On the Susceptibility of *Anopheles sinensis* to the Larvae of *Wuchereria bancrofti* and a Note on the Feeding Habit of the Mosquito in Kin Area, Okinawa Main Island

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(Received for Publication February 14, 1967)

Abstract

The susceptibility of *Anopheles sinensis* to the *Wuchereria bancrofti* was examined experimentally at Kin area, Okinawa Main Island with the result that the Okinawa strain of this mosquito is fairly susceptible to the parasite, though it may have no bearing on the spread of filariasis there in recent years because of its being not fond of human blood. Upon comparison of the result with those of the other authors in Japan and China, it is concluded that the susceptibility is varied with local strains and more fundamentally with individual mosquitoes and accordingly the susceptibility of a local strain appears to depend on the number of susceptible individuals within the population of the strain. On the other hand, it is presumed that adaptation of the parasite to the local strain may occur under the condition in which mosquitoes of the strain are abundant frequenting houses and feeding readily on man.

Introduction

Regarding the susceptibility of *Anopheles sinensis* to the larvae of *Wuchereria bancrofti*, it seemed to the present author that it differs with the local strains of the species. Mochizuki (1911) and Fujisaki (1959) working respectively in Fukuoka and Nagasaki, Kyushu, failed to obtain mature larvae in mosquitoes in laboratory, while Yamada (1927) working in Tokyo succeeded to get mature larvae in 18.8% of the mosquito.

On the other hand, Feng (1931) found the mosquito being a principal natural transmitter of filariasis due to *W. bancrofti* in country villages near Woosung, China, and Simpson (1951) reported natural infection with mature larvae in the mosquito from the Okinawa Main Island.

Hereupon, experiments were projected to make clear in the laboratory the susceptibility of the local strain to the parasite at Kin

Contribution No. 502 from the Research Institute of Endemics, Nagasaki University and No. 160 from the Department of Medical Zoology, Nagasaki University School of Medicine.

area, Okinawa, and also to examine the host preference of the mosquito in nature.

The author wishes to express his sincere appreciation to professor N. Omori for many helpful suggestions and criticisms during the course of this work and for aid in the preparation of the manuscript and to Dr. S. Ito for her help in learning the laboratory techniques.

Material, Place and Method

The mosquito, *Anopheles sinensis* Wiedemann, 1828 used in this experiment was reared as adults from wild caugnt older larvae and pupae in ricefields and ditches near Kin village which was located in the middle part of Okinawa Main Island, the Ryukyus. The rearing, feeding, dissecting and staining methods followed those having been adopted in the Department of Medical Zoology, Nagasaki University School of Medicine. The donors on which the female mosquitoes were fed were selected among the microfilarial carriers of bancroftian filariasis found in the village of Kin the microfilarial prevalence of which were about nine percent.

Developmental stages and sub-stages of filarial larvae in the mosquito

The developmental periods for the succeeding stages were examined at 27°C dissecting the infected mosquitoes at adequate intervals with the results that : In a mosquito among 262 infected ones, a larva reached the 3rd stage (IIIa sub-stage) in the shortest period of 11 days, while in other 3 mosquitoes they reached IIIa sub-stage on the 14th day and in some others they reached IIIb sub-stage or the infective larvae in the 14th day after the infective blood meal. The period in days for the lst and 2nd stages appeared to require 6 and 5 days in the shortest case, while those in the other cases 7 and 6 days respecitvely.

The lst, 2nd, and 3rd stages were subdivided, for convenience, into Ia, Ib, Ic, Id; IIa, IIb, IIc; and IIIa, IIIb sub-stages respectively, after Omori (1957) as shown in Table 1.

The IIIa sub-stage larvae are thicker and a little shorter than IIIb sub-stage ones. These two sub-stage larvae are said inclusively as the 3rd stage ones and vaguely as mature larvae. Strictly speaking, however, IIIa larvae are less active and living yet in the thoracic muscles of the host mosquito for a while, a half or a day, during the time they become slender, very much active and migratory, and to be called as infective larvae. In this paper, unless otherwise stated, IIIa and IIIb sub-stage larvae are called inclusively as the 3rd stage or mature larvae.

The outline of infection experiments

Thirteen experiments were carried out during from February 6, 1965 to April 27, 1966 in our laboratory at Kin village to examine the susceptibility of the mosquito to the parasite. The rearing temperature before and after the feeding ; the number of microfilariae per *ml* of each donor at the time of feeding ; the number of mosquitoes

Table 1. Diagnosis of developmental stages and sub-stages of filariae in mosquitoes (After N. Omori, 1957)

Stage	Substage	Body length in mm	Remarks
I	a	0.24-0.31	Microfilariae
Exsheath			
1	b	0.31-0.19	Shortening and thickening take place
	c	0.17-0.14-0.17	Shortest sausage stage
	d	0.19-0.34	Elongation and thickening take place
lst ecdysis			
II	a	0.34-0.68	Rapid elongation take place; three sub-stages are separated by length for convenience
	b	0.68-1.02	
	c	1.02-1.19	
2nd ecdysis			
III	a	1.19-1.36	Thick and shorter, less active
	b	1.19-1.87	Thin and longer, very active

Remarks : IIIa sub-stage larvae are found in the thorax which become thiner, longer and more active in a half or a day and then migrate within the body cavity including legs, head and proboscis. In this paper, IIIa and IIIb sub-stage larvae are denominated inclusively as the 3rd stage larvae or the mature larvae

Table 2. The susceptibility of *Anopheles sinensis* to the larvae of *Wuchereria bancrofti*

Exp. No.	Date of taking infective blood meal	Rearing temp. °C	No. Mf per *ml* blood of donor	No. of mosquitoes			% feeding	% infection to the engorged
				allowed to feed	engorged	infected		
I	Feb. 6, '65	27	3.167	25	10	5	40.0	50.0
II	Apr. 1, '65	27	0.900	83	16	2	19.3	12.5
III	Apr. 11, '65	27	2.933	30	14	11	46.7	78.6
IIII	Apr. 25, '65	27	3.600	140	54	49	38.6	90.7
V	Apr. 22, '65	27	2.900	20	4	2	20.0	50.0
VI	Jun. 25, '65	27	6.750	50	19	17	38.0	89.5
VII	Jul. 9, '65	27	5.717	60	22	21	36.7	95.5
VIII	Jul. 13, '65	27	1.767	60	34	22	56.7	64.7
IX	Jul. 15, '65	27	1.733	30	16	12	53.3	75.0
X	Dec. 3, '65	25	8.000	100	37	37	37.0	100.0
XI	Jan. 3, '66	24	6.370	80	25	21	31.3	84.0
XII	Apr. 23, '66	23.0[1)]	2.700	150	74	44	49.3	59.5
XIII	Apr. 27, '66	23.2 [1)]	2.700	120	61	19	50.8	31.1
Total				948	386	262	40.7	67.9

1) : Means of natural air temperatures

allowed to feed, engorged, and infected; the percentage feeding of mosquitoes; and the percentage infection of mosquitoes to the engorged are tabulated in Table 2.

The percentage feeding varied greatly with experiments. The variation appeared to be related to the ages of mosquito larvae reared in the laboratory to adults and ages of adult females from emergence to feeding and some other unknown factors.

The percentage infection of mosquitoes to the engorged was varied from 12.5% to 100.0%. The variation seemed to have intimate relation to the microfilarial count of donors. The relation is illustrated in the Lower Fig. of Fig. 1 which appears to show that the percentage infection of mosquitoes becomes higher with nagative acceleration with the increase in the number of microfilarial count of the donors.

The process of development, death, and chitinization of filarial larvae in the mosquito

As the larvae taken up by the females were expected to be killed or chitinized in a great number before reaching maturity, the engorged females on the microfilarial carriers were dissected at adequate intervals to examine the process of development and the fate of undeveloped larvae with the lapse of time after the infective meal. The whole experimental period during which the dissection of mosquitoes were carried out were subdivided into three : The first period from the feeding to the 7th day is one in which most larvae were in the lst stage; the 2nd period from the 8th to 13th day is one in which most of the larvae which passed the lst ecdysis were growing; the 3rd period from the 14th to the 20th day is one in which some larvae were expected to be in the 3rd stage after passing the 2nd ecdysis. In each experiment, the number of filarial larvae of each sub-stage found in a group of mosquitoes were examined for life or death for each period as shown in Table 3.

Before going further to explain the Table 3, some comment will be made on the process of chitinization in the lst stage and on the living conditions of 2nd and 3rd stage larvae. In the mosquitoes dissected in several days after the infective meal, it is difficult to make sure of the life or death of filarial larvae, though on some days later partially chitinized Ib or Ic and Id sub-stage larvae become visible. With the lapse of time the chitinization advance and the number of larvae chitinized become larger. The process of chitinization seems as follows : The moribund lst stage larvae in mosquitoes undergo gradual chitinization or are covered partially at first and entirely at last by black coating. The larvae are naturally killed in the course of advance in chitinization. In the case of 2nd stage larvae when killed in mosquitoes they gradually change in color from yellowish to greyish and undergo gradual disintegration for a long time. The 3rd stage larvae are in all cases living and active in the mosquito.

Susceptibility of Okinawa strain to the parasite

In mosquitoes (Table 3) dissected on from the lst to 7th day after the infective meal the lst stage larvae were only found, among which some were found chitinized, some

Table 3. The number of filariae and that of (chitinized) or (dead) for each sub-stage found in a gronp of mosquitoes which were dissected for each experiment on from lst to 7th, 8th to 13th, and 14th to 20th days after the infective blood meal

Exp. No.	No. Mf per *ml* blood of donor	Dissection dates after infective blood meal, No. of infected mosquitoes dissected within the dates, No. of filariae of different substages found in them. (and No. of filariae chitinized or dead)																			Total of		
		lst-7th				8th-13th								14th-20th									
		No. mosqs.	Substage			No. mosqs.	Substage							No. mosqs.	substage						Mosqs.	Larvae (Dead and chiti-nized)	Larvae per mosq.
			Ib	Ic	Id		Ib	Ic	Id	IIa	IIb	IIc	IIIa		Ib	Ic	Id	IIa	IIb	IIc	IIIa	IIIb			
I	3.167	4	15	4	3	1		4	2	2													5	30	6.00
II	0.900	2			3 (1)																		2	3 (1)	1.50
III	2.933	6	14	3		5	13 (12)	10 (3)	22 (21)	1 (1)													11	63 (37)	5.73
IV	3.600	13	46 (21)	2 (2)	7 (6)	20	12 (12)	35 (34)	79 (79)	34 (10)	8 (1)	2	1	16	12 (12)	31 (30)	57 (54)	11 (9)	8 (1)	3 (1)	4	6	49	358 (272)	7.31
V	2.900					2			10 (3)	7	2	1											2	20 (3)	10.00
VI	6.750	11	12 (4)	21 (2)	17 (2)	6		43 (11)	32 (32)														17	125 (51)	7.35
VII	5.717					21	4 (4)	27 (17)	28 (24)	6	1												21	66 (45)	3.14
VIII	1.767					16	2 (2)	12 (1)	16 (5)	5				6	4 (4)	7 (3)	5 (5)						22	51 (20)	2.32
IX	1.733					8	4 (4)	17 (6)	10 (4)	3				4		9 (7)							12	43 (21)	3.58
X	8.000	3	1	14 (1)	11	1		20 (14)	6 (6)					33	13 (13)	319 (209)	286 (258)	21 (5)	8		4	3	37	706 (506)	19.08
XI	6.370													21	26 (26)	185 (56)	55 (43)	12	24	5	10	26	21	343 (125)	16.33
XII	2.700													44		56 (30)	54 (33)	5	2	1			44	118 (63)	2.68
XIII	2.700	7	3	2	3	5		3	5 (1)			1		7		4 (1)	7 (7)		1	1		1	19	31 (9)	1.63
Total		46	91 (25)	46 (5)	44 (9)	85	35 (34)	171 (86)	210 (175)	58 (11)	11 (1)	4	1	131	55 (55)	611 (336)	464 (400)	49 (14)	43 (1)	10 (1)	18	36	262	1957 (1153)	7.47
% chitinized or dead in each substage			27.5	10.9	20.5		97.1	50.3	83.3	19.0	9.1	0.0	0.0		100.0	55.0	86.2	28.6	2.3	10.0	0.0	0.0			
% chitinized or dead in each stage			21.5				70.9			16.4			0.0		70.0			15.7			0.0				

were living, and some others were intact but could not be distinguished their life or death.

Eighty five mosquitoes dissected on from the 8th to 13th day had 490 larvae of which the lst, 2nd, and 3rd stage ones were respectively 416, 73 and 1 in number or 84.9, 14.9, and 0.2 in percentage. About 71% of the lst stage larvae were chitinized or dead and the remainder were moribund or seemed to die shortly. The second stage larvae were dead in 16.4%, while the remainer appeared alive or virtually were moving. A single active 3rd stage larva was found in a mosquito dissected on the 11th day in Exp. IV. The above shows that the great majority of larvae are killed in this mosquito in their lst stage, while the 2nd stage larvae which could pass the lst ecdysis can develop normally, excepting some of them which may be killed mostly in the very early IIa sub-stage. A mature larva which reached IIIa sub-stage was as active as in the most suitable intermediate host, *Culex pipiens fatigans* in the area at which this experiment was carried out.

One hundred and thirty one mosquitoes dissected on from the 14th to 20th day had 1286 larvae of which the lst, 2nd, and 3rd stage larvae were respectively 1130, 102, and 54 in number or 87.9, 7.9, and 4.2 in percentage. About 70% of the lst stage larvae were chitinized or dead. The percentage was nearly the same as that in the lst stage larvae found in mosquitoes dissected on from the 8th to 13th day suggesting that the larvae left behind in the lst stage must be killed even if they appeared intact or alive at the time of the dissection. About 16% of the 2nd stage larvae were found dead. The percentage was also nearly the same as that found in the previous period, and higher mortality which was found only in the early IIa sub-stage was similar to the previous case, suggesting that the 2nd stage larvae can mostly develop further when they could pass safely the lst ecdysis. All of 54 mature larvae were very active, some in proboscis, some in thorax, and others in abdomen. Throughout the whole period, the number of infected mosquitoes and filarial larvae found in them are totalized and the number of larvae per mosquito are computed for each experiment and given in the last column of Table 3.

The relation between the number of larvae per mosquito to the microfilarial count of the donors on which mosquitoes were fed is illustrated in Upper Fig. of Fig. 1 which shows that the relation appears to be linear.

To examine how many mosquitoes had the larvae of being left behind in younger sub-

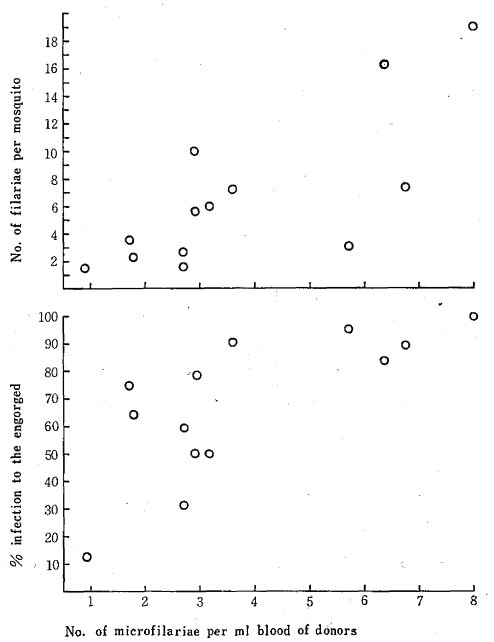

Fig. 1. Relation of the number of filariae per infected mosquito (Upper Fig.) and that of the percentage infection of mosquitoes against the engorged ones (Lower Fig.), to the number of microfilariae per *ml* blood of donors

Table 4. Number of mosquitoes which had the larvae of any one of the indicated sub-stages for the most advanced sub-stage at the time of dissections from the 14th to 20th day after the infective meal

Exp. No.	No. Mf per ml blood of donor	No. mosq. having different stage larvae							
		Ⅰb	Ⅰc	Ⅰd	Ⅱa	Ⅱb	Ⅱc	Ⅲ	Total mosq.
Ⅳ	3.600	1	0	7	3	0	1	4	16
Ⅷ	1.767		3	3					6
Ⅸ	1.733		4						4
Ⅹ	8.000		2	23	3	3		2	33
Ⅺ	6.370		3	10	2	1		5	21
Ⅻ	2.700		14	22	5	2	1		44
ⅩⅢ	2.700		1	3		1	1	1	7
Total		1	27	68	13	7	3	12	131
% to grand total		0.8	20.6	51.9	9.9	5.3	2.3	9.2	100.0

stages on the days of dissections carried out on from the 14th to 20th day after the infective meal, the number of mosquitoes which had the larvae of any one of six sub-stages from Ib to IIc and the 3rd stage are presented in Table 4.

Of 131 mosquitoes, 96 (73.3%), 23 (17.5%), and 12 (9.2%) ones had the lst, 2nd and 3rd stage larvae respectively. This shows that in how many mosquitoes the larvae are killed in their lst stage especially in the Id and Ic sub-stages. Table 4 also shows that the numbers of mosquitoes with IIa, IIb, and IIc decrease gradually, though that of those with mature larvae become larger suggesting that at least IIb and IIc sub-stage larvae shall have reached maturity when dissections were made some days later.

With each of 13 mosquitoes which had active mature larvae (cf. Tables 3 and 4) at the time of dissections on from the 11th to 20th day after the infective meal, the number and fate of concurrently found larvae are shown in Table 5.

Of the 105 lst stage larvae found in 13 females shown in Table 5, 58 or 55.2% were found chitinized and 47 or 44.8% appeared intact or even alive at the time of dissections. However, that the all larvae which remained unchitinized will be killed in the lst stage, must be accepted by the reason as presumed above. The 2nd stage larvae were alive and moving excepting some dead ones especially in the early IIa sub-stage just after the ecdysis. IIb and IIc sub-stage larvae were expected to reach maturity as mentioned above.

It is of great interest that the states of development of the filarial larvae in mosquitoes were subjected to a great variation with individual mosquitoes : In mosquitoes No. 49 and No. 12, most larvae developed well and majority of them reached maturity; while in many other mosquitoes the larvae were killed mostly in their lst stage. The percentage of mosquitoes which had the mature larvae also vaired greatly with experiments as shown in Table 6.

In 9 experiments shown in Table 3, the percentage of mosquitoes with mature larvae was zero. In only 4 experiments shown in Table 6 some larvae could reach maturity. However, interesting to say, the percentages

Table 5. The states of development of the larvae of *Wuchereria bancrofti* in *Anopheles sinensis* females in which one or more larvae of the 3rd stage were found on dissection. The number of larvae shown in parentheses shows that of chitinized ones and those in double parentheses does that of apparently dead ones

Exp. No.	No. Mf per *ml* blood of donor	Mosq. No.	Days after the infective blood meal	Number of filarial larvae of each substage								
				Ⅰb	Ⅰc	Ⅰd	Ⅱa	Ⅱb	Ⅱc	Ⅲa	Ⅲb	Total
Ⅳ	3.60	26	11				1 ((1))			1		2
		42	14	1 (1)	1 (1)	1 (1)		2	1		2	8
		44	14		3 (3)	4 (4)	2 ((2))	3 ((1))			1	13
		45	14	2 (2)	5 (5)	11 (11)	5 ((5))	2	1		1	27
		49	14					1		4	2	7
Ⅹ	8.00	27	19		10 (1)	8 (4)	2			2	3	25
		32	19		12 (9)	4 (3)	3	2		2		23
Ⅺ	6.37	7	19								1	1
		12	19				3	11	3	7	22	46
		18	19	2 (2)	25 (7)	2 (1)	4	11	2	1	1	48
		22	20		7			1		1	2	11
		25	20	2 (2)	4 (1)	1				1		8
ⅩⅢ	2.70	52	19								1	1
Total		13		7 (7)	67 (27)	31 (24)	20 ((8))	33 ((1))	7	19	36	220
Total in each stage				105(58 or 55.2%)			60 ((9 or 15.0%))			55		220
No. larvae per mosq.				8.1(4.5)			4.6 ((0.7))			4.2		16.9

greatly varied with experiments ranging from 5.3 to 23.8%.

The above shows that in this local population of *Anopheles sinensis* there existed a few numder of mosquitoes which were susceptible, and a small numder of those which were moderately or less susceptible, and a great majority of those which were quite unsusceptible to the parasite.

Considerations on the susceptibility of *Anopheles sinensis* to *Wuchereria bancrofti*

In Japan, several authors had carried out infection experiments to the parasite with *Anopheles sinensis* but the results were not the same and accordingly the present author intended to carry out similar experiments with the mosquitoes of the same species in Okinawa. Mochizuki(1911) carried out the experiment using the local strain at Fukuoka City, Kyushu. Yamada (1927) did his experiment at Tokyo using the Tokyo strain. Fujisaki(1959) made his experiment with the local strain obtained near Nagasaki, Kyushu. The present author carried out the experiment using the local

Table 6. Variation in the percentage of mosquitoes with mature larvae in the experiments in which one or more mosquitoes were found having mature larvae

Exp. No.	Total No. mosqs. infectad	No. and % mosqs. with mature larvae	
		No.	%
IV	49	5	10.2
X	37	2	5.4
XI	21	5	23.
XIII	19	1	5.3

Remarks : As for the sub-stage of mature larvae compare Table 5.
In other nine experiments the percentages were zeros.

strain at Kin Village which is located at about the middle part of Okinawa Main Island, the Ryukyus. Here, it is to be noted that the mosquitoes found in the four localities are not different in morphology and therefore it may be not necessarily appropriate to use the term "strain" for them. However, in this report each local population is regarded for convenience as different strain of the species because the strains seemed different in the susceptibility to the parasite. The data of the four authors are given for comparison in Table 7 (7.1 and 7.2).

Tokyo strain was highest in percentages of mosquitoes having 2nd stage and also 3rd stage or mature larvae and Okinawa strain was next in these percentages as shown in Table 7.1. Fukuoka strain (Mochizuki, 1911) and Nagasaki strain

Table 7. Comparison of the data of different authors on the experimental infection of *Anopheles sinensis* with *Wuchereria bancrofti*

1. The number and percentage of mosquitoes infected

Author	Place of Exp.	Dissection period in days after feeding	No. mosquitoes		% infection	Infection with 1st stage larvae		Infection with 2nd stage larvae		Infection with mature larvae	
			dissected	infected		No. of mosqs.	% to the infected	No. of mosqs.	% to the infected	No. of mosqs.	% to the infected
Mochizuki (1911)	Fukuoka	11—13	28	23	82.1	22	95.7	1	4.3	0	0.0
Yamada (1927)	Tokyo	12—19	16	16	100.0	15	93.8	6	37.5	3	18.8
Fujisaki (1959)	Nagasaki	11—32	247	205	83.0	203	99.0	3	1.5	0	0.0
Present author	Okinawa	14—20	188	131	69.7	125	95.4	32	24.4	12	9.2

Remarks. The sum of the number of mosquitoes with each stage larvae becomes larger than the number of mosquitoes infected because a single mosquito frequently has the larvae of different stages at the same time.

2. The percentage of chitinized or dead larvae in the mosquito

Author	Place of Exp.	No. mosqs. infected	1st stage larvae			2nd stage larvae			Mature larvae		
			Total	Chitinized or dead	%	Total	Chitinized or dead	%	Total	Chitinized or dead	%
Mochizuki	Fukuoka	23	163	143	87.7	3	2	66.7			
Yamada	Tokyo	16	203	107	52.7	46	0	0.0	12	0	0.0
Fujisaki	Nagasaki	205	634	590	93.1	5	0	0.0			
Present author	Okinawa	131	1130	791	70.0	102	16	15.7	54	0	0.0

(Fujisaki, 1959) were very much low even in the percentage of mosquitoes with the 2nd stage larvae and were zeros in that of those with mature larvae. Particularly speaking, however, Mochizuki found one actively moving larvae just prior to the second ecdysis (IIc sub-stage of the present author) and two dead larvae of being probably in IIa sub-stage in a single female dissected 11 days after the infective meal among 23 infected females, and Fujisaki found three very active larvae of IIc sub-stage in a single female dissected 15 days after the infective meal among 205 infected ones. Regarding the fate of the living IIc sub-stage larvae the two authors expected that they shall have reached maturity if the mosquitoes were dissected for a few days later. The above seems to show that there exists no strain of *Anopheles sinensis* which is perfectly unsusceptible to the parasite although the degree of the susceptibility is subjected to a great variation.

The percentages of mosquitoes with the lst stage larvae were very high in every strain, though those in Tokyo and Okinawa strains appeared a little lower than in the other two.

Rearging the fate of the great number of larvae left behind in the lst stage till the day of reaching or nearly reaching maturity of some larvae concurrently taken up by mosquitoes at the initial infective blood meal, Fujisaki (1959) made a couclusion that the lst stage larvae which were left behind in that stage for a long time were immobilized and most of them were chitinized to death and the rest, even though appeared intact at the time of dissection, would be killed shortly. The present author arrived at the same conclusion. Mochizuki made no reference to the fate of the intact and not chititized lst stage larvae which seemed to the present author to be killed mostly in that stage shortly. However, the result of the observation made by Yamada (1927) was interesting to say, quite different from those of the others. His data was that out of 203 lst stage larvae, 52.7% were chitinized or dead, 18.7% were those to which he made no mention, and 28.6 % were living. To the fate of the living lst stage larvae which were very much being delayed in development, Yamda made no reference and the present author can not made any presumption about the matter. The 2nd and mature larvae in Yamada's experiment were living and very active.

Thus, the 2nd stage larvae were mostly living and expected by the authors to develop further. The 3rd stage larvae were ascertained by all of them to be very active without exception. From the data given in Table 7 the comparision on the susceptibility of the strains will be made on the next table.

It is clearly seem from the table that the susceptibility is the lowest in Nagasaki and Fukuoka strains, while become higher in Okinawa and highest in Tokyo starin. Thus, the susceptibility of *Anopheles sinensis* differs clearly with local strains and moreover, as

Strain	Author	% of lst stage larvae to all stage larvae	% larvae chitinized or dead in the lst stage	% mosquitoes with mature larvae
Nagasaki	Fujisaki	99. 2	93. 1	0
Fukuoka	Mochizuki	98. 2	87. 7	0
Okinawa	Uemura	87. 9	70. 0	9. 2
Tokyo	Yamada	77. 8	52. 7	18. 8

already mentioned, greatly with individual mosquitoes. In other words, the susceptible strain can be said to contain a larger number of susceptible mosquitoes.

On the other hand, in nature, Feng (1931) examined the natural infection of *Anopheles sinensis* in small farm villages scattered around Woosung Town near Shanghai, China. In the villages bancroftian filariasis was endemic and *Anopheles sinenis* as well as *Culex tritaeniorhynchus* were abundant frequenting houses and biting readily on man, while *Culex pipiens* seemed to be not abundant. In 17 such villages he caught 230 females of *An. sinensis* in 54 houses and found infected in 36 or 15.7%, of which 6 or 16.7% females were found with mature larvae. From the results, he concluded that in the villages *An. sinensis* is a very good natural intermediate host of *W. bancrofti.* The percentage of 15.7 in natural infection with mature larvae can be said to be very high for the rate in nature. Experimentally, he collected 67 engorged females of the mosquito in a mosquito-net in which a boy of bancroftian microfilarial carrier have slept. Out of 5 females which lived for 7 to 11 days after the infective meal, in two females dissected on the 11th day he found mature larvae and in 3 females which died in less than 11 days some number of 2nd stage larvae. The results of Feng's experiments seem to show that the local strain he used is much more highly susceptible than the Tokyo strain which showed the highest susceptibility to the parasite in Japan. Simpson (1951) found 4 infected mosquitoes out of 49 females of *Anopheles sinensis* caught in nature and dissected on the period July 13-20, 1945 at Sedake village located in the middle part of the Okinawa Main Island. At that time many rural communities (including the Sedake village) along the coastal areas north to middle parts of the Main Island had been greatly overcrowded by the war refugees. His statement is as below: "The species was found to feed voraciously at night on the exposed huamn population, which was the most available source of blood meals in the almost complete absence of domestic animals." In one of the 4 infected mosquitoes he found a single mature larvae lying within the proboscis. From the result of his observation he concluded that "*Anopheles sinensis* should be regarded as a possible vector of bancroftian filariasis in Okinawa and neighboring islands and that under conditions similar to those encountered at the time of the study, it may prove to be of relative importance."

The results of examinations on the susceptibility of *Anopheles sinensis* to the parasite carried out by many authors in the laboratory and in nature, tell us that there exists no strain of *Anopheles sinensis* which is perfectly not susceptible to the parasite although the degree of the susceptibility is subjected to a great variation. The variation in susceptibility existing among strains and more fundamentally among individual mosquitoes seems innate for locality because the variation has no connection with the filariasis endemicity of the locality. In fact, Tokyo where the local strain was highest in susceptibility, and Nagasaki and Fukuoka cities where the strains were scarcely susceptible, were places where filariasis was absent, while, Kin area (in Okinawa) where the strain was moderately susceptible was an area where filariasis was moderately endemic (about 9%).

Apart from the apparently innate variation

in the susceptibility among strains, adaptation of the parasite to the local strain of *Anopheles sinensis* may occur under the condition in which mosqutoes of the strain are abundant frequenting houses and readily feeding on man as in the case of small farm villages near Woosung.

Host preference and epidemiological significance of *Anopheles sinensis* in Kin area, Okinawa Main Island

Anopheles sinensis is the 3rd species in the order of abundance in Kin area but it can be scarcely found in dwelling houses as shown in Table 8. The species is only found in a very small number in stables and in a relatively large number in pigstys. The mosquito, therefore, can be said to have no significance on the epidemiology of bancroftian filariasis in the area in recent years.

Table 8. Relative abundance and host preference of famale mosquitoes collected at Kin village, Okinawa Main Island. The catches were made one hour after the sunset for 20 minutes by a man, once a week from June to November, 1965 usually in 3 to 6 mosquito-nets ; within 4 houses ; in a stable ; and in 2 pigstys. R. A. : Relative abundance. Mosqs. : Mosquitoes.

Place / No. & R. A. / Species	In 29 mosquito-nets in total		In 71 dwelling houses in total		In 26 stables in total		In 35 pigstys in total	
	No. of mosqs.	R. A. (%)	No. of mosqs.	R. A. (%)	No. of mosqs.	R. A. (%)	No. of mosqs.	R. A. (%)
C. P. fatigans	231	99. 1	565	76. 4	28	6. 2	8	0. 7
C. tritaeniorhynchus	0	0	144	19. 5	393	86. 4	863	72. 3
C. vishnui	0	0	4	0. 5	5	1. 1	52	4. 4
C. bitaeniorhynchus	0	0	0	0	0	0	4	0. 3
C. sitiens	0	0	0	0	0	0	1	0. 1
An. sinensis	0	0	1	0. 1	5	1. 1	140	11. 7
Ar. subalbatus	0	0	18	2. 4	22	4. 8	27	2. 3
M. uniformis	2	0. 9	0	0	0	0	66	5. 5
Ae. vexans	0	0	3	0. 4	2	0. 4	31	2. 6
Ae. albopictus	0	0	5	0. 7	0	0	2	0. 2
Total	233	100. 0	740	100. 0	455	100. 0	1, 194	100. 0

Summary

Laboratory experiments were carried out to examine the susceptibility of the local strain of *Anopheles sinensis* to the larvae of *Wuchereria bancrofti* at Kin village, Okinawa Main Island, the Ryukyus, during from February, 1965 to April, 1966. The village in which the experiments were made was about mine percent in filariasis prevalence.

The infection rate of mosquitoes appeared to become higher with negative acceleration with the increase in microfilarial count of donors, while the number of filariae per infected mosquito appeared to increase proportionally with the increase in microfilarial count.

Of 131 mosquitoes dissected on from the 14th to 20th day after the infective meal, 96 (73.3%), 23 (17.5%), and 12 (9.2%) ones respectively had the lst, 2nd, and 3rd stage larvae. Of 1286 larvae found in the 131 mosquitoes, the lst stage, 2nd stage, and 3rd stage or mature larvae were respectively 1130 (87.9), 102 (7.9), and 54 (4.2) in number and percentage.

About 70% of the lst stage larvae were found chitinized or dead and the remainder seemed to be killed shortly. About 16% of the 2nd stage larvae were found dead at early IIa sub-stage, while the remainder seemed to develop further and at least IIb and IIc sub-stage larvae were expected to reach maturity. The 3rd stage larvae i. e. mature larvae were living and very active in every case. The percentage of mosquitoes infected with mature larvae was 9.2% in an average as stated above but it varied greatly with experiments ranging from zero to 23.8 percent, and moreover, detailed examinations showed that the local strain which was used in the present experiments was consisted of a few number of mosquitoes which were highly susceptible, and some number of those which were moderately or less susceptible, and a great majority of those which were quite unsusceptible to the parasite.

When compared the present data with those by other Japanese authors, it seemed that there exists no strain which was perfectly unsusceptible to the parasite, although the degree of the susceptibility was subjected to a great variation with strains. The variation in susceptibility existing among strains and more fundamentally among individual mosquitoes seemed innate for each local strain or individual mosquito because the variation had no connection with the filariasis endemicity of the locality.

Apart from the innate variation in the susceptibility among strains, adaptation of the parasite to the local strain may occur under the condition in which mosquitoes of the strain are abundant frequenting houses and biting readily on man, as in farm villages near Woosung, China where Feng (1931) had proved *Anopheles sinensis* to be an important vector mosquito of bancroftian filariasis there.

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沖縄本島金武地方産シナハマダラカのバンクロフト糸状虫に対する感受性及び吸血嗜好性について．上村昭栄，長崎大学医学部医物学教室，長崎大学風土病研究所衛生動物部．

摘　　要

沖縄本島金武地方産のシナハマダラカのバンクロフト糸状虫幼虫に対する感受性を調べるために1965年2月から1966年4月迄の間に13回の感染実験を行なって次の結果を得た．蚊体内で成熟幼虫が発育してくると思われる時期即ち感染血摂取後14日以後に剖検した131個体の蚊体内で発見されたフィラリア幼虫の発育，生存，死亡状態についてみると，蚊に摂取されたフィラリア幼虫の約88％はⅠ期幼虫期に死亡しその約70％はキチン化される．Ⅱ期幼虫に迄進み得るものは約8％でその内若干は初期に死亡するが残余のものは尚発育を続けて成熟幼虫に到達するものも可成あると考えられる．成熟幼虫に迄進み得たものは約4.2％と非常に少ないが，アカイエカ体内に於けると同様長く生存し極めて活潑である．

成熟幼虫保有蚊の率は131♀中の9.2％であるが，感染蚊全体について個体別にみると，発育した成熟幼虫の多数と若干の元気なⅡ期中・末期のみを持った極く少数の蚊と，成熟幼虫の他にⅡ期幼虫及び多数の死亡したⅠ期幼虫を持った（その令構成は蚊の個体により色々である）若干の蚊と，死亡した或るいは死亡すると思われるⅠ期幼虫のみを持った極めて多数の蚊とが混っている事が判る．即ち，本 strainは感受性の極めて高い少数の蚊と，中程度又は低度の感受性を持った若干の蚊と，全く感受性のない極めて多数の蚊とから成立している事が判る．この事を一般的に云えば，strain の感受性の強弱は，その中に含まれる感受性個体

の多少によってきめられるものと云える.

金武地方での本 strain の吸血嗜好性を調べた結果では, 発生個体数としては, コガタアカイエカ, ネッタイイエカに次いで多いが, 人家内では殆んど採集されず特に蚊帳内では全く発見できない, 馬小舎で若干豚小舎で可成り採集される程度なので, 本strain は実験的には, シナハマダラカとしては, 中程度の感受性のあるものであるが疫学的には, 少なくとも現状では全く問題とはならないものと思われる.

本実験結果を望月 (1911), 山田 (1927), 及び藤崎 (1959) の感染実験の結果と比較すると, 成熟幼虫保有蚊の率は望月(福岡),藤崎 (長崎)の使用した strain では何れも零であるが, 著者 (沖縄) の場合は 9.2%, 山田 (東京) の場合は実に18.8%である. 然し藤崎は205個体の感染蚊中1♀に, 望月は23 個体中の1♀に成熟幼虫になる直前即ちIIc 期の元気な幼虫を発見しており, 数日後に剖検すれば成熟幼虫になったのではないかと述べている事を考えると *Anopheles sinensis* には感受性の全くない strain はあり得ないように思われる. その感受性の程度はstrain によって, 更に基本的には個体によって著しく異なるが, strain 間の感受性の程度はその strain に特有であろうと考えられる. と云うのは, この感受成の強弱はその地方に於けるフィラリアの有無及び浸淫率とは平行的な関係が全く認められないからである.

感受性の高低がその土地の strain に特有であると云う事とは別に, 或る地方でその土地の strain の発生量が大で, 侵屋性が強く, 好んで人から吸血するような条件下ではフィラリア幼虫がその strain に対して適応してくる事もあり得ることが,支那の Woosung 近郊ではアカイエカが最適伝搬者であるのにその近郊の農村ではその地方産のシナハマダラカが最も重要なバンクロフト糸状虫の伝搬者であるとの報告などからも推定できる.