Comparative Studies on the Role of the *Culex pipiens molestus* and *Culex pipiens pallens* Mosquitoes in Transmitting Dog Filaria, *Dirofilaria immitis*, in Nagasaki City

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Abstract: The vector potential to *Dirofilaria immitis* of *Culex pipiens molestus*, a member of the *Culex pipiens* complex, was compared with that of *Culex pipiens pallens*, another member of the complex. In the laboratory, both mosquitoes were fed on a dog with various levels of microfilaremia. The mean number of microfilariae taken up by the mosquitoes and the rate of migration to Malpighian tubules for *Cx. p. molestus* was similar to that for *Cx. p. pallens*. However, the survival rate of *Cx. p. molestus* after feeding on the dog was lower than that of *Cx. p. pallens*. The lower survival rate of *Cx. p. molestus* was the main cause of the lower index of experimental infection. By a light trap, females of *Culex pipiens* complex were collected at two sites in Nagasaki City in 1986 and 1987. *Cx. p. molestus* was distinguished from *Cx. p. pallens* by the number of ommatidia in the compound eyes. Mosquitoes were dissected and examined for the presence of *D. immitis* larvae in proboscis, thorax, abdomen and Malpighian tubules. Natural infection rate of *Cx. p. pallens* was distinctly higher than that of *Cx. p. molestus*. Results of experimental infection and field survey clearly indicate that *Cx. p. molestus* is apparently not as important as *Cx. p. pallens* in the transmission of *D. immitis*.

Key words: *Dirofilaria immitis, Culex pipiens molestus, Culex pipiens pallens*, Vector potential

INTRODUCTION

The dog filaria, *Dirofilaria immitis*, which is transmitted by mosquitoes, is prevailing among house dogs in all parts of Japan (Ohishi, 1986). Recently *D. immitis* offers a public health problem, and so far more than 20 human cases infected with this worm have been reported in Japan (Yoshimura *et al.*, 1980; Yoshimura, 1985).

By examining microfilariae in the blood of dogs, Suenaga *et al.* (1971) found that about 30% of the house dogs were infected with *D. immitis* and Suenaga and Itoh (1973) suggested that its main vector in Nagasaki City was the house mosquito, *Culex pipiens*

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pallens, a member of the *Culex pipiens* complex. Moreover, Webber and Hawking (1955) and Suenaga (1972a) reported that the first stage larvae of *D. immitis* could develop to the infective stage in *Culex pipiens molestus*, another member of the *Culex pipiens* complex. *Cx. p. molestus* commonly attack humans in houses in Nagasaki City (Oda *et al.*, 1986). From these findings, it may be supposed that *Cx. p. molestus* is a vector of this parasite. However, there have been few reports that compared the susceptibility of *Cx. p. molestus* to *D. immitis* with that of *Cx. p. pallens* and the infection of *Cx. p. molestus* with *D. immitis* has not been studied in the field.

The purpose of the present paper is to compare the importance of *Cx. p. molestus* as the vector of *D. immitis* with that of *Cx. p. pallens* by examination of experimental and natural infections.

Materials and Methods

Experimental Infection

In the present experiments I used the females of *Cx. p. molestus* in the 54−68th generation and those of *Cx. p. pallens* in the 34−55th generation. They were reared in the laboratory with 25℃ and 70% RH under the photoperiod of 16:8 (L:D), which had been established from adults collected in a house and in an overwintering place in Nagasaki City, respectively. Larvae from 400 to 500 in number were reared with an equally mixed powder of Brewer's yeast and mouse pellets in a white plastic tray (30×40×7cm) with tap water of 3000ml. Adults were kept in a 20×20×30cm cage. Cotton pads soaking 2% sugar solution were given as a nutrient source to the mosquitoes.

Mosquitoes were infected by feeding on the same male dog with microfilariae of *D. immitis*, throughout the experiment. The seasonal change of microfilarial density in the dog provided mosquitoes with the chance to feed on blood with different levels of microfilaremia. Mosquitoes were allowed to take blood from a dog kept in a metal cage (44×51×61cm) inside a mosquito net from 17:00 to 03:00 the next morning. Before each experimental feeding, 30mm^3 blood sample was drawn for microfilarial counts from the capillary of the dog ear at about 16:30. Females of *Cx. p. pallens* were permitted to feed on blood of a dog at day 5−8 after emergence. In the case of *Cx. p. molestus*, females used were at the same age as in *Cx. p. pallens*, but they had finished the autogenous oviposition.

After feeding, fully blood-fed and unfed females were separately placed with an aspirator to cages. In each experiment, 20 to 40 blood-fed mosquitoes were dissected immediately after feeding to examine the number of microfilariae ingested. The number of mosquitoes in a cage was limited to 100. They were examined daily for survival, and dead individuals were counted and removed. Dead blood-fed females were kept in a freezer (−20℃) for later dissection. Observation for survival was continued until day 15 after infective feeding, because this period was long enough for microfilariae to develop to the 3rd stage larvae at 25℃ (Suenaga, 1972a, b). Surviving mosquitoes were dissected on day

15 after infective feeding. Mosquitoes were dissected in 0.7% saline for the detection of *D. immitis* larvae. Then their Malpighian tubules were examined in 1% acetic acid. I was able to find the larvae in the dead females as well as in the live females. The number and developmental stage of the larvae were recorded with each infected mosquito.

Natural Infection

For the study of natural infection in mosquitoes, two collection sites were selected in Nagasaki City. One was at the campus of Nagasaki University School of Medicine (Site A), and the other at an apartment house, which is situated 2km southwest of Site A, in a residential district (Site B). It should be noted that on the ground around these sites, feeding and oviposition activities of *Cx. p. molestus*, which is essentially an underground breeder, had been reported previously (Oda and Ueda, 1979; Oda *et al.*, 1984; Oda *et al.*, 1986; Zaitsu *et al.*, 1987). Mosquitoes were sampled daily, as a rule, by a light trap (black light type, Fujihira Industry Co., Ltd.) operated from 17:00 to 09:00 at each site from April to early November in 1986 and 1987.

Specimens of the *Culex pipiens* complex were sorted out and stocked in a freezer (−20℃). They were dissected for filarial infection by the same method as for the experimental infection. However, the head was not dissected except for the proboscis, because it was used for the determination of either *Cx. p. pallens* or *Cx. p. molestus*. The identification of *D. immitis* was based on the location of larval development in mosquitoes and the length of larvae.

Cx. p. pallens or *Cx. p. molestus* was determined by counting the number of ommatidia in the compound eyes. Noguchi and Asahina (1966) reported that *Cx. p. molestus* females had 8 ommatidia and *Cx. p. pallens* 9 ommatidia in the 4th row of their compound eyes. Oda (personal communication) conversely suggested that *Cx. p. molestus* females, which were confirmed by autogenous oviposition, scarcely had 9 or 10 ommatidia in the 4th, 5th and 6th rows, and few *Cx. p. pallens* females had 8 or 7 in these rows. Accordingly, in this study, females with 8 or 7 ommatidia in the 4th, 5th and 6th rows of both compound eyes were regarded as *Cx. p. molestus* and those with 9 or 10 ommatidia as *Cx. p. pallens*. To which strain the females with 8 (*molestus* type) and 9 (*pallens* type) ommatidia mingling in both compound eyes belonged could not be identified.

RESULTS

Experimental Infection

Fig. 1 shows the relation between the density of microfilariae in the dog blood and the numbers of microfilariae taken up by mosquitoes. In both *Cx. p. pallens* and *Cx. p. molestus*, the number of microfilariae taken up by individual mosquitoes varied greatly, but tended to increase when the density of microfilariae in the dog became high. At similar levels of microfilarial density in the dog blood, the difference in microfilarial number per mosquito was not clear between *Cx. p. pallens* and *Cx. p. molestus*. It was thus indicated that both mosquitoes take up similar numbers of microfilariae.

Fig. 2 illustrates the survival curves for females of *Cx. p. pallens* and *Cx. p. molestus* exposed to a dog infected with *D. immitis*. In *Cx. p. pallens*, the survival rate of the blood-fed group was lower than that of the unfed control group throughout the experimental period up to 15 days after infective feeding irrespective of the microfilarial density in the dog, and the difference between the two groups was greater at a high microfilarial density than a low density. In *Cx. p. molestus*, the difference between blood-fed and unfed groups was also observed when they were fed on a dog with a high microfilarial density, but the two groups had a similar survival rate when the dog had a low microfilarial density. It was clear that *Cx. p. molestus* is short-lived compared to *Cx. p. pallens*.

Table 1 shows the numbers of developing larvae of *D. immitis* in *Cx. p molestus* and *Cx. p. pallens* females that died before and survived up to day 15 after infective feeding. In *Cx. p. pallens*, more larvae of *D. immitis* were found when mosquitoes were fed on a dog with a higher microfilarial density, but the increase in the number of larvae with the microfilarial density was far greater in dead mosquitoes than in live mosquitoes. The general tendency for *Cx. p. molestus* was similar to that for *Cx. p. pallens*. The discrepancy between dead and live mosquitoes can be explained by the fact that the mosquitoes

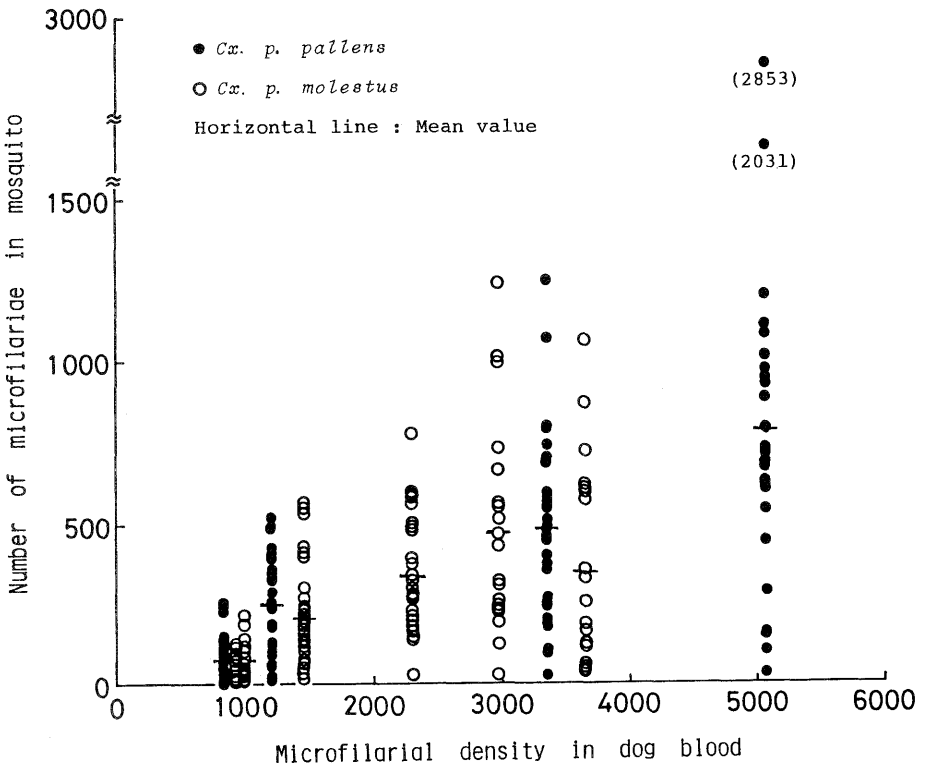

Fig. 1. Comparison between the numbers of microfilariae ingested by *Culex pipiens pallens* and *Cx. p. molestus* when exposed to a dog infected with *Dirofilaria immitis*.

infected with many larvae hardly survived for 15 days.

Table 2 shows the results on the loss rate of *D. immitis* larvae in mosquitoes for 15 days after infective feeding. The percentage of larvae that failed to migrate to Malpighian tubules was calculated by the following formula:

$$\left(1-\frac{\text{Total No. of larvae in live and dead mosquitoes}}{\text{Theoretical No. of microfilariae ingested by all mosquitoes}}\right)\times 100,$$

where the theoretical No. was given by (Mean No. of microfilariae ingested by mosuqitoes) × (Total No. of mosquitoes dissected). More than 95% of microfilariae taken up in the midgut were estimated to be lost without entering Malpighian tubules in both mosquitoes of *Cx. p. molestus* and *Cx. p. pallens*.

Next, it was estimated what percentage of the larvae having entered Malpighian tubules was lost by death of mosquitoes during the 15 days after feeding. The percentage was calculated as follows:

$$\frac{\text{Total No. of larvae in dead mosquitoes}}{\text{Total No. of larvae in live and dead mosquitoes}}\times 100$$

The loss rate by mosquito death was a little higher in *Cx. p. molestus* than in *Cx. p. pallens*. A tendency of slightly higher loss rate in both mosquitoes was also indicated, when mosquitoes were fed on a dog with a higher microfilarial density.

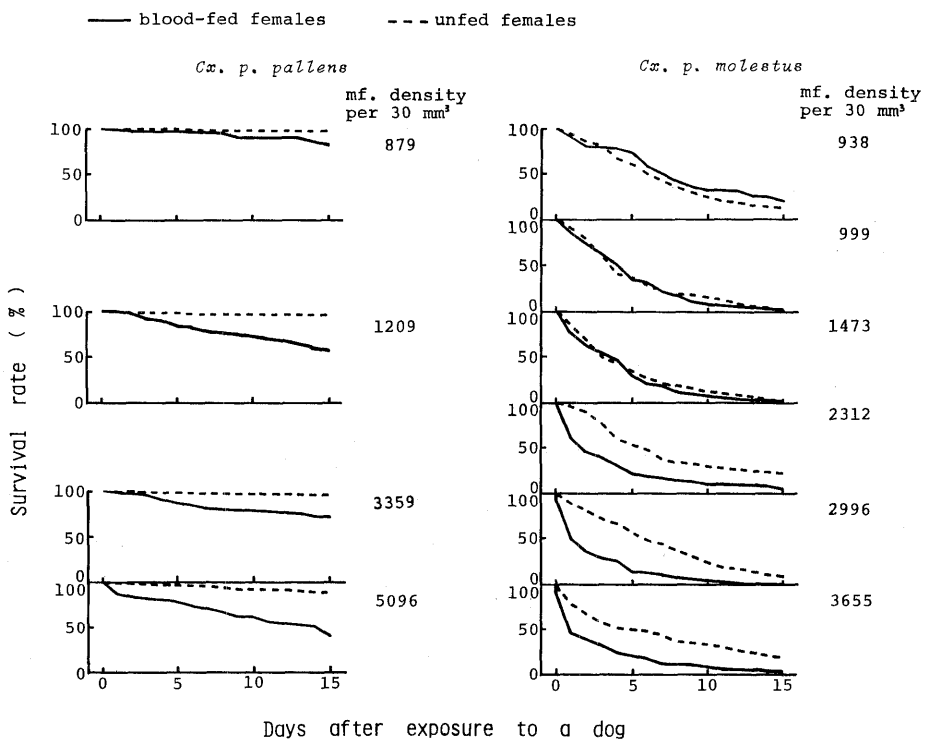

Fig. 2. Survival curves of *Culex pipiens pallens* and *Cx. p. molestus* exposed to a dog infected with *Dirofilaria immitis* whose microfilariae (mf.) varied in density.

The total loss rate of *D. immitis* larvae both by failure of migration and death of mosquitoes was extremely high, being more than 99%. The slightly higher total loss rate in *Cx. p. molestus* than in *Cx. p. pallens* is ascribable to the lower survival rate in the former mosquito.

Table 3 shows the developmental stages of larvae found in mosquitoes that have survived for 15 days after infective feeding on a microfilaremic dog. In *Cx. p. pallens*, both the number of mosquitoes with 3rd stage larvae and the number of 3rd stage larvae in mosquitoes increased when the number of microfilariae in the dog became large. However, a clear relation was not demonstrated in *Cx. p. molestus* between these two numbers because of the low survival rate.

Fig. 3 shows the relation between the number of larvae of all stages and the rate of the 3rd stage larvae to all stages in individual mosquitoes surviving for 15 days after infective feeding. This figure indicates that the rate of 3rd stage larvae in *Cx. p. pallens* did

Table 1. Comparison of the numbers of developing larvae of *Dirofilaria immitis* in mosquitoes of *Culex pipiens pallens* and *Cx. p. molestus* that died before and survived up to day 15 after infective feeding on a dog

	No. of mf* in 30mm^3 of dog blood	No. of mosquitoes dissected		No. of mosquitoes with							Mean No. ±S. E.** of larvae
				0	1−10	11−20	21−30	31−40	41−50	>50 larvae	
Cx. p. pallens	879	dead	18	5	10	1	2	0	0	0	6.2±2.0
		live	81	46	35	0	0	0	0	0	0.8±0.1
	1209	dead	39	15	19	0	3	0	1	1	7.3±2.8
		live	58	31	27	0	0	0	0	0	1.2±0.2
	3359	dead	27	5	11	3	0	0	4	4	21.0±5.4
		live	73	26	43	3	1	0	0	0	3.2±0.5
	5096	dead	56	14	17	8	5	2	2	8	23.1±5.0
		live	40	12	25	3	0	0	0	0	3.1±0.6
Cx. p. molestus	938	dead	36	26	8	1	1	0	0	0	1.3±0.7
		live	11	6	5	0	0	0	0	0	1.2±0.7
	999	dead	86	61	22	3	0	0	0	0	1.4±0.3
		live	1	1	0	0	0	0	0	0	0.0
	1473	dead	81	50	29	2	0	0	0	0	1.7±0.4
		live	5	3	2	0	0	0	0	0	0.8±0.6
	2312	dead	79	27	31	6	6	4	0	5	11.2±2.1
		live	4	2	2	0	0	0	0	0	0.5±0.3
	2996	dead	59	7	25	9	9	1	3	5	16.2±2.5
		live	0	—	—	—	—	—	—	—	————
	3655	dead	58	12	19	9	10	2	3	3	16.2±2.7
		live	1	1	0	0	0	0	0	0	0.0

* mf.: Microfilariae. ** S. E.: Standard error.

Table 2. Loss of *Dirofilaria immitis* larvae in mosquitoes of *Culex pipiens pallens* and *Cx. p. molestus*

	No. of mf. in 30mm³ of dog blood	Mean No. of mf*. ingested by mosquitoes	No. of mosquitoes dissected			Theoretical** No. of mf*. ingested by all mosquitoes	Tota No. of larvae in			Loss rate (%) of larvae		
			live	dead	total		live mosquitoes	dead mosquitoes	total	by failure** of migration	by death of** mosquitoes	total†
Cx. p. pallens	879	76.1	81	18	99	7534	63	112	175	97.68	64.00	99.16
	1209	246.3	58	39	97	23891	68	286	354	98.52	80.79	99.72
	3359	483.9	73	27	100	48390	231	566	797	98.35	71.01	99.52
	5096	781.5	40	56	96	75024	124	1292	1416	98.11	91.24	99.83
Cx. p. molestus	938	54.3	11	36	47	2552	13	46	59	97.69	77.97	99.49
	999	72.7	1	86	87	6325	0	120	120	98.10	100.00	100.00
	1473	202.9	5	81	86	17449	4	138	142	99.19	97.18	99.98
	2312	335.5	4	79	83	27847	2	881	883	96.83	99.77	99.99
	2996	472.4	0	59	59	27872	0	955	955	96.57	100.00	100.00
	3655	346.8	1	58	59	20461	0	938	938	95.42	100.00	100.00

*mf.: Microfilariae. **See text for details. † $\left(1-\frac{\text{Total No. of larvae in live mosquitoes}}{\text{Theoretical No. of mf. ingested by all mosquitoes}}\right)\times 100$

Table 3. Results on dissection of mosquitoes of *Culex pipiens pallens* and *Cx. p. molestus* surviving for 15 days after feeding on an infected dog

	No. of mf*. in 30mm³ of dog blood	No. of mosquitoes dissected	No. of mosquitoes with larvae	%	No. of mosquitoes with 3rd stage larvae	%	Mean No. of larvae in dissected mosquitoes (Mean±S. E.**)		
							1st stage larvae	2nd stage larvae	3rd stage larvae
Cx. p. pallens	879	81	35	43.2	21	25.9	0.02±0.02	0.25±0.06	0.51±0.12
	1209	58	27	46.6	21	36.2	0.00	0.31±0.09	0.86±0.18
	3359	73	47	64.4	46	63.0	0.00	0.22±0.06	2.95±0.48
	5096	40	28	70.0	28	70.0	0.20±0.17	0.60±0.24	2.30±0.41
Cx. p. molestus	938	11	5	45.5	2	18.2	0.00	1.00±0.63	0.18±0.12
	999	1	0	0.0	0	0.0	—	—	—
	1473	5	2	40.0	2	40.0	0.00	0.00	0.80±0.58
	2312	4	2	50.0	1	25.0	0.00	0.25±0.25	0.25±0.25
	2996	0	—	—	—	—	—	—	—
	3655	1	0	0.0	0	0.0	—	—	—

* mf.: Microfilariae. ** S. E.: Standard error.

not differ with larval density in mosquitoes. In *Cx. p. molestus*, females dissected were not large in number, but a similar relation was implied.

Among the indices proposed for the evaluation of experimental infection of mosquitoes with *D. immitis*, three were calculated for *Cx. p. pallens* and *Cx. p. molestus* (Table 4). The index used by Kartman (1954) can be calculated from the survival rate of mosquitoes, the infection rate with 3rd stage larvae and the host efficiency, where the host efficiency is the ratio of mean number of 3rd stage larvae in surviving mosquitoes to mean number of microfilariae in ingested mosquitoes. Wharton (1957) modified Kartman's index and his index represents an estimate of the number of mature larvae produced by each fed mosquito. The index by Pichon *et al.* (1974) is the parasite yield, which is the survival rate of ingested microfilariae until development to infective larvae in mosquitoes (Kurihara and Maeda, 1980). That is, the parasite yield is also the product of the rate of larvae not

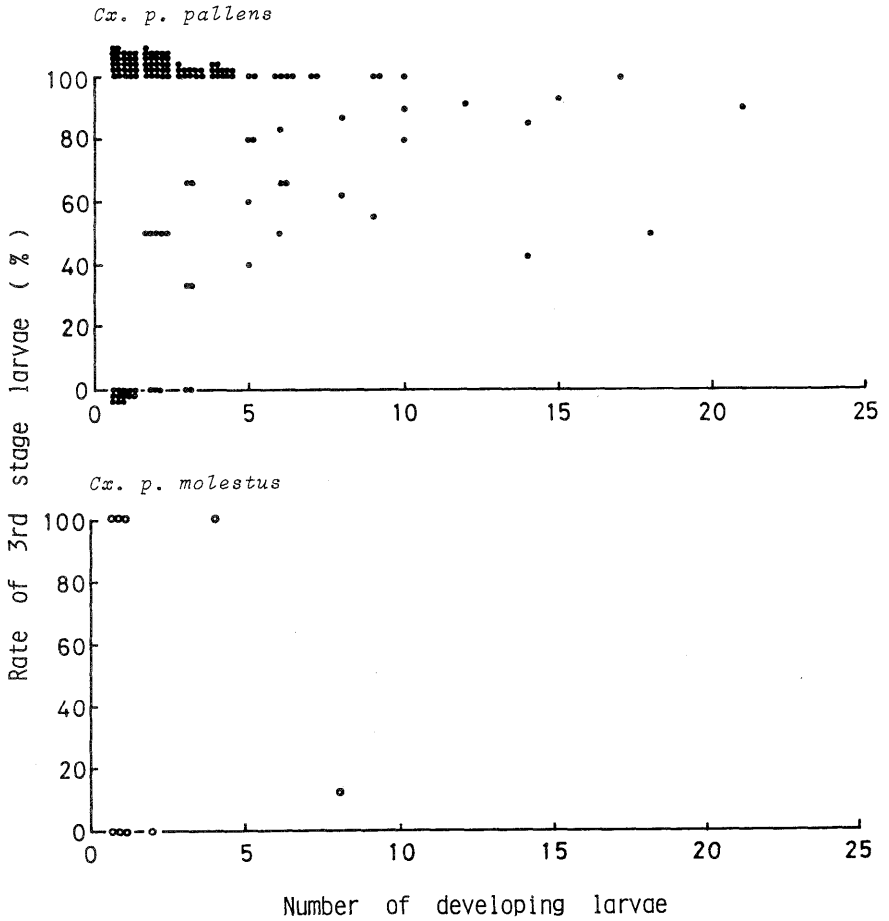

Fig. 3. Relation between the rate of 3rd stage larvae and the number of developing larvae in individual mosquitoes of *Culex pipiens pallens* and *Cx. p. molestus* at day 15 after infection with *Dirofilaria immitis*.

Table 4. Indices for experimental infection of mosquitoes with *Dirofilaria immitis*

	No. of mf*. in 30mm^3 of dog blood	Mean No. of mf*. ingested by mosquitoes (A)	Rate of mosquitoes with 3rd stage larvae (B)	Mean No. of 3rd stage larvae		Survival rate of mosquitoes till day 15 (E)	Index by		
				in surviving mosquitoes (C)	in mosquitoes with 3rd (D)		Pichon *et al.* (1974) C/A×E×100	Kartman (1954) C/A×B×E	Wharton (1957) D×B×E
Cx. p. pallens	879	76.1	0.259	0.51	1.95	0.818	0.548	0.00142	0.413
	1209	246.3	0.362	0.86	2.38	0.586	0.205	0.00074	0.505
	3359	483.9	0.630	2.95	4.67	0.730	0.445	0.00280	2.148
	5096	781.5	0.700	2.30	3.29	0.412	0.121	0.00085	0.949
Cx. p. molestus	938	54.3	0.182	0.18	1.00	0.208	0.069	0.00013	0.038
	999	72.7	0.000	0.00	0.00	0.010	0.000	0.00000	0.000
	1473	202.9	0.400	0.80	2.00	0.051	0.020	0.00008	0.041
	2312	335.5	0.250	0.25	1.00	0.039	0.003	0.000007	0.010
	2996	472.4	—	—	—	0.000	—	—	—
	3655	346.8	0.000	0.00	0.00	0.014	0.000	0.00000	0.000

* mf.: Microfilariae.

Table 5. The composition of members of the *Culex pipiens* complex collected by the light trap, determined by the number of ommatidia in compound eyes

Collection site	Year	No. (%) of mosquitoes				
		Cx. p. pallens	*Cx. p. molestus*	not determined[3)]	not examined[4)]	total
A[1)]	1986	298	70	98	35	501
	(%)	(59.5)	(14.0)	(19.6)	(7.0)	(100.0)
	1987	103	30	34	6	173
	(%)	(59.5)	(17.3)	(19.7)	(3.5)	(100.0)
B[2)]	1986	37	75	21	34	167
	(%)	(22.2)	(44.9)	(12.6)	(20.4)	(100.0)
	1987	28	78	15	10	131
	(%)	(21.4)	(59.5)	(11.5)	(7.6)	(100.0)

1) The campus of Nagasaki University School of Medicine.
2) An apartment house in a residential district in Nagasaki City.
3) The number of ommatidia was intermediate; see text for details.
4) Heads were missing.

Table 6. Natural infection with *Dirofilaria immitis* in members of the *Culex pipiens* complex collected by the light trap

Collection site	Year	No. (%) of mosquitoes				
		Cx. p. pallens	*Cx. p. molestus*	not determined[3]	not examined[4]	total
A[1]	1986	14	1	2	2	19
	(%)	(73.7)	(5.3)	(10.5)	(10.5)	(100.0)
	1987	9	0	0	1	10
	(%)	(90.0)			(10.0)	(100.0)
B[2]	1986	2	0	0	0	2
	(%)	(100.0)				(100.0)
	1987	0	0	0	1	1
	(%)				(100.0)	(100.0)

See Table 5 for 1), 2), 3) and 4).

Table 7. Comparison of the natural infection rate in *Culex pipiens pallens* and *Cx. p. molestus*

Collection site	Year	No. of *Cx. p. pallens*[3]					No. of *Cx. p. molestus*[4]				
		dissected	infected	%	with 3rd stage larvae	%	dissected	infected	%	with 3rd stage larvae	%
A[1]	1986	298	14	4.7	2	0.7	70	1	1.4	0	0.0
	1987	103	9	8.7	0	0.0	30	0	0.0	0	0.0
B[2]	1986	37	2	5.4	0	0.0	75	0	0.0	0	0.0
	1987	28	0	0.0	0	0.0	78	0	0.0	0	0.0
	Total	466	25	5.4	2	0.4	253	1	0.4	0	0.0

1) The campus of Nagasaki University School of Medicine.
2) An apartment house in a residential district in Nagasaki City.
3), 4) Determined by the number of ommatidia in compound eyes; see also text.

lost (1−Total loss rate in Table 2) by the rate of larvae successfully developing to infective stage in 15 days. All indices of *Cx. p. pallens* were higher than those of *Cx. p. molestus*. The lower indices of *Cx. p. molestus* were apparently caused by the lower survival rate.

Natural Infection

Females of the *Culex pipiens* complex were collected by a light trap at two sites in Nagasaki City. They were identified as either *Cx. p. pallens* or *Cx. p. molestus* by the number of ommatidia (Table 5). At site A, the number of females of *Cx. p. pallens* was larger than that of *Cx. p. molestus*, but at site B, *Cx. p. molestus* females were dominant, and this dominance is conceivable as the breeding place of *Cx. p. molestus* was located near the collection site. Table 6 shows the natural infections with *D. immitis* in mosquitoes. At site A, 14 and 9 females of *Cx. p. pallens* had the larvae of *D. immitis* (all stages) in 1986 and 1987, respectively, and one *Cx. p. molestus* in 1986. At site B where *Cx. p. molestus* was dominant, two females only of *Cx. p. pallens* had the larvae in 1986. Comparison of the natural infection rate was made between *Cx. p. pallens* and *Cx. p. molestus* in Table 7. The total infection rate was 5.4% in *Cx. p. pallens*, but in *Cx. p. molestus* it was only 0.4%. Therefore, the role in transmitting *D. immitis* in the field is much lower in *Cx. p. molestus* than in *Cx. p. pallens*.

DISCUSSION

Microfilariae of *D. immitis* do not multiply in the vector mosquito. Generally, only some part of microfilariae ingested develop to infective larvae in the mosquito. Thus, the parasite in a good vector has a low loss rate of larvae in the development to infective larvae. The loss of larvae appears in the following ways: (1) failure of the migration into Malpighian tubules; (2) arrested development of larvae; (3) death of mosquitoes with larvae (Kurihara and Maeda, 1980; Buxton and Mullen, 1981). The present experiment showed that in *Cx. p. molestus* the parasite yield, which is defined as the rate of microfilariae successfully developing to the infective stage, was lower than in *Cx. p. pallens*. The difference in the parasite yield between the two mosquitoes was due mainly to the different death rates of mosquitoes.

Christensen (1978) suggested that there was a strong negative correlation between parasite burden and mosquito survival. The present results also indicated that the heavy infection of parasite caused the short longevity of the mosquitoes. However, the longevity of the unfed group of *Cx. p. molestus* is shorter than that of *Cx. p. pallens*. This suggests that heavy infection is not always a major cause of mosquito death.

Cx. p. molestus females used in the present experiment were those that finished the first oviposition in autogenous state. On the other hand, *Cx. p. pallens* used in the experiment were at the same calendar age as *Cx. p. molestus* but they had not oviposited. Both mosquitoes were reared and fed on a dog otherwise in the same conditions. In view of

these facts, the oviposition in autogenous state may be a cause of the shorter longevity of *Cx. p. molestus* females.

Oda *et al.* (1986) investigated the physiological age of *Cx. p. molestus* females collected in a house. All of the females collected were uniparous and biparous. Therefore, they were considered to have come to bite after the first oviposition. The biparous/uniparous ratio in October and November was calculated as 0.02 from their data. Oda (1968) reported 0.1 as the biparous/uniparous ratio in the same season for *Cx. p. pallens* attracted to humans and dogs. These facts suggested that the shorter longevity of *Cx. p. molestus* than that of *Cx. p. pallens* is observed in the field, too.

In the *Culex pipiens* complex caught by light traps, several *Cx. p. pallens* females with 3rd stage larvae of *D. immitis* were found, but no *Cx. p. molestus* were infected with 3rd stage larvae. This is assumed to be due mainly to the shorter longevity in *Cx. p. molestus* females in the field than in *Cx. p. pallens* females.

Judging from these data on the longevity, experimental and natural infections, it is concluded that *Cx. p. molestus* is extremely low in the importance as a vector of *D. immitis* in comparison with *Cx. p. pallens*. The present results support that *Cx. p. pallens* is the main vector of *D. immitis* in Nagasaki City.

The present results are in agreement with those of Inoue (1937) and Suenaga (1972a, b) in that the rate of larval migration into Malpighian tubules was low and the larval development to the infective stage was normal in *Cx. p. pallens* and *Cx. p. molestus*. This was observed also in *Culex pipiens quinquefasciatus* (=*Culex quinquefasciatus*) and *Culex pipiens pipiens* (=*Culex pipiens*), other members of the *Culex pipiens* complex (Hu, 1931; Yen, 1938; Travis, 1947; Kartman, 1953; Intermill and Frederick, 1970; Nayar and Sauerman, 1975). The low migration rate and the good development of *D. immitis* larvae may be common to the *Culex pipiens* complex. Yen (1938) and Nayar and Sauerman (1975) regarded the low migration rate as the demerit in transmitting *D. immitis*, because it decreases the rate of mosquitoes with 3rd stage larvae. However, in the present experiments with high microfilarial density, the rate of *Cx. p. pallens* with 3rd stage larvae did not become so low. Therefore, when mosquitoes are fed on a dog with high microfilarial density, the low migration rate might be the merit in securing the development of an appropriate number of *D. immitis* larvae in Malpighian tubules of mosquitoes.

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REFERENCES

1) Buxton, B. A. & Mullen, G. R. (1981): Comparative susceptibility of four strains of *Aedes aegypti* (Diptera: Culicidae) to infection with *Dirofilaria immitis*. J. Med. Entomol., 18, 434−440.

2) Christensen, B. M. (1978): *Dirofilaria immitis*: Effect on the longevity of *Aedes trivittatus*. Exp. Parasitol., 44, 116−123.

3) Hu, S. M. K. (1931): Studies on host-parasite relationships of *Dirofilaria immitis* Leidy and its Culicine intermediate hosts. Amer. J. Hyg., 14, 614−629.

4) Inoue, T. (1937): Development of *Dirofilaria immitis* in 9 species of mosquitoes in Tokyo. Keio-Igaku., 17, 1097−1128 (in Japanese).

5) Intermill, R. W. & Frederick, R. M. (1970): A study of potential mosquito vectors of *Dirofilaria immitis* Leidy, on Okinawa, Ryukyu islands. J. Med. Entomol., 7, 455−461.

6) Kartman, L. (1953): Factors influencing infection of the mosquito with *Dirofilaria immitis* (Leidy, 1856). Exp. Parasitol., 2, 27−78.

7) Kartman, L. (1954): Suggestions concernig an index of experimental filaria infection in mosquitoes. Am. J. Trop. Med. Hyg., 3, 329−337.

8) Kurihara, T. & Maeda, R. (1980): Entomological factors affecting the trasmission of mosquito-borne filariasis. Jpn. J. Sanit. Zool., 31, 231−242 (in Japanese).

9) Nayar, J. K. & Sauerman, Jr., D. M. (1975): Physiological basis of host susceptibility of Florida mosquitoes to *Dirofilaria immitis*. J. Insect Physiol., 21, 1965−1975.

10) Noguchi, Y. & Asahina, S. (1966): Ommatidial number as a diagnostic character for Japanese autogenous *Culex molestus*. J. Med. Entomol., 3, 146−148.

11) Oda, T. (1968): Studies on the follicular development and overwintering of the house mosquito, *Culex pipiens pallens* in Nagasaki area. Trop. Med., 10, 195−216.

12) Oda, T. & Ueda, M. (1979): Seasonal changes in the oviposition activity of *Culex pipiens molestus* Forskal in the above-ground water. Trop. Med., 21, 139−144 (in Japanese).

13) Oda, T., Mori, A. & Fujita, K. (1984): Annual changes in number of egg-rafts of *Culex pipiens* complex in above-ground water in Nagasaki City. pp 655. XVII International Congress of Entomology, Abstract Volume, Hamburg.

14) Oda, T. Mori, A. & Fujita, K. (1986): Observations on seasonal changes in feeding activity of *Culex pipiens molestus* in a house in Nagasaki City. Z. Angew. Zool., 73, 129−134.

15) Ohishi, I. (1986): [*Dirofilaria immitis* −from the parasitological point of view] 1st ed., pp27−87, Buneido, Tokyo (in Japanese).

16) Pichon, G., Perrault, G. & Laigret, J. (1974): Rendement parasitaire chez les vecteurs de filarioses. Bull. Wld Hlth Org., 51, 517−524 (in French).

17) Suenaga, O. (1972a): Studies on the filarial prevalence among dogs and the mosquito vectors in Nagasaki City, western Japan. 3. On the susceptibility of *Aedes albopictus* and *Culex pipiens molestus* to the larvae of *Dirofilaria immitis* in Nagasaki City. Trop. Med., 14, 144−150 (in Japanese).

18) Suenaga, O. (1972b): Studies on the filarial prevalence among dogs and the mosquito vectors in Nagasaki City, western Japan. 2. On the susceptibility of four common mosquitoes to the larvae of *Dirofilaria immitis* in Nagasaki City. Trop. Med., 14, 32−40 (in Japanese).

19) Suenaga, O. & Itoh, T. (1973): Studies on the filarial prevalence among dogs and the mosquito vectors in Nagasaki City, western Japan. 5. On the vector mosquitoes of *Dirofilaria immitis* in Nagasaki City. Trop. Med., 15, 131−140 (in Japanese).

20) Suenaga, O., Itoh, T. & Nishioka, T. (1971): Studies on the filarial prevalence among dogs and the mosquito vectors in Nagasaki City, western Japan. 1. On the microfilarial prevalence among house dogs in Nagasaki City. Trop. Med., 12, 169−178 (in Japanese).

21) Travis, B. V. (1947): Relative efficiency of six species of mosquitoes from Guam, M. I., as developmental hosts for *Dirofilaria immitis*. J. Parasitol., 33, 142−145.

22) Webber, W. A. F. & Hawking, F. (1955): Experimental maintenace of *Dirofilaria repens* and *D. immitis* in dog. Exp. Parasitol., 4, 143−164.

23) Wharton, R. H. (1957): Studies on filariasis in Malaya: Observation on the development of *Wuchereria malayi* in *Mansonia* (*Mansonioides*) *longipalpis*. Ann. Trop. Med. Parasitol., 51, 278−296.

24) Yen, C. H. (1938): Studies on *Dirofilaria immitis* Leidy, with special reference to the susceptibility of some Minnesota species of mosquitoes to infection. J. Parasitol., 24, 189−205.

25) Yoshimura, H., Akao, N., Kondo, K., Ohnishi, Y., Kitagawa, M. & Kamimura, K. (1980): Human dirofilariasis in Japan case report and review of literature. Int. J. Zoon., 7, 107−114.

26) Yoshimura, H. (1985): Human dirofilariasis in Japan. Minophagen Medical Review, 30, 136 (in Japanese).

27) Zaitsu, M., Oda, T., Ueda, M., Kurokawa, K., Mori, A. & Fujita, K. (1987): Seasonal changes in the number of *Culex pipiens molestus* egg-rafts collected in ovitraps set on the ground. −Results obtained from 1977−. Trans. Nagasaki Biol. Soc., 32, 15−19 (in Japanese).

長崎市内での犬フィラリア伝搬におけるチカイエカとアカイエカの役割の比較

在津　誠（長崎大学医学部医動物学教室）

チカイエカの犬フィラリアに対する伝搬能力を同じアカイエカ群に属し，長崎市内での犬フィラリアの主要伝搬蚊であるアカイエカと比較した．実験室内において，種々の程度のミクロフィラリア密度（800−5000/30mm^3）を持つ犬から長崎産のチカイエカとアカイエカを吸血させた．吸血した蚊は，気温25℃，湿度70%，16時間照明の条件下で15日間飼育した．その結果，蚊でのミクロフィラリアの中腸へのとり込み方と，ミクロフィラリアのマルピーギ管への移動の割合には，両者の蚊で差は認められなかった．しかし，吸血蚊の15日目の生存率はチカイエカ（0.0−20.8%）の方がアカイエカ（41.2−81.8%）にくらべ著しく低かった．また，チカイエカの実験感染指数は，どのミクロフィラリア密度の実験でも，アカイエカより低かった．さらに，野外においてライトトラップを用いてアカイエカ群成虫を採集し，それらについて犬フィラリア感染率をしらべた．調査は長崎大学医学部構内とそこから約2㎞離れたアパートで，1986年と1987年の4月から11月上旬に行なった．チカエイカとアカイエカは個眼数の違いによって判別した．2ヶ年の結果を合計すると，チカイエカの自然感染率は0.4%，アカイエカでは5.4%で，チカイエカの方がアカイエカにくらべて著しく低いことがわかった．以上の実験感染と自然感染の結果から，野外におけるチカイエカの犬フィラリア伝搬に対する役割は，アカイエカにくらべて極めて低いものと結論された．

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