable for laboratory colonization.

Laboratory colonization of an insect of medical importance is essential to critical studies on many aspects of its biology and of its relation to disease transmission. Colonization of the Simuliidae have been attempted with many species, but only a few species of blackflies have been successfully colonized in the laboratory (reviewed by Mokry *et al.*, 1981). The main reason for this difficulty lies on the fact that most of the simuliid species do not mate in captivity. Until now, laboratory matings have been observed only in 4 palaearctic (Friedrichs, 1922; Wenk, 1965a, b), 3 North American (Davies and Peterson, 1956; Nicholson, 1945; Hocking and Pickering, 1954; Snow *et al.*, 1958) and 2 African (Wenk and Raybould, 1972) species. However, laboratory mating (*i.e. stenogamy*) has not been investigated in Japanese simuliids.

This paper reports the results of observations on mating, blood feeding and oviposition of *Simulium takahasii* (Rubtsov) in the laboratory.

Materials and Methods

All males and females of *S. takahasii* used in this investigation were reared from pupae collected from a small stream running in the rice fields at Yufuin, Oita, Japan.

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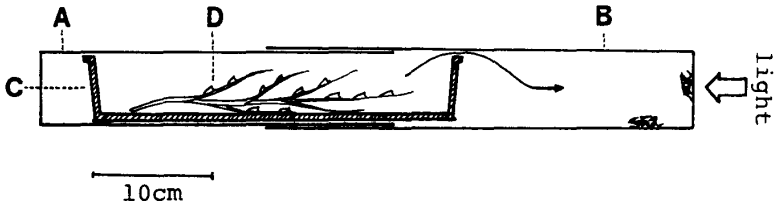

Fig. 1 A simple apparatus (side view) for collecting adults emerging from pupae in the laboratory, showing polyethylene bags (A and B), tray (C), and pupae attached to plant material (D). Direction of emerging blackflies into bag B is indicated by an arrow.

Collections of the pupae were carried out in the afternoon, and the pupae attached to aquatic plants were transported to the laboratory in polyethylene bags.

In the laboratory, the pupae were placed in 4 plastic trays (Fig. 1) (30 cm long × 20 cm wide × 5 cm deep) for adults to emerge. These trays were each enclosed into double transparent polyethylene bags (37 cm long × 32 cm wide), inner bag (A) for maintaining humidity in the tray and the outer one (B) for collecting emerging adults. To facilitate gatherings of adults in the outer bag, these trays were set on a table facing towards the windows. The adults entered the outer bag due to their positive phototaxis. The adults gathered were used, unless otherwise mentioned, for mating experiments and subsequent blood feeding and oviposition trials.

Mating experiment

In the mating experiment I, observations were made on less than 2-hr-old males and females in the outer bags. For this purpose, all blackflies which emerged from 06:00 to 09:00 in the morning were first removed and fixed in 70% ethanol. Thereafter, the adults in the outer bags were removed every 2 hr from 11:00 to 17:00. The number of adults was counted according to sex. This collection was repeated for subsequent 2 days.

The females were microscopically examined for a spermatophore which was attached to the genital fork, if present, and was readily removable with a dissecting needle. To detect the presence of sperms, the females were then dissected in a drop of water on a glass slide and the spermathecae were examined under a microscope.

In the experiment II, observations were made in a polystyrene tube (7.5 cm long × 1 cm in diameter) in which 1 male and 1 female were put together. For this experiment, pupae attached to an aquatic plant were individually kept in a similar tube until adults emerged. Pairing of both sexes was carried out on the day of emergence. The mating behavior was observed and the duration required for copulation was measured. All copulated females were dissected to ensure the observation.

Further, small scale mating experiment III was performed in a tube to measure the insemination capacity of individual males. Four males were rendered to mate each with 5 to 7 females one by one in sequence. All females were dissected in the similar manner to check the spermathecae.

Blood feeding

Six trials of blood feeding of inseminated or non-inseminated females on a man's hand or a rabbit's ear were carried out on the day of emergence. In 5 trials a man's hand was inserted in the outer bags containing 11–34 female blackflies, and in 1 trial a rabbit's ear was exposed to 16 females in a similar bag for about 30 minutes. Blood-fed females were replaced individually into tubes, and maintained at a temperature of 20–24°C, using the same method as previously described (Takaoka *et al.*, 1982).

Inducement of oviposition

On the 2nd to 9th days after the blood feeding, all gravid females were transferred each into a polystyrene tube (10 cm long × 1.4 cm in diameter) containing ca. 3 ml of distilled water, and a strip of white paper

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(water proof, 7 cm long × 0.5 cm wide) half immersed in water. Under the room temperature of about 22°C, all blackflies were allowed to stay in these tubes for 4 hr in the afternoon. The method of oviposition and the site of eggs laid were recorded and, in some cases, the duration required for oviposition was measured. After their oviposition was completed, the number of eggs oviposited in the tube and that retained in their ovaries were counted under a microscope. Females which did not oviposit were returned to the maintaining tube in the evening. Further inducement of oviposition was attempted with these blackflies on the subsequent days.

The eggs laid in a mass on the paper were kept in water (about 20°C) of the same tube. Thereafter, hatching of eggs were monitored and the numbers of hatched and unhatched eggs were recorded.

Results

Mating

The result of the mating experiment I with *S. takahasii* is shown in Table 1. Mating took place soon after emergence throughout the daytime. In 22 of 24 outer bags, 51 of 75 females which emerged during the period from 09:00 to 17:00, carried a spermatophore and were inseminated. Remarkably, insemination had been completed in all 288 females that emerged during the time from 06:00 to 09:00 in the morning.

Table 1 Proportions of *S. takahasii* females inseminated within 2 hr post-emergence kept under various sex rates in a polyethylene bag (37 cm long×32 cm wide).

Time	Tray No.	Day 1			Day 2			Day 3		
		No. male	No. female	No. (%) inseminated	No. male	No. female	No. (%) inseminated	No. male	No. female	No. (%) inseminated
~0900	1,2,3,4 *	76	37	37 (100)	193	161	161 (100)	138	90	90 (100)
0900~1100	1	1	2	1 (50)	—	—	— (—)	—	—	— (—)
	2	—	—	— (—)	2	4	3** (75)	—	—	— (—)
	3	1	1	1 (100)	—	—	— (—)	2	2	1 (50)
	4	—	—	— (—)	2	8	4** (50)	3	2	1 (50)
1100~1300	1	2	3	2 (67)	3	7	3 (43)	1	3	1 (33)
	2	6	6	5 (83)	1	1	1 (100)	2	4	4 (100)
	3	4	1	1 (100)	—	—	— (—)	1	3	2 (67)
	4	1	2	2 (100)	—	—	— (—)	—	—	— (—)
1300~1500	1	7	2	2 (100)	—	—	— (—)	—	—	— (—)
	2	5	4	4 (100)	—	—	— (—)	—	—	— (—)
	3	—	—	— (—)	1	3	0 (0)	—	—	— (—)
	4	11	4	4 (100)	—	—	— (—)	1	3	1 (33)
1500~1700	1	4	2	0 (0)	—	—	— (—)	—	—	— (—)
	2	5	1	1 (100)	—	—	— (—)	—	—	— (—)
	3	9	2	2 (100)	—	—	— (—)	—	—	— (—)
	4	9	5	5 (100)	—	—	— (—)	—	—	— (—)

* The numbers of males and females in this line represent the total of adults emerging from the 4 trays during the period from 06:00 to 09:00 in the morning.

** Each of these figures includes 1 female in copulation.

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Table 2 Number of female *S. takahasii* mated in sequence by a male.

Male No.	No. females			
	Tested	Copulated	With spermatophore	With sperms in spermatheca
1	6	6	3	3
2	5	5	4	4
3*	7	4	3	3
4*	6	3	2	2

* Male No. 3 and 4 failed to copulate with the last 3 females, probably due to mechanical obstruction of a spermatophore remaining attached to the outer surface of their genitalia.

In all 43 trials of the mating experiment II, the adults readily mated in the tubes, and 11 min on average (range, 6.6–27 min) was required for copulation (based on 14 copulations).

Table 2 shows the result of the mating experiment III. It was found that single male can inseminate 2 to 4 females in succession. Further, the male, if given a chance, continued to be stimulated by a female and often copulation took place, but without insemination.

Blood feeding

Table 3 shows the result of their blood feeding. In all 5 trials with a man's hand, 15 of 102 females (14.7%) took blood. On the other hand, 6 of 16 females (37.5%) fed on a rabbit's ear. Both inseminated and non-inseminated females were found to take blood even on the day of emergence. The duration required for blood feeding of 6 females which fed on the back of a man's hand averaged 23.7 min, ranging from 15.3 min to 28.9 min.

Oviposition

Of 21 females which had fed on blood, 8 died within 48 hr after the blood feeding. The remaining 13 females survived beyond 72 hr, which was long enough for their ovaries to develop, and 11 of them (84.6%) laid eggs 3 or 4 days after their blood feed. Two females which had not been inseminated, but fed on a man's hand failed to oviposit, although 174 and 190 mature eggs were retained each in the two females on the 9th day post-feeding.

The result of the 11 females that oviposited in the tubes is given in Table 4. Most of the eggs were laid in a mass on the strip paper at or just below the waterline, while eggs of 3 females were laid scattered either on the paper or on the inside wall of the tube. Oviposition took a mean of 3.3 min when the eggs were laid in a mass. Dissection revealed that all females except one laid eggs completely. The man-fed females laid 158 eggs on average, whereas the rabbit-fed females laid 195 eggs.

Table 3 Blood feeding of female *S. takahasii* in the laboratory.

Blood source	Trial No.	No. females used*	No. (%) females fed on blood
Man's hand	1	16	3 (18.8)
	2	30	5 (16.7)
	3	34	4 (11.8)
	4	11	1 (9.1)
	5	11	2 (18.2)
Total		102	15 (14.7)
Rabbit's ear	6	16	6 (37.5)

* All females used were less than 8-hr-old and were inseminated except females used in trial No. 5.

Only the egg batches laid in a mass were induced to hatch. In all egg batches, embryonic development took place, and as early as 3 days after oviposition the eye spots became visible. Larval emergence was observed 5 to 12 days after oviposition. However, about 60% of the total larvae emerged in the first 2 days (*i.e.* on days 5 and 6). In all, 80.9–97.9% of eggs hatched, except the eggs laid by female No. 2 which

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Table 4 Method, site and duration of oviposition, and number of eggs laid, by female *S. takahasii* which had fed on man and rabbit.

Blood source	Female No.	Oviposition			No. eggs	
		Method	Position	Duration (min)	Oviposited	Remaining
Man	1	Massed	On w.l.*	—	191	0
	2	Massed	In water	—	183	0
	3	Massed	On w.l.	—	165	0
	4	Massed	On w.l.	—	123	73
	5	Scattered	In air	—	160	0
	6	Scattered	In air	5.55	125	0
	Mean				158	
Rabbit	7	Massed	On w.l.	2.83	208	0
	8	Massed	In w.l.	3.91	188	0
	9	Massed	On w.l.	3.67	187	0
	10	Massed	In water	2.83	236	0
	11	Scattered	In air	—	154	0
	Mean			3.31	195	

* w.l.: waterline.

Table 5 Number and proportion of hatching of eggs laid by female *S. takahasii*, under a water temperature of about 20°C.

Blood source	Female No.	No. eggs laid	Hatched eggs	
			No.	%
Man	1	191	177	92.7
	2	183	70	38.3
	3	165	150	90.9
	4	123	109	88.6
	Total	662	506	76.4
Rabbit	5	208	200	96.2
	6	188	179	95.2
	7	187	183	97.9
	8	236	191	80.9
	Total	819	753	91.9

was as low as 38.3% (Table 5).

Discussion

Simulium takahasii, an anautogeneous, multivoltine blackfly, has been observed to mate on vegetation at breeding sites under natural conditions (Takaoka, unpublished data). In this investigation, it was clearly shown that this species readily mate in confinement. The males mated during the day and was polygamous, being capable of inseminating more than two females. The high insemination rates proved that mating is not an obstacle to the colonization of this species.

In addition, blood feeding was successfully induced with a man's hand and a rabbit's ear. This finding is also noteworthy, because this species is zoophilic, attacking cattle in nature. Their feeding rates on a man and a rabbit were not so high. The feeding rate will be influenced by many factors. One of these is the age of adult females used (McMahon, 1968; Mokry, 1976). For example, McMahon (1968) showed that the 5-day-old *S. ornatum* which emerged in the laboratory fed at a higher rate (55%) than the 1-day-old (4.5%). Therefore, it is likely that the feeding rate is improved with further experiments using females of an older age, as well as with the modification of feeding techniques.

Out of 13 inseminated females which had fed on man, 7 died within 48 hr, but the remaining 6 females (46%) survived beyond 3 days. The reason for this high mortality is unknown. However, a high survival rate (83%) obtained in the rabbit-fed females encourages for further experimentation.

Furthermore, it is of special interest that

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successful oviposition of viable eggs was obtained in all the 11 inseminated females tested. Unlike *S. damnosum* s.l. in Africa (Lewis *et al.*, 1961), *S. takahasii* was induced to lay eggs without any artificial stress, such as immersion in water or decapitation. And, nearly all the females oviposited completely in the tubes. The average numbers of eggs laid by females which had fed on man and rabbit (158 and 195, respectively) tended to be lower than the number laid by 6 wild-caught gravid females (250 eggs—Takaoka, unpublished data). However, it is unlikely that perpetuating of colonies in the laboratory would be hampered by these somewhat reduced numbers of eggs, because this could be compensated for by the high hatching rates of eggs.

In summary, the successful mating, blood feeding and oviposition of viable eggs of *S. takahasii* in the laboratory suggest that this species could be colonized. It is also suggested that, as already utilized by other investigators (Raybould and Yagunga, 1969; Wenk and Raybould, 1972; Grunewald and Wirtz, 1978), a rabbit will be a good blood source for this species.

A proportion of hatched larvae was successfully reared up to the adult stage in the laboratory, using a stir-bar system (Takaoka, unpublished data). An improvement of larval rearing techniques, coupled with those of blood feeding, is now in progress.

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References

Davies, D. M. and B. V. Peterson (1956): Observations on the mating, feeding, ovarian development, and oviposition of adult black flies (Simuliidae, Diptera). *Can. J. Zool.*, **34**: 615–655.

Friedrichs, K. (1922): Untersuchungen über Simuliiden (Teil II). *Z. Angew. Entomol.*, **8**: 31–92.

Grunewald, J. and H. P. Wirtz (1978): Künstliche Blutfütterung einiger afrikanischer und paräarktischer Simuliiden (Diptera). *Z. Angew. Entomol.*, **85**: 425–435.

Hocking, B. and L. R. Pickering (1954): Observations on the bionomics of some northern species of Simuliidae (Diptera). *Can. J. Zool.*, **32**: 99–119.

Lewis, D. J., G. R. L. Lyons and J. D. M. Marr (1961): Observations on *Simulium damnosum* from the Red Volta in Ghana. *Ann. Trop. Med. Parasitol.*, **55**: 202–210.

McMahon, J. P. (1968): Artificial feeding of *Simulium* vectors of human and bovine onchocerciasis. *Bull. Wld. Hlth. Org.*, **38**: 957–966.

Mokry, J. E. (1976): A simplified membrane technique for feeding blackflies (Diptera: Simuliidae) on blood in the laboratory. *Bull. Wld. Hlth. Org.*, **53**: 127–129.

Mokry, J. E., M. H. Colbo and B. H. Thompson (1981): Laboratory colonization of blackflies. *In*: *Blackflies,* pp. 299–306, Academic Press, London.

Nicholson, H. P. (1945): The morphology of the mouthparts of the nonbiting blackfly, *Eusimulium dacotense* D. and S., as compared with those of the biting species, *Simulium venustum* Say. *Ann. Entomol. Soc. Am.*, **38**: 281–297.

Raybould, J. N. and A. S. K. Yagunga (1969): Artificial feeding of East African Simuliidae (Diptera) including vectors of human onchocerciasis. *Bull. Wld. Hlth. Org.*, **40**: 463–466.

Snow, W. E., E. Pickard and J. B. Moor (1958): Observations on blackflies (Simuliidae) in the Tennessee River Basin. *J. Tenn. Acad. Sci.*, **33**: 5–24.

Takaoka, H., J. O. Ochoa A., E. L. Juares and K. M. Hansen (1982): Effects of temperature on development of *Onchocerca volvulus* in *Simulium ochraceum* and longevity of the simuliid vector. *J. Parasitol.*, **68**: 478–483.

Wenk, P. (1965a): Über die Biologie blutsaugender Simuliiden. II. Schwarmverhalten, Geschlechterfindung und Kopulation. *Z. Morphol. Öekol. Tiere,* **55**: 671–713.

Wenk, P. (1965b): Über die Biologie blutsaugender Simuliiden. III. Kopulation, Blutsaugen und Eiablage von *Boophthora erythrocephala* De Geer im Laboratorium. *Z. Tropenmed. Parasitol.*, **16**: 207–226.

Wenk, P. and J. N. Raybould (1972): Mating, blood-feeding and oviposition of the "Kibwezi" form of *Simulium damnosum* Theobald (Diptera: Simuliidae) in the laboratory. *Bull. Wld. Hlth. Org.*, **47**: 627–634.

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摘　要

ウマブユ（*Simulium takahasii*）の交尾，吸血および産卵に関する室内観察

ブユの室内累代飼育は，蛹から羽化した成虫に交尾および吸血行動を起させることの困難さから欧米産の2, 3のブユ種を除いて成功していない．今回，本邦産のウマブユ（*Simulium takahasii*）について室内飼育の可能性を探る目的で室内観察を行った．

本種は，羽化後すぐ，ポリエチレン袋やポリスチレン小試験管内で容易に交尾する，いわゆる狭所交尾性を有することがわかった．交尾は日中どの時刻に羽化した雌雄にも認められ，交尾率および受精率は，小試験管に雌雄各1個体を入れた場合は，100%と高率であった．また1個体の雄は2～4個体の雌を連続して受精せしめることもわかった．羽化当日，11～34個体の受精雌をポリエチレン袋に入れ，約30分間人の手を与えたところ，14.3% (13/91) が吸血した．未受精雌は18.2% (2/11) が吸血した．また，16個体の受精雌に約30分間ウサギの耳を与えたところ6個体（37.5%）に吸血が認められた．吸血後2日目から，水を3分の1ほど入れた試験管に成虫を移し毎日産卵の機会を与えた結果，途中死亡した雌を除いてすべての受精雌（11個体）が吸血後3～4日目に産卵した．2個体の未受精雌は体内に卵の成熟は認められたが産卵しなかった．産卵数は，人およびウサギを吸血したブユで異なり，おのおの1個体の雌当り平均158と195であった．産下された卵のうち，一カ所にまとめて産み付けられた8個の卵塊をおのおの水中に放置したところ，産卵後5～12日目に80.9～97.9%（38.3%の1例を除く）の卵が孵化した．このように，室内で困難とされている交尾・吸血，および産卵が容易に認められたことから，本邦産ウマブユは室内累代飼育に好適なブユ種と思われ，今後が期待される．

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