

Exsheathment and Migration of Microfilariae of *Brugia malayi* (Che-ju Strain) in Mosquitoes

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Abstract: Observations were made on the exsheathment and migration of microfilariae of *Brugia malayi* in mosquitoes. In *Aedes togoi* and *Aedes albopictus*, the microfilariae ingested into the midgut commonly exsheathed and migrated to the thorax. The larvae which moved to the thorax in the former mosquito developed to the 3rd stage, but in the latter the larvae did not grow even to the 2nd stage. The microfilariae in *Armigeres subalbatus* also cast off the sheath in the stomach at a moderate rate, whereas, those which moved to the thorax were small in number, and did not develop any further. On the other hand, in *Culex pipiens pallens*, most of the microfilariae did not exsheathe and died without migrating to the thorax. Therefore, it can be said that the exsheathment of microfilariae of *B. malayi* in the midgut of mosquitoes has a close relation with their migration to the thorax.

Malayan filariasis is found in the South-and-East Asian countries, such as Ceylon, Burma, Thailand, North Vietnam, the Philippines, Malaysia, Indonesia, China, South Korea, and Japan (Sasa, 1976). It is transmitted by mosquitoes. The mosquitoes such as *Aedes togoi*, *Mansonia uniformis* and *Anopheles sinensis* are known to play an important role as the vectors, since they have generally a high susceptibility to *Brugia malayi* larvae in experimental as well as natural infections, while other mosquitoes such as *Culex pipiens pallens* and *Armigeres subalbatus* are said to be only poor vectors, because they show a low susceptibility to this filarial larvae (Feng, 1934 ; Hayashi, 1954 ; Hu, 1940 a, b, 1941 ; Kim and Seo, 1968 ; Nakajima et al., 1976 ; Sasa et al., 1952 ; Sasa, 1976 ; Wada et al., 1973 ; Wharton, 1962). However, it is not quite clear why susceptibilities of these mosquitoes to the filarial larvae vary with the mosquito species.

As the first step to elucidate this problem, we have observed the extent of the exsheathment and migration of microfilariae of *B. malayi* in the mosquitoes, and also the development of the filarial larvae after migration to the thorax. The present paper reports the results of these observations.

MATERIALS AND METHODS

The mosquitoes used in the present experiment were the following species of Nagasaki strain : *Aedes togoi*, *Aedes albopictus*, *Armigeres subalbatus*, and *Culex pipiens pallens*. The mosquitoes were maintained at a temperature of 25°C and were allowed to feed on a cat infected with *Brugia malayi* of Che-ju strain for one night (4 PM to 8 AM). With these mosquitoes, two following experiments were done independently: (1) the mosquitoes were killed immediately and 24 hours after the end of blood feeding (8 AM). These times mean 8 and 32 hours after midpoint of feeding time, respectively. The midgut of the infected females was separated from the mosquito body and dissected in a drop of 0.9% saline solution, and the numbers of unsheathed and sheathed microfilariae were recorded with a phase contrast microscope; (2) in the other experiment, mosquitoes were dissected at various times after blood feeding. Each mosquito was divided into head, thorax, and abdomen, which were teased apart in a drop of the same solution and stained by Giemsa after drying. The number and developmental stages of larvae found in each part were examined with a compound microscope.

RESULTS

1. Exsheathment of microfilariae in the midgut

Table 1 shows the results of the exsheathment of microfilariae in the midgut of *Ae. togoi*, *Ae. albopictus*, *Ar. subalbatus* and *Cx. p. pallens*. In *Ae. togoi*, most of microfilariae exsheathed already 8 hours after blood feeding. In *Ae. albopictus* and *Ar. subalbatus*, rates of exsheathment were moderate or low 8 hours after blood feeding, but high 32 hours or later. However, in *Cx. p. pallens*, only a very few microfilariae exsheathed in 8 and 32 hours.

Table 1. Exsheathment of *Brugia malayi* microfilariae in the midgut of mosquitoes

Mosquito species	Hours after infection					
	8 hours			32 hours		
	No. females dissected	Microfilariae		No. females dissected	Microfilariae	
Total No. observed		% ex- sheathed	Total No. observed		% ex- sheathed	
<i>Ae. togoi</i>	12	53	96.7	5	35	91.4
<i>Ae. albopictus</i>	6	15	60.0	10	21	100.0
<i>Ar. subalbatus</i>	11	51	13.7	18	56	85.8
<i>Cx. p. pallens</i>	10	135	7.4	9	137	9.7

2. Number and developmental stages of larvae in thorax and other parts

After taking blood meal of a cat infected with *B. malayi*, females of each mosquito species were dissected at irregular intervals, and the number of larvae and their developmental stages were examined. Tables 2 and 3 show infection rates of mosquitoes with larvae, and the numbers of larvae which migrated to the thorax and those which developed following the migration into the thorax.

Table 2 indicates that when females were dissected immediately after taking blood meal of the infected cat, microfilariae were usually found. In *Ae. togoi*, many females retained the larvae for 15 days, however, in *Ar. subalbatus*, *Ae. albopictus*, and *Cx. p. pallens* females with larvae diminished in rates with time, and no larvae were found 15 days after infection. As shown in Table 3, in *Ae. togoi*, the larvae of *B. malayi* migrated to the thorax in one day after blood feeding, and developed to the 3rd stage in 15 days. In *Ae. albopictus* and *Ar. subalbatus*, the larvae also migrated to the thorax in one or three days after taking blood meal, but all the larvae in the thorax were at the 1st stage.

Table 2. Infection rates of mosquitoes with larvae of *Brugia malayi*

Mosquitoes	0			1			3			15		
	No. dissected	No. with larvae	%	No. dissected	No. with larvae	%	No. dissected	No. with larvae	%	No. dissected	No. with larvae	%
<i>Ae. togi</i>	8	8	100.0	22	14	63.6	12	7	58.3	13	10	76.9
<i>Ae. albopictus</i>	10	9	90.0	10	7	70.0	27	0	0.0	27	0	0.0
<i>Ar. subalbatus</i>	28	23	82.1	33	9	27.3	29	13	44.8	12	0	0.0
<i>Cx. p. pallens</i>	7	7	100.0	7	5	71.4	14	1	7.1	3	0	0.0

Table 3. Average number of *Brugia malayi* larvae in the mosquitoes shown in Table 2

Microf. per mm ³ of cat blood	Mosquito	Part of mosquito body	Days after infection			
			0	1	3	15
4.3	<i>Ae. togoi</i>	Midgut	8.4			0.0
		Thorax,		2.9	2.6	3.5*
4.0	<i>Ae. albopictus</i>	Midgut	6.8	7.7		0.0
		Thorax,	6.1	7.1	2.4	0.0
5.2	<i>Ar. subalbatus</i>	Midgut	8.6	3.5		0.0
		Thorax,			4.2	0.0
3.6	<i>Cx. p. pallens</i>	Midgut	95.1	5.2	1.0	0.0
		Thorax,		0.2		0.0

* the 3rd stage larvae in thorax and mouth part.

Absence of any larvae 15 days after infection seems to indicate the extremely low susceptibility of these mosquitoes. In *Cx. p. pallens*, the migrated larvae were much smaller in number than in the 3 species mentioned above, and any larvae of the 3rd stage were not found in this mosquito species.

In Table 4, results presented in Tables 1 to 3 are summarized. In *Ae. togoi* and *Ae. albopictus*, the microfilariae ingested into the midgut commonly exsheathed and migrated to the thorax. The larvae which moved to the thorax in *Ae. togoi* developed to the 3rd stage, but in *Ae. albopictus* the larvae did not grow even to the 2nd stage. The microfilariae in *Ar. subalbatus* also exsheathed in the stomach at a moderate rate, however, those which moved to the thorax were small in number, and did not develop more. On the other hand, in *Cx. p. pallens*, most of the microfilariae did not exsheathe and died without migrating to the thorax.

Table 4. Summary of results given in Tables 1 to 3

Mosquito	Rate of exsheathment in might	Rate of migration to thorax	Rate of development to 3rd stage
<i>Ae. togoi</i>	High	High	High
<i>Ae. albopictus</i>	High	High	0
<i>Ar. subalbatus</i>	Medium	Medium	0
<i>Cx. p. pallens</i>	Very low	Very low	0

DISCUSSION

In *Ae. togoi*, the microfilariae ingested into the midgut commonly moved to the thorax and developed to the 3rd stage, when females were fed on a patient or a cat infected with *B. malayi* of periodic form and were reared for reasonably long time (Kim and Seo, 1968 ; Nakajima et al., 1976). In our experiments, this fact was confirmed, and as Kim and Seo (1968) observed, the microfilariae were found to exsheathe usually in the stomach of this mosquito. In other words, the microfilariae can easily shed their sheath in the midgut in *Ae. togoi* which is highly susceptible to the *B. malayi* larvae.

Our present experiment showed that when *Ae. albopictus* females ingested microfilariae into the midgut, the exsheathment occurred commonly, and most of larvae which cast off the sheath soon migrated to the thorax, but they did not develop to the 2nd stage. Accordingly, it seems that the mosquito of *Ae. albopictus* can not be an efficient vector.

Hu (1941) found that when 149 *Ar. subalbatus* (as *Ar. obturbans*) females were fed on a patient with a large number of microfilariae of *B. malayi*, 123 mosquitoes had the dead larvae of the 1st stage, and 11 or 7.4 % of 149 females retained infective larvae which were about 2 in number in a female. Whaton (1962) reported that the vast majority

of larvae died at the 1st stage in the thorax, and a few developed normally to the 3rd stage in *Ar. subalbatus* females which were fed on a cat with *B. malayi* of periodic form. Nakajima et al. (1976) wrote that in *Ar. subalbatus* females infected with *B. malayi* (Che-ju strain), most of larvae died in the stomach and only a few moved to the thorax, but they did not develop and died 2 or 3 days later. About similar results were obtained in the present experiment. From our data on exsheathment of microfilariae and the results written above, it can be said that generally in *Ar. subalbatus* mosquito which has low susceptibility to *B. malayi*, microfilariae cast off sheath at moderate rate in midgut and migrate to the thorax at a low rate, and a very few of the larvae may develop to the 3rd stage. Therefore, this mosquito also will not play an important role as a vector in nature, as reported already (Wharton, 1962).

It is interesting that microfilariae of *B. pahangi* develop to the infective stage in *Ar. subalbatus* females which show the extremely low susceptibility of *B. malayi* larvae (Nakajima et al., 1976 ; Wharton, 1962). According to our unpublished data, it was also found that in *Ar. subalbatus*, the microfilariae of *B. pahangi* shed the sheath in a higher rate than those of *B. malayi*.

Although *Cx. p. pallens* did not produce any 3rd stage larvae of *B. malayi* in our experiments, Hu(1940) reported that 5 or 2.1% of 242 *Cx. p. pallens* females which were allowed to feed on a heavy case of *B. malayi* produced 1 to 3 infective larvae. Hayashi (1954) also reported almost the same results. Therefore, it is considered that only a few, if any, of a great number of microfilariae ingested into midgut of *Cx. p. pallens* females develop to the 3rd stage.

Our experiment revealed that 8 and 32 hours after infection, most of microfilariae in the midgut of *Cx. p. pallens* still had sheath and were very slow in their activities. The same fact has been observed also in *B. pahangi* microfilariae in the midgut of *Cx. p. quinquefasciatus* (Ewert, 1965). Aoki(1971) supposed that some factors necessary for microfilariae to exsheathe are lacking in the stomach of mosquitoes in which larvae can not complete their development, from observations on the exsheathment of microfilariae of *B. pahangi* and *Wuchereria bancrofti* in vitro. His supposition can be accepted in the case of *B. malayi*.

From the present result and those mentioned above, it will be concluded that *B. malayi* microfilariae exsheathe generally in a higher rate in the midgut of mosquitoes with high susceptibility than in those with low susceptibility, and the exsheathment of the microfilariae of *B. malayi* has a close relation with their migration to the thorax, as observed in *B. pahangi* (Ewert, 1965 ; Owen, 1978).

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蚊体内におけるマレー糸状虫（済州島系）のマイクロフィラリアの脱鞘と移動
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済州島系のマレー糸状虫に感染したネコから長崎系のトウゴウヤブカ，ヒトスジシマカ，オオクロヤブカ，及びアカイエカに吸血させ，マイクロフィラリアの蚊の胃内での脱鞘の程度，胸筋への移動とその後の幼虫の発育の状況を調べた。トウゴウヤブカ及びヒトスジシマカでは，マイクロフィラリアは胃内で高率に脱鞘して胸筋に移動した。これらの幼虫はトウゴウヤブカ体内ではⅢ期幼虫にまで発育したが，ヒトスジシマカではⅢ期幼虫まで発育したものはなかった。オオクロヤブカの胃の中でも，マイクロフィラリアはかなり脱鞘し，少数のものは胸筋に移動した。しかし，これら幼虫はすべてⅠ期で死亡した。アカイエカでは，胃の中にとりこまれたマイクロフィラリアの極く少数が脱鞘したが，胸筋に移行するものも極めて少数であり，Ⅲ期幼虫まで発育したものはなかった。以上のことから，本種のマイクロフィラリアの蚊の胃内での脱鞘は胸筋への移動と密接な関連を持つことがわかる。

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