Susceptibility of *Culex pipiens fatigans* to *Wuchereria bancrofti* in the Ryukyus, in comparison with that of *C. p. pallens* in Japan*

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ABSTRACT : The intake of microfilariae, and experimental and natural infection of the mosquito in relation to the susceptibility of *Culex pipiens fatigans* to *Wuchereria bancrofti* was investigated on Miyako Island, in the Ryukyus. The results of observations were compared with those of *C. p. pallens* in Japan.

Introduction

The senior author has investigated for long years on the susceptibility of *C. p. pallens* to *W. bancrofit* in Japan concerning the microfilariae uptake by the female (Omori 1958a) and the nature of experimental and natural infections under various conditions and in different locales (Omori 1958b, c, 1962, 1963). Studies on these subjects were done with *C. p. fatigans* on Miyako Island in the Ryukyus in October 1961 and May 1962. The results of these investigations with *C. p. fatigans* on Miyako Island are reported here and compared with *C. p. pallens* in Nagasaki.

Place and Method

Miyako Island (24°47′N, 125°17′E) is in the southern part of the Ryukyus, subtropical in climate, and highest in bancroftian microfilarial incidence in the archipelago. Investigations were conducted in the village of Hisamatsu. The topography and mean air temperatures of Miyako Island and the detailed account of microfilarial incidence in Hisamatsu were reported in a previous paper (Omori et al. 1962). The methods of

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investigation were similar to those employed in the experiments with *C. p. pallens* in Nagasaki. Further particulars will be explained under the headings below.

Number of microfilariae taken up by a female *fatigans*

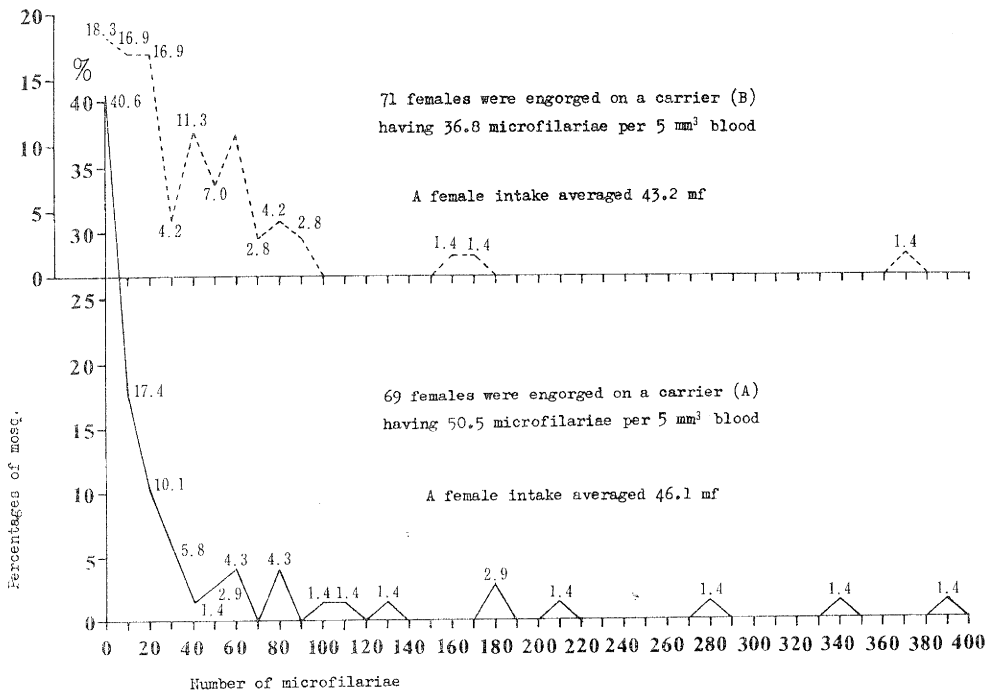
Females of *C. p. fatigans* emerged from wild caught larvae and pupae in a farm village on Miyako Island were fed on two donors on 26 October, 1961 from 9 : 40 to 10 : 40 p. m. Mean microfilarial count of donor A before and after feeding of mosquitoes was 202 per 20mm^3 blood taken from his ear lobe, or 50.5 per 5 mm^3 blood (the quantity is nearly equal to a full blood meal of the mosquito). The mean microfilarial count of donor B was 147 per 20mm^3, or 36.8 per 5mm^3 blood.

A number of engorged females were smeared, each on a slide, just after feeding to examine microfilaria intake, while others were reared to examine the states of larval development.

The percentages of female *C. p. fatigans* infected with different numbers of microfilariae are illustrated in Fig. 1. In the case of A, 69 females were infected with a total of 3179 microfilariae or an average of 46.1; while in B, 71 females were infected with 3066 or 43.2 microfilariae per female. Thus in A, the mean microfilaria intake was slightly less; while in B, the mean was slightly greater than the expected number.

As seen in Fig. 1, the frequency distribution differed in A and B, and individual females also took up widely varying numbers: In A, two females picked up no micro-

Fig. 1. Percentage frequeney of females infected with the indicated number of microfilariae



filaria, whereas one had as many as 394; in B, two females had none and one had 379. This great variation seemed to be caused by the distribution pattern of microfilariae being of a Pólya-Eggenberger type in the peripheral blood stream of the carrier. The same trend was observed by Omori (1958a) with *C. p. pallens*, and by Nakamura (1964) with *Aedes togoi*.

Generally speaking, however, the average number of microfilariae found in a large sampling of engorged female mosquitoes might approach the number of microfilariae found in the same quantity of blood taken from an ear lobe of the carrier rupturing perhaps several blood vessels.

Results of experimental infection of *C. p. fatigans*

Females of *C. p. fatigans* reared from wild caught larvae and pupae in a village on Miyako Island were fed on donors A and B at night on 26 October, 1961. They were reared under natural air temperatures of 23 −26°C for 8 days on Miyako Island, 21−25°C for 4 days at Naha City, and 20−23°C for 3 days from Naha through Kagoshima to Nagasaki. Upon arrival at Nagasaki (15 days after the infective meal), the females were found to have mature larvae. On and after that day, they were kept at 25°C. The females were dissected 15−20 days after the feeding with the results shown in I of Table 1. Results of experiments carried out at Tokyo by Yamada (1927), at Fukuoka by Mochizuki (1911), and at Nagasaki by Omori (1962) are presented in II of Table 1 for comparison with *C. p. pallens*.

Table 1 shows that the percentages of

Table 1 Results of experimental infection of *C. p. fatigans* with *W. bancrofti* on Miyako Island, Ryukyus, in comparison with those of *C. p. pallens* in Kyushu and Tokyo

Locale	Donor	No. of microfilariae per mm³	No. of mosq. dissected	Dissection period in days after feeding	No. (& %) of mosquitoes with larvae	No. (& %) of mosquitoes with infective larvae	No. of all larvae	No. (&%) of infective larvae	Infective larvae per dissected	Max. No. infective larvae in a female
I. Experiments with *C. p. fatigans* by Omori et al. (1961) on Miyako Island, Ryukyus										
Miyako I. Ryukyus (1961)	B	7.350	140	15−22	75(53.6)	64(45.7)	194	138(71.1)	1.0	9
	A	10.100	25	15−21	22(88.0)	18(72.0)	71	55(77.5)	2.2	13
II. Experiments with *C. p. pallens*: Yamada in Tokyo; Mochizuki in Fukuoka; Omori in Nagasaki										
Tokyo (1927)	*		17	16−19	17(100.0)	17(100.0)	400	326(81.5)	19.2	52
			25	13−19	25(100.0)	25(100.0)	647	557(86.1)	22.3	62
Fukuoka (1911)	**	3.735	58	11−17	58(100.0)	57(98.3)	1236	1110(89.8)	19.1	50
Nagasaki (1962)	90.1,2	1.090	99	14−48	94(94.9)	91(91.9)	554	523(94.4)	5.3	26
	91.2,3,4	1.165	104	14−92	101(97.1)	100(96.2)	1009	909(90.1)	8.7	44
	94.3,4	1.540	110	14−85	104(94.5)	102(92.7)	838	745(88.9)	6.8	24
	72	4.600	49	12−49	44(89.8)	44(89.8)	869	559(64.3)	11.4	85
	71	6.125	24	12−44	21(87.5)	21(87.5)	189	134(70.9)	5.6	21
	Total		386		364(94.3)	358(92.7)	3459	2870(83.0)	7.4	

* The number of microfilariae of the donor is not given in the report.

** The number of microfilariae is uncertain because the author only examined the blood 48 days before the feeding experiment.

females of *fatigans* having filaria larvae and of those having infective larvae are lower, and that the number of infective larvae per female dissected and the maximum number of infective larvae found in a female are much smaller than in *pallens*. This appeared to be caused partly by the discharge of some of microfilariae by the female *fatigans* and partly by the death of younger stage larvae in the mosquito, considering that the female *fatigans* were found to have taken up as many microfilariae as *pallens*.

Contrarily, *pallens* was highly susceptible to the parasite and was much greater in number of infective larvae per female dissected, and also in the maximum number of mature larvae in a female. However, it is to be remembered that by the author (1958c) the higher mortality of female *pallens* was found to be caused by the heavier parasitism of the parasite on those days of reaching maturity of filariae in them.

Results of natural infection of *C. p. fatigans*

Table 2. Use of mosquito-nets and natural infection of *C. p. fatigans* with *W. bancrofti* in Hisamatsu Village, Ryukyus

Beginning of dry season, October 21 to 26, 1961

Use of mosquito-nets	Mosquitoes found (+) or not found (−)	No. of houses	No. of mosquitoes collected	dissected	infected	% infection to dissected mosquitoes
no	(−) in houses	5	0			
	(+) in houses	44	306	306	141	46.1%
yes	(−) in houses, (−) in mosq. nets	1	0			
	(+) in houses, (−) in mosq. nets	4	21	21	11	52.4%
	(−) in houses, (+) in mosq. nets	0				
	(+) in houses, (+) in mosq. nets	2	41	41	30	73.2%
Total		56	368	368	182	49.5%
Average per house			6.6	6.6	3.3	

Beginning of wet season, 28 May to 1 June, 1962

Use of mosquito-nets	Mosquitoes found (+) or not found (−)	No. of houses	No. of mosquitoes collected	dissected	infected	% infection to dissected mosquitoes
no	(−) in houses	2	0			
	(+) in houses	9	75	68	16	23.5%
yes	(−) in houses, (−) in mosq. nets	2	0			
	(+) in houses, (−) in mosq. nets	15	124	124	23	18.5%
	(−) in houses, (+) in mosq. nets	13	104	104	22	21.2%
	(+) in houses, (+) in mosq. nets	15	190	171	47	27.5%
Total		56	493	467	108	23.1%
Average per house			8.8	8.3	1.9	

Surveys of natural infection of *C. p. fatigans* were carried out at Hisamatsu Village on Miyako Island from 21 to 26 October, 1961 and from 28 May to 1 June, 1962. The topography, number of houses, population, and microfilarial incidence in this village were presented in a previous report (Omori et al., 1962).

In Hisamatsu there were no rivers, wells, or public water supply and, therefore, water for domestic use came exclusively from rain water. Consequently, the villagers had to economize extremely in water usage, and only small amounts of foul

Table 3. Results of field survey of natural infection of *C. p. fatigans* with *W. bancrofti* on Miyako Island, Ryukyus, in comparison with those of *C. p. pallens* in Nagasaki

Name of village	Date	Mf incidence	No. of mosq.		No. of larvae per female having different stage larvae			
			dissected	infected	Total	I stage	II stage	III stage
I. Studies on Miyako Island where *C. p. fatigans* is the vector								
Hisamatsu	Oct. '61	37.9	368	182	1737/182 = 9.5	1611/169 = 9.5	81/22 = 3.7	45/12 = 3.8
	May '62	37.9	467	108	342/108 = 3.2	249/85 = 2.9	82/30 = 2.7	11/7 = 1.6
II. Studies in Nagasaki Prefecture where *C. p. pallens* is the vector								
Yaburoki	Aug. '58	20.6	249	59	270/59 = 4.6	250/57 = 4.4	16/3 = 5.3	4/3 = 1.3
Setobatake	Jun. '60	20.6	97	19	70/19 = 3.7	49/16 = 3.1	21/5 = 4.2	—
〃	Jul. '60	〃	92	16	55/16 = 3.4	47/14 = 3.4	7/1 = 7.0	1/1 = 1.0
Hyugashi	Jun. '60	15.8	28	7	39/7 = 5.6	35/5 = 7.0	4/2 = 2.0	—
〃	Jul. '60	〃	63	18	119/18 = 6.6	81/15 = 5.4	38/5 = 7.6	—
Ota	Jun. '60	21.7	52	16	237/16 = 14.8	189/10 = 18.9	48/8 = 6.0	—
〃	Jul. '60	〃	62	16	55/16 = 3.4	34/14 = 2.4	17/3 = 5.7	4/1 = 4.0
Nagate	May to Nov. '61	14.0	555	43	176/43 = 4.1	155/38 = 4.1	21/5 = 4.2	—
Abumize	〃 '61	8.3	445	51	342/51 = 6.7	334/50 = 6.7	7/1 = 7.0	1/1 = 1.0
〃	〃 '62	〃	268	13	97/13 = 7.5	62/13 = 4.8	35/2 = 17.5	—
Total			1911	258	1460/258 = 5.7	1236/232 = 5.3	214/35 = 6.1	10/6 = 1.7
III. Studies in Okubo, Nagasaki Prefecture where *C. p. pallens* is the vector								
Okubo	Jun. to Aug. '64	26.4	655	279	3937/279 = 14.1	3857/271 = 14.2	65/12 = 5.4	15/10 = 1.5

water collected in the small sinks in each house. This created a favorable breeding place for *C. p. fatigans*. The number of mosquitoes breeding in these sinks was not so numerous, and most villagers passed the nights without mosquito-nets. The results of examinations for natural infection of *C. p. fatigans* carried out in houses and mosquito-nets early in the morning or at night, are tabulated in Table 2.

At the beginning of the dry-season, about 46% of *fatigans* collected were found infected in houses without mosquito-nets; about 52% were positive in houses where only parents and young children slept in mosquito-nets (older boys and youths were doing without them); about 73% were positive in houses where mosquitoes were rather numerous but the usage of mosquito-nets was as above.

At the beginning of wet season, although mosquitoes were not yet so numerous, in many houses mosquito-nets were used but the older children were sleeping without them as usual. The infection rate in this season (23.1%) was markedly lower than in the dry season. The reason for this could not be clearly determined but may have been partly caused by the changing of houses in which some collections were made, and partly to the increase in use of mosquito-nets because of some increase in number of newly emerged females.

The number of mosquitoes (collected and) dissected, that of those infected, and the number of filaria larvae per female *fatigans* having all stages, first, second, and third or infective stage larvae in nature are tabulated in I of Table 3, and those with *C. p. pallens* in Nagasaki Prefecture are in II and III of the same table. As seen in the table, the number of filaria larvae per female varied greatly. Generally speaking, however, the first stage larvae per female was larger in number than the second and especially infective stage larvae. Also, the number of infective larvae per female *fatigans* appeared a little larger than that of *p llens*. In the survey carried out with *fatigans* in October 1961, 12 females had 45 infective larvae : 3 females had 1 each; 4 had 2 each; and the remaining 5 had 3, 4, 5, 7, and 15 respectively. In the survey of May 1962, 7 females had 11 infective larvae : 4 had 1 each; 2 had 2 each; 1 had 3. The above suggested that at the beginning of dry (and cooler) season some females had larger number of mature larvae than at the beginning of wet (and warmer) season.

In Nagasaki, however, from the end of June through early September, or from wet and warm to dry and warm months, 16 femles had 25 infective larvae: 11 females had 1 each; 3 had 2 each; 2 had 4 each.

The above facts indicated that *fatigans* on Miyako Island, might have had, in some cases, a larger number of mature larvae per female than did *pallens* in Nagasaki.

Comparison of results of experimental and natural infection in *fatigans* and *pallens*

In order to compare the susceptibility of the two species to *W. bancrofti*, chief points of the results of experiments in the laboratory (Table 1) and in nature (Table 3) are given in Table 4.

Under experimental conditions, the percentage of female *fatigans* having mature larvae to the infected was lower (that of those to the dissected was much lower as 49.7% in *fatigans* against 92.7% in *pallens*), and the number of mature larvae per female was much smaller than in *pallens*, suggesting that

Table 4. Comparison of the susceptibility of *C. p. fatigans* and *C. p. pallens* to *W. bancrofti*

Species	Locale	% of ♀ having Ⅰ, Ⅱ, and Ⅲ stage larvae to the infected			No. of each stage larvae per ♀		
		Ⅰ	Ⅱ	Ⅲ	Ⅰ	Ⅱ	Ⅲ
Experimental infection (cf. Table 1)							
fatigans	Miyako			85.4%			2.4
pallens	Nagasaki			98.4%			8.0
Natural infection (cf. Table 3)							
fatigans	Miyako	87.6%	17.9%	6.6%	7.3	3.1	2.9
pallens	Nagasaki	93.7%	8.8%	3.0%	10.1	5.9	1.6

fatigans is less susceptible, while, *pallens* is highly susceptible to the parasite.

In nature, on the contrary, the percentage of female *fatigans* having mature larvae was significantly higher and the number of mature larvae per female appeared even larger than in *pallens*. It is to be noted, however, that the percentage of the female *pallens* having the first stage larvae is higher, and that the numbers of the first and second stage larvae per female are also higher in *pallens*, showing that *pallens* is naturally high in susceptibility as observed in the laboratory experiments. The marked decrease in the percentages of female *pallens* having the second and infective stage larvae, and in the number of mature larvae per female, is probably due to the reduction in number of female *pallens* infected heavily with late second and infective stage larvae. This, again, seems to be due to the high mortality of females infected heavily with premature and mature larvae on those days of reaching maturity of filariae in mosquitoes.

In short, it seems that *fatigans* is less susceptible to the parasite and permits the development of smaller number of filariae but less affected by premature and mature larvae, while, *pallens* is highly susceptible and permits the development of larger number of filariae but much affected mechanically by developed filariae with a high mortality on those days of reaching maturity of filariae in mosquitoes.

Summary

Engorged females of *C. p. fatigans* which were fed nearly simultaneously on the same carrier took up greatly varying number of microfilariae in their full blood meals (about 5mm^3). The average number, however, may roughly approximate the number to be found in 5mm^3 blood taken from an ear lobe of the carrier. The situation is similar in the case of *C. p. pallens* in Japan.

In experiments, the infection rate of female *fatigans* having mature larvae was much lower and the number of mature larvae per female was much smaller than in *pallens*. On the contrary, in nature, the rate was significantly higher and the number of mature larvae per female appeared even larger in *fatigans*. The reason seems to be that *fatigans* is less susceptible to the parasite and permits the development of smaller number of filariae but less affected by

premature and mature larvae, while, *pallens* is highly susceptible and permits the development of larger number of filariae but much affeeted by developed filariae with a high mortality of heavily infected mosquitoes on those days of reaching maturity of filariae in them.

References

1) MOCHIZUKI, D. : On the relation between various species of mosquitoes and bancroftian filaria larvae. Fukuoka Ikadaigaku Zassi, 4 (3) : 384—444, 1911. (in Japanese).

2) NAKAMURA, Y. : Experimental studies on the role of *Aedes togoi* in the transmission of bancroftian filariasis. 1. Number of microfilariae taken up by the female and their movement in her body. Endem. Dis. Bull. Nagasaki, 6 (1) : 25—33, 1964. (in Japanese with English summary).

3) OMORI, N. : Experimental studies on the role of the house mosquito, *Culex pipiens pallens* in the transmission of bancroftian filariasis. 2. On the pattern of spatial distribution of microfilaria in the peripheral blood stream of the carrier. Nagasaki Med. J., 33 (8) : 1045—1053, 1958a. (in Japanese with English summary).

4) OMORI, N. : Experimental studies on the role of the house mosquito, *Culex pipiens pallens* in the transmission of bancroftian filariasis. 4. Development and longevity in days of filariae in mosquitoes kept at a series of constant temperatures. Nagasaki Med. J., 33 (11), Suppl. : 61—70, 1958b.

5) OMORI, N. : Experimental studies on the role of the house mosquito, *Culex pipiens pallens* in the transmission of bancroftian filariasis. 5. On the distribution of infective larvae in mosquito and the effect of parasitism of filariae upon the host insect. Nagasaki Med. J., 33 (11) ; Suppl. : 143—155, 1958c.

6) OMORI, N. : A review of the role of mosquitoes in the transmission of malayan and bancroftian filariasis in Japan. Bull. Wld Hlth Org., 27 : 585—594, 1962.

7) OMORI, N. : A role of mosquitoes in the transmission of filariasis in Japan. The 16th General Assembly of the Japan Medical Congress. April 1—5, 1963, Osaka, Vol. Ⅱ : 759—776, 1963. (in Japanese).

8) OMORI, N., SUENAGA, O. and NAKACHI, K. : Epidemiology of bancroftian filariasis in Hisamatsu Village, Miyako Island, the Ryukyus. 1. Results of a survey made in October, 1961. Endem. Dis. Bull. Nagasaki, 4 (3) : 194—205, 1962.

9) YAMADA, S. : An experimental study on twentyfour species of Japanese mosquitoes regarding their suitability as intermediate hosts for *Filaria bancrofti* Cobbold. Sci. Rep. Gov. Inst. Inf. Dis., 6 : 559—622, 1927.

琉球に於けるネッタイイエカのバンクロフト糸状虫に対する感受性と日本に於けるアカイエカのそれとの比較。大森南三郎・末永敏。上村昭栄・伊志嶺亮，長崎大学医学部医動物学教室，長崎大学風土病研究所衛生動物部（主任：大森南三郎教授）仲地国夫・上原直三，琉球衛生研究所。

総　　括

ネッタイイエカの♀成虫を，同一仔虫保有者から略同時的に吸血させ，満腹蚊の吸血直後に於ける摂取仔虫数をみると，個体によってその数が著しく異なる事から人末梢血流中の仔虫の分布が一様ではな

い事が推測される．然し，極めて大まかに云えば，満腹蚊の平均摂取仔虫数の多寡は被吸血者の保有仔虫数の多寡と平行的である．これらの事はアカイエカでの場合と略同様である．

実験的感染率即ち感染幼虫出現以後の剖検蚊に対する感染幼虫保有蚊の割合はネッタイイエカの場合に非常に低く，１♀当たりの感染幼虫数も極めて少ない．従ってネッタイイエカの感受性は非常に低いと云わねばならない．

然し，自然感染率即ち感染蚊に対する感染幼虫保有蚊の割合はネッタイイエカが有意的に高い．又，感染幼虫保有蚊１♀当たりの感染幼虫数もアカイエカの場合と比較して多少多い様に見える．

以上の様に両種の蚊共，大体に於いては，仔虫保有者の保有する仔虫数と平行的に仔虫を摂取すると思われるが，ネッタイイエカの場合には，実験的には，感染率が低く，１♀当たりの感染幼虫数も少ない．然し自然感染では逆に感染率が有意的に高く，１♀当たりの感染幼虫数も多少は多い。この事実は，ネッタイイエカでは感受性が低く，摂取された仔虫が排泄されたり，幼弱期に死亡するものが多いようであるが，発育した幼虫によっては殆んど悪影響を受けないのではないか，逆にアカイエカの場合には極めて感受性が高く摂取された幼虫は揃って発育し，感染幼虫が出現してくる時期に，多寄生を受けた蚊ほど早期に死亡する結果，感染幼虫保有蚊の出現率も，１♀当たりの感染幼虫数も少なくなるのではないかと考えられる．