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Experimental Infection of the Blackfly *Simulium takahasii* with *Brugia pahangi*

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Among various arthropods of medical importance, a blackfly (Diptera: Simuliidae) has been known to transmit several species of filarial nematodes (e.g. *Onchocerca volvulus, Mansonella ozzardi, Ornithofilaria fallisensis, Wehrdikmansia cervipedis*), as reviewed by Crosskey (1973) and Schacher (1973). Other than these four genera, there is no record of any kind of filariae transmitted by this biting gnat.

However, developing and/or infective larvae of unknown filariae were often found in wild-caught females of several blackfly species including vectors for human onchocerciasis in tropical Africa and Central America (Crosskey, 1957; Crosskey and Crosskey, 1958; Nelson and Pester, 1962; Duke, 1967; Garms and Voelker, 1969; Garms, 1975). These field observations may suggest that some species of blackflies play a role in the transmission of much wider range of filarial worms in nature.

Up to date, it was difficult to carry out the experimental feeding of blackflies in the laboratory due to the reluctancy of the newly-emerged flies to feed a blood meal in captivity. Our recent finding on blood-feeding habit of *Simulium takahasii* (Rubtsov) in the laboratory (Takaoka, 1985) has overcome this problem and enabled us to investigate the susceptibility of this blackfly to various kinds of filarial parasites maintained in laboratory animals.

The present study deals with the experimental infections of *S. takahasii* with *Brugia pahangi* by feeding newly-emerged female flies directly on the infected jirds (*Meriones unguiculatus*). The aim of this study was to determine whether or not *S. takahasii* is susceptible to infection with this exotic parasite, which is quite different from all the known simuliid-transmitted filariae by having the sheathed microfilariae.

Materials and Methods

The filarial worm, *B. pahangi*, used in this experiment has been maintained for many years at Institute for Tropical Medicine, Nagasaki University, by jird-to-jird transmission through *Aedes aegypti*. Two *B. pahangi*-infected jirds were exposed to the bites of blackflies. One of them showed moderate microfilarial density (MfD) (223 mf/40 mm^3 of blood) and the other, high MfD (699 mf/40 mm^3 of blood). Females of *S. takahasii* used in this study were all reared from pupae collected from a small stream at Yufuin, Oita.

In a polyethylene bag (about 20 cm in diameter), a batch of 80–120 newly-emerged female flies (less than 8 hour old) were allowed to feed to repletion on the shaved tail and hip of animals, which were held immobile by wire net to facilitate feeding. These feeding trials were made for four hours from 14.00 to 18.00 under a room temperature of 22–24°C, and

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repeated for four days. In total, 24% of 370 flies successfully fed blood meal from a jird with moderate MfD, and 28% of 410 flies from a jird with high MfD. Twenty flies fed on either animal were killed immediately after feeding to determine if microfilariae were ingested alive. The remaining blood-fed flies were kept individually in a polypropylene tube under a constant temperature of 26°C. The methods of fly maintenance in the laboratory followed those of Takaoka *et al* (1982), with a slight modification of the diet of 35% sucrose given at 48-hour intervals. Every day after feeding, all the flies were checked for mortality. The dead or moribund flies, if any, were removed and dissected for larval development. From the ninth to 14th day, live flies, as well as dead ones, were dissected.

Dissections were made in a drop of 0.9% saline solution on a glass slide under a dissecting microscope. Each fly was separated into head, thorax and abdomen, each of which was teased and then searched for parasites. The number of larvae in each part of the body was counted. The developmental stages of larvae were determined by the morphological characteristics, defined by Schacher (1962). Larvae, if vigorously moving, were immobilized by heat, and measured with the aid of a calibrated ocular micrometer in the eyepiece of a compound microscope.

Results

The live microfilariae of *B. pahangi* were recovered from the stomach of 85% (17/20) and 75% (15/20) of female *S. takahasii* which had fed on jirds with moderate and high MfD, respectively. The number of microfilariae ingested per positive fly from jird with modeate MfD varied from 1 to 21 (mean 6.5), and that from jird with high MfD from 2 to 119 (mean 25).

A total of 163 flies which fed on jirds, 69 fed with moderate MfD and 94 with high MfD, were dissected during the period of 1 to 14 days after ingestion of blood meal. Thirty seven flies, seven from moderate MfD and 30 from high MfD, harboured either microfilariae or developing larvae in the thorax (Table 1). The number of larvae recovered per fly ranged from 1 to 11.

In the fly group which fed on jird with moderate MfD, no larvae were recovered on days 3–8 after feeding. Between the 9th and 14th days, 13 larvae were recoverd, six of which were in the second stage. The second-stage larvae were first found on day 9. However, no third-stage larvae were obtained in this fly group. Whereas, a total of 107 larvae were recovered from the flies which had fed on jird with high MfD. All the larvae found on days 5–8 remained in the first stage. No larvae

Table 1 Results of dissections of *S. takahasii* females which fed on jirds infected with *B. pahangi*, and maintained at a constant temperature of 26°C

Days post feeding	Fly group fed with moderate-MfD blood			Fly group fed with high-MfD blood		
	No. flies dissected	No. (%) flies with larvae in thorax	No. larvae & developmental stage*	No. flies dissected	No. (%) flies with larvae in thorax	No. larvae & developmental stage*
1– 2	5	2 (40)	3mf	3	1 (33)	4mf
3– 4	4	0 (0)		5	0 (0)	
5– 6	1	0 (0)		2	1 (50)	$4L_1$
7– 8	4	0 (0)		3	2 (67)	$8L_1$
9–10	14	1 (7)	$1L_1+2L_2$	20	5 (25)	$22L_1+8L_2$
11–12	19	3 (16)	$6L_1+3L_2$	27	9 (33)	$8L_1+13L_2+1L_3$
13–14	22	1 (5)	$1L_2$	34	12 (35)	$25L_1+9L_2+5L_3$
Total	69	7 (10)	$3mf+7L_1+6L_2$	94	30 (32)	$4mf+67L_1+30L_2+6L_3$

*mf = microfilariae; L_1 = first-stage larva(e); L_2 = second-stage larva(e); L_3 = third-stage larva(e)

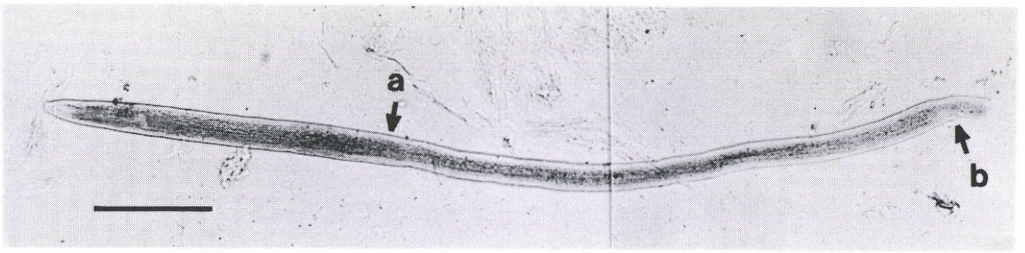

Fig. 1 A third-stage larva of *B. pahangi* recovered from *S. takahasii.* a, esophago-intestinal junction; b, anus. Scale 100 μm.

were found in three flies examined on day 9. On the next day, 22 (73%) of 30 larvae recovered were still in the first stage, but remaining 8 (27%) developed to the second stage. Third-stage larva (Fig. 1) was first found on day 12 post-infection. During the period of 13–14 days after feeding, the majority of larvae recovered were in the first stage, followed by second- and third-stage larvae. Some of the first-stage larvae recovered 9–14 days after ingestion of blood meal, were deformed. On the other hand, all the second- and third-stage larvae but one did not show any remarkable degeneration. The body lengths of six third-stage larvae found were 764–1,262 μm (mean 938 μm) and the body widths at nerve ring were 22–30 μm (mean 25 μm). All the six larvae were found in the thorax, two from one fly and the others each from four flies.

Discussion

In the present study, microfilariae of *B. pahangi* were found to be ingested by *S. takahasii.* The mean microfilarial intake in flies which fed on a jird with moderate MfD was fewer than that in flies which fed on a jird with high MfD, as might be expected. Further, microfilariae of *B. pahangi,* though sheathed, were found to be able to migrate from the midgut to the thorax in this blackfly species.

There was no previous report on the blackfly's susceptibility to infection with filarial parasites other than *Onchocerca* spp., *Mansonella ozzardi, Ornithofilaria fallisensis* and *Wehrdikmansia cervipedis,* as already mentioned. It was proven in our study that *B. pahangi* could develop to the third-stage larvae in the blackfly, too. However, larval development was mostly retarded or arrested and only a small number of larvae reached the third stage.

Our results do not necessarily indicate that *S. takahasii* is capable of transmitting *B. pahangi.* It seems doubtful if all the third-stage larvae found were infective, because they were short in body length, as compared with the average size (1,558 μm) of the same stage of larvae which had developed in the mosquito, *Anopheles quadrimaculatum* (Schacher, 1962), and were not found to migrate from the thorax to the head region. Further studies are needed to determine whether the third-stage larvae of *B. pahangi* recovered from *S. takahasii* develop to the adult worms in the vertebrate host animals.

On the other hand, Macdonald and Ramchandran (1965) reported that the susceptibility of *Ae. aegypti* to *B. pahangi,* as well as *B. malayi* and *Wuchereria bancrofti,* was controlled by a sex-linked recessive gene. It will be interesting to study whether a similar genetic control of infection with the same filaria is exerted in *S. takahasii,* of which mating and larval rearing are feasible in the laboratory (Takaoka, 1985).

Summary

In order to determine whether a blackfly is susceptible to infection with *Brugia pahangi,*

experimental infections were performed by feeding newly-emerged *Simulium takahasii* females on the infected jirds with moderate microfilarial density (MfD) (223 mf/40 mm^3 of blood) and high Mfd (699 mf/40 mm^3 of blood).

The live microfilariae of *B. pahangi* were successfully ingested by 85% of flies fed on jird with moderate MfD and by 75% of flies fed with high MfD. The mean microfilarial intake per positive fly (25 mf) in the latter fly group was, however, higher than that (6.5 mf) in the former group.

A total of 163 flies which fed on infected jirds were maintained at 26°C and examined during the period of 1–14 days post-infection. As a result, 37 (22.7%) of these flies harboured 1–11 larvae per fly. The larval development of *B. pahangi* microfilariae to the third stage was observed to occur in the thorax of *S. takahasii.* The moultings from the first to second and from the second to third stage took place as early as day 9 and day 12 post-infection, respectively. However, the proportion of larvae developing to the third stage was very low, due to the retarded or arrested development found in most larvae.

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Brugia pahangi のウマブユへの実験感染

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ブユは，*Onchocerca* 属の数種，*Mansonella ozzardi*, *Ornithofilaria fallisensis* および *Wehrdikmansia cervipldis* を媒介することはよく知られているが，その他のフィラリアに対する感受性の有無についてはよく判っていない．この理由の一つは，ほとんどのブユ種が室内で吸血しないため感染実験が行えなかったことによる．今回，我々は，ウマブユ(*Simulium takahasii*)が室内でも吸血する点に注目し，本種の*Brugia pahangi* に対する感受性について検討した．実験では，羽化当日のブユに，*B. pahangi* の血中仔虫密度が各々223mf/血液40㎜^{3} および699mf/血液40㎜^{3} の2頭のスナネズミを吸血させ，仔虫とりこみ，および発育を観察した．その結果，スナネズミ血中の *B.pahangi* の仔虫は，ウマブユの吸血に際し摂取され，一部の仔虫はさらに中腸から胸筋へ移行することが判った．そして，26℃の恒温下で *B.pahangi* の仔虫は摂取された後，早くて9日目に第2期幼虫に，さらに12日目に第3期幼虫になることが観察された．しかしながら，多くの幼虫で発育の遅延や異常がみられ，第3期幼虫まで発育したのは僅か6隻であった．今回の結果により，ブユは有鞘仔虫をもつ *B.pahangi* にも感受性があることが明らかとなった．