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 *

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This is the fifth report of the theses and deals with the effect of parasitism of immature filariae in the host animal; the movement, distribution, and migrating and penetrating habits of infective larvae; and the effect of parasitism of infective larvae upon the life of the host.

MATERIAL AND METHOD

A lot of infected mosquitoes reared at 25°C or 27°C from the day of feeding on microfilarial carrier were transferred on the day of reaching maturity of filariae to costant temperatures of 33, 30, 27, 25, 24, 22, 19, 18, 16, 13, and 10°C during from 1953 to 1955, and variable temperatures in our laboratory (W. L.) and those at the north side passage of the laboratory (W. P.) in the winter of 1953−1954.

Mosquitoes which were dead within 24 hours or dying or morbid at the time of daily inspection or killed in rare case if necessary, were dissected for filariae. The routine work was continued from the next day of the feeding to the day of the natural death of the last mosquito.

Explanation of Tables and Figures Given in This paper

The number of filariae taken up by a female mosquito is subjected to a great variation as already mentioned in the second report and the longevity in days of mosquitoes greatly vary with individuals, and therefore it came to the author's notice that it is better to compare the number or the states of distribution of filariae found in a group of mosquitoes which died naturally within a certain period of time, with that of the other groups. The period of times in each experiment, are divided as below: a period from the day of infective meal to the previous day of reaching maturity of filariae; every successive ten days (or twenty days when necessary) after the maturity of filariae; and the last period of days during which the last five to ten mosquitoes died. To each group of mosquitoes which died within each successive

* Contributions from the Research Institute of Endemics, Nagasaki University, No. 293

period of time divided as above, numerical order or group number is given. It must be remembered therefore that the group No. 1 shown in the tables and figures in this paper contains some number of mosquitoes which died prior to the day of reaching maturity of filariae and the group No. 2 contains those dead within the first ten days after the maturity of filariae and so on.

As the results of experiments I and II shown in Table 1 were already explained in Report 1, merely regarding the figures in parentheses given on lines marked with *, explanations will be made. In Exp. I, a female No. 12 displayed abnormal behavior (the meaning of which will be explained later) on the 9th day and was found on dissection harboring 116 immature larvae, that is, 3, 5, 89 and 19 filariae of Ib, Ic, Id and IIa stages.

In Exp. II, a living female No. 1 was killed at random and found harboring 34 Ic stage larvae.

In Exp. III in Table 1, 59 infected mosquitoes were reared at 27°C for 11 days during which 10 females were dissected. Of which two living

Table 1 The number of filariae before and after their reaching maturity, found in naturally dead or dying or exceptionally killed mosquitoes (regarding the figures in parentheses on lines marked with*, see text)

Experiment I (Lot No. 71 ; Expected No. Mf to be taken up by a female is 40.8)

Rearing temp. before and after the reaching maturity of filariae	Group No.	Days after infective meal		No. mosq. dissected	Number of filariae in mosqnitoes						Remarks
		Interval	Days		I	II	III	Total	Per ♀	Its ratio to mean	
27°C, before	1	1−11	11	14	203	36	0	239	17.1	1.51	
	* 1)		(9)	(No. 12)	(97)	(19)	0	(116)			killed (abnormal)
27°C, after	2	12−21	10	6	5	34	67	106	17.7	1.57	
	3	22−31	10	10	7	8	52	67	6.7	0.59	
	4	32−44	13	8	0	1	15	16	2.0	0.18	
Total or mean	1−4	1−44	44	38	215	79	134	428	11.3	1.00	

* 1) When except the killed one which had 116 immature larvae, the remaining 13 females of group 1 had 9.5 filariae per ♀ or 0.84 times as much as the mean (11.3)

Experiment II (Lot No. 94. 3 & 4 ; Expected No. Mf to be taken up by a female mosquito is 10.3)

Rearing temp.	Group No.	Interval	Days	No. mosq. dissected	I	II	III	Total	Per ♀	Its ratio to mean	Remarks
25°C, before	1	1−13	13	6	52	5	0	57	9.5	1.23	
	* 2)		(5)	(No. 1)	(34)			(34)			killed (alive)
25°C, after	2	14−23	10	62	0	66	494	560	9.0	1.17	
	3	24−33	10	28	2	19	199	220	7.9	1.03	
	4	34−43	10	14	0	5	36	41	2.9	0.38	
	5	44−85	42	6	0	1	16	17	2.8	0.36	
Total or mean	1−5	1−85	85	116	54	96	745	895	7.7	1.00	

* 2) When except the killed one which had 34 Ic stage larvae, the remaining 5 females of group 1 had 4.6 filariae per ♀ or 0.60 times as much as the mean (7.7)

Table Ⅰ (Continued)

Experiment Ⅲ (Lot No. 72; Expected No. Mf to be taken up by a female mosquito is 30.6)

Rearing temp. before and after the reaching maturity of filariae	Group No.	Days after infective meal		No. mosq. dissected	Number of filariae in mosquitoes						Remarks
		Interval	Days		Ⅰ	Ⅱ	Ⅲ	Total	Per ♀	Its ratio to mean	
27°C, before	1	1−11	11	10	127	25	0	152	15.2	0.88	
	* 3)		(3)	(No. 1)	(32)			(32)			killed (alive)
			(3)	(No. 2)	(24)			(24)			killed (alive)
24°C, after	2	12−21	10	10	13	18	66	97	9.7	0.56	
	3	22−31	10	8	0	1	43	44	5.5	0.32	
	4	32−41	10	6	2	1	17	20	3.3	0.19	
	5	42−49	8	6	0	1	4	5	0.8	0.05	
The following 19 females, as they displayed abnormal behaviour on the 12th day or the day of maturity of filariae, were transferred to a thermostat at 16°C											
16°C, after	2′	12−21	10	5	18	124	178	320	64.0	3.70	
	3′	22−31	10	8	15	82	182	279	34.9	2.02	
	4′	32−70	39	6	16	19	69	104	17.3	1.00	
Total or mean	1−5−2′−4′	1−70	70	59	191	271	559	1021	17.3	1.00	

* 3) When except the two killed ones which had 32 and 24 I stage larvae, the remaining 8 females of group 1 had 12.0 filariae per ♀ or 0.69 times as much as the mean (17.3)

Table Ⅰ (Continued)

Experiment Ⅳ (Lot No. 79. 1, 2; Expected No. Mf to be taken up by a female mosquito is 51.4)

Rearing temp. before and after the reaching maturity of filariae	Group No.	Days after infective meal		No. mosq. dissected	Number of filariae in mosquitoes						Remarks
		Interval	Days		Ⅰ	Ⅱ	Ⅲ	Total	Per ♀	Its ratio to mean	
25°C, before	1	1−13	13	24	154	154	0	308	12.8	1.05	
	* 4)		(12)	(No. 21)	(5)	(59)		(64)			killed (abnormal)
10°C, after	2	14−23	10	26	38	192	291	521	20.0	1.64	
	3	24−33	10	20	17	8	182	207	10.4	0.85	
	4	34−43	10	10	4	4	67	75	7.5	0.61	
	5	44−53	10	11	2	6	68	76	6.9	0.57	
	6	54−73	20	8	2	12	73	87	10.9	0.89	
	7	74−93	20	10	1	7	97	105	10.5	0.86	
	8	94−180	87	11	6	1	79	86	7.8	0.64	
Total or mean	1−8	1−180	180	120	224	384	857	1465	12.2	1.00	

* 4) When except the killed one which had 64 immature larvae, the remaining 23 females had 10.6 filariae per ♀ or 0.87 times as much as the mean (12.2)

females, No. 1 and No. 2 were killed and found having 32 or 30 Ib + 2 Ic and 24 or 19 Ib + 5 Ic larvae respectively. On the 12th day, that is, on the day of reaching maturity of filariae 19 females were found displaying abnormal behavior and seemed to die within one or two days and therefore were exposed to 16°C. The remaining 30 females were exposed to 24°C.

In Exp. IV in Table 1, 120 infected mosquitoes were reared at 25°C for 13 days. A female No. 12 displayed abnormal behavior on the 12th day and was found on dissection harboring 64 filariae i.e. 5, 30, 22 and 7 of Id, IIa, IIb and IIc stages.

Table 2 Distribution of infective larvae in the body of mosquitoes in each successive group which died or were dying naturally or killed in very rare case if necessary, from the day of reaching maturity of filariae

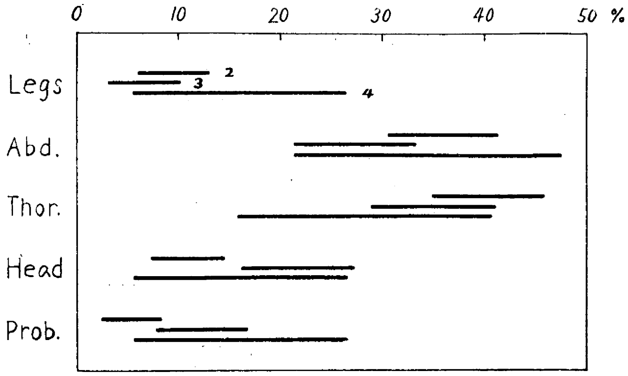
Group No.	Days after infective meal: Interval	Days after infective meal: Days	No. mosq. dissected	Number of infective larvae in 5 parts of the body of mosquitoes: Legs	Abd.	Thor.	Head	Prob.	Total	cf.
(I) The states of distribution observed during subjection to 27°C (Lot No. 71)										
2	12−21	10	6	6	24	27	7	3	67	Exp. I of Table 1
3	22−31	10	10	3	14	18	11	6	52	
4	32−44	13	8	2	5	4	2	2	15	
(II) The states of distribution observed during subjection to 25°C (Lot No. 94.3 & 4)										
2	14−23	10	62	70	83	141	89	111	494	Exp. II of Table 1
3	24−33	10	28	20	48	42	54	35	199	
4	34−43	10	14	4	6	11	8	7	36	
5	44−85	42	6	0	5	10	0	1	16	
(III) The particular states of distribution in group 2 of the preceediag table (II)										
2.1	14−17	4	14	13	25	43	28	15	124	Exp. II of Table 1
2.2	18−19	2	15	25	19	39	15	41	139	
2.3	20	1	12	16	14	9	17	22	78	
2.4	21−22	2	11	10	8	16	11	23	68	
2.5	23	1	10	6	17	34	18	10	85	

In Table 2, the number of infective larvae in each successive group in the five parts of the body of mosquitoes was tabulated. (I) shows the states of distribution of infective larvae in mosquitoes kept at 27°C. (II) shows those in mosquitoes kept at 25°C. (III) shows those in group 2 of (II).

In order to inquiry into the variation in distribution of infective larvae in mosquitoes, with the changes in contact temperatures and with the lapse of time of the exposure, the 60% confidence intervals for population percentage number of the infective larvae in the five parts of the body of mosquitoes were obtained.

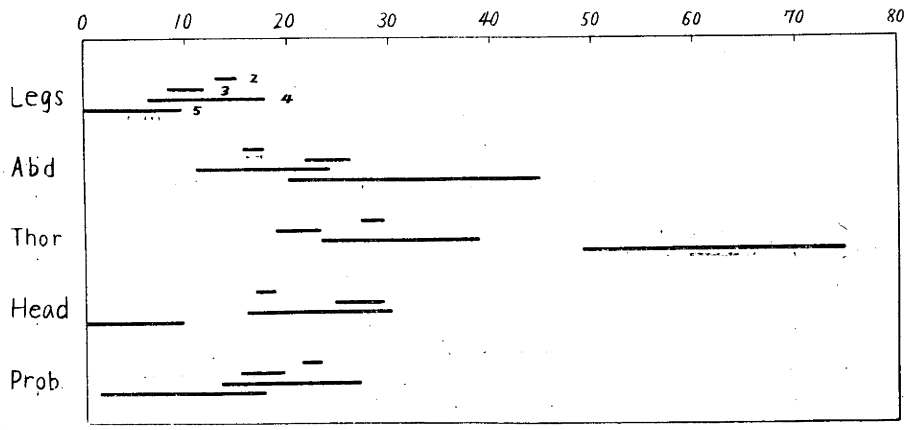
The confidence intervals obtained for the results of experiments made at 27°C, 25°C and for that of group 2 in the experiment at 25°C i.e. for (I), (II) and (III) in Table 2 were illustrated in Fig. 1, 2 and 3. The confidence intervals obtained for the results of experiment made at 10°C and at winter variable temperatures at the north side passage were illustrated in Fig. 4 and 5.

Fig. 1 60% confidence intervals for population percentage number of infective larvae in five parts of the body of mosquitoes kept at 27°C



Remarks : 2, 3 and 4 reprsesnt the intervals in legs and so on, in the successive groups shown in (I) of Table 2

Fig. 2 60% confidence intervals for population percentage number of infective larvae in five parts of the body of mosquitoes kept at 25°C

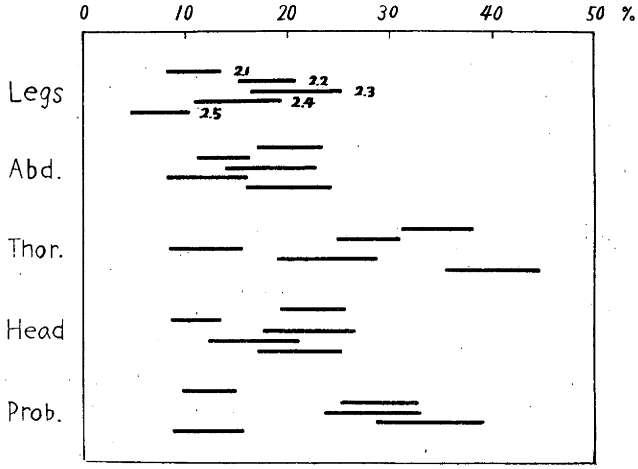


Remarks : 2, 3, 4 and 5 represent the intervals in legs and so on, in the successive groups shown in (II) of Table 2

Those for the other experiments are omitted as the variation in the states of distribution are being well demonstrated in the above figures.

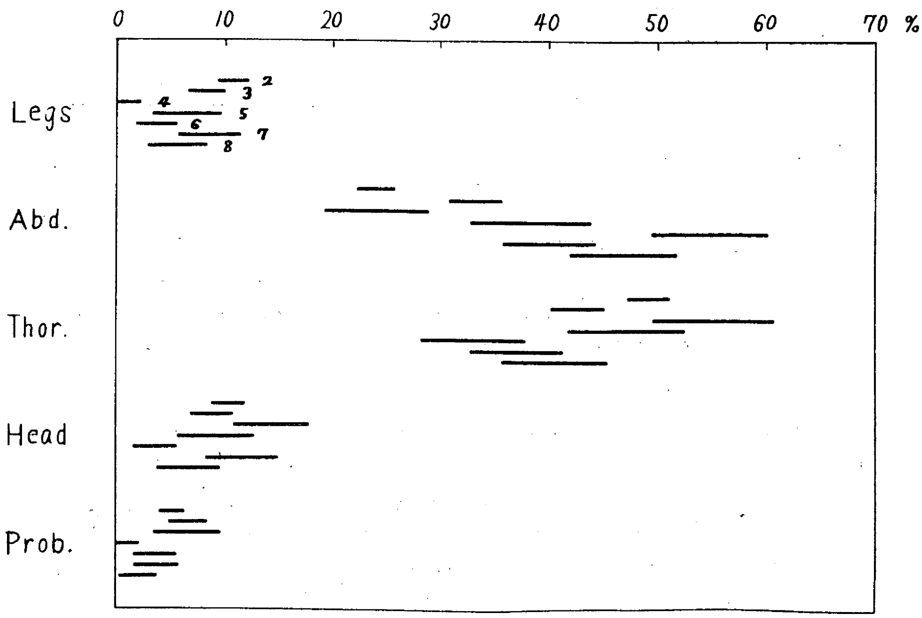
To see the general trend of distribution of infective larvae in five parts of the body of mosquitoes, the total numbers of those found in each of the five parts by each of the experiments are tabulated in Table 3 (see page 151) and the 60% confidence intervals for population percentage number of those are illustrated in Fig. 6. In Table 3, the number of infective larvae per female in each experiment and the expected number of microfilariae to be taken up by a female mosquito from the carrier are added.

Fig. 3 60% confidence intervals for population percentage number of infective larvae in five parts of the body of mosquitoes of **group 2** shown in (II) of Table 2



Remarks : 2.1, 2.2, 2.3, 2.4 and 2.5 represent the intervals in legs and so on, in the successive groups shown in (III) of Table 2

Fig. 4 60% confidence intervals for population percentage number of infective larvae in five parts of the body of mosquitoes exposed to 10°C



Remarks : Figures from 2 to 8 represent the intervals in legs and so on, in the suecessive groups showe in IV of Table 1

Fig. 5 60% confidence intervals for population percentage number of infective larvae in five parts of the body of mosquitoes exposed to winter temperatures at the north side passage of our laboratory, in 1953–54

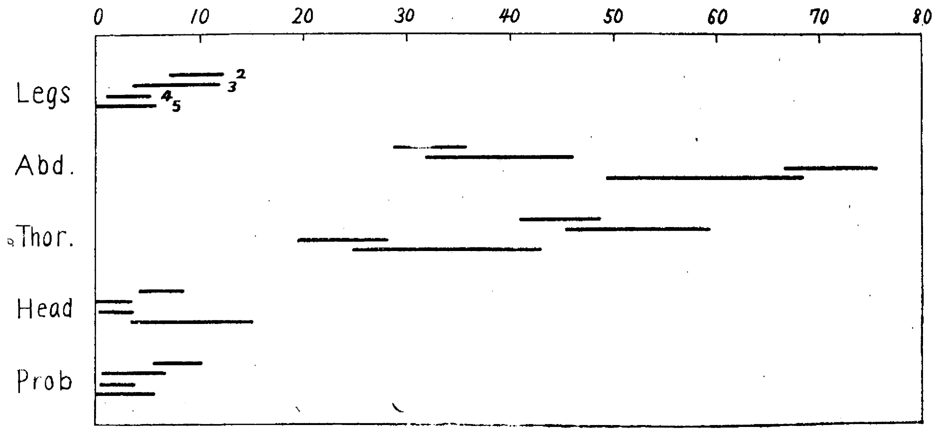
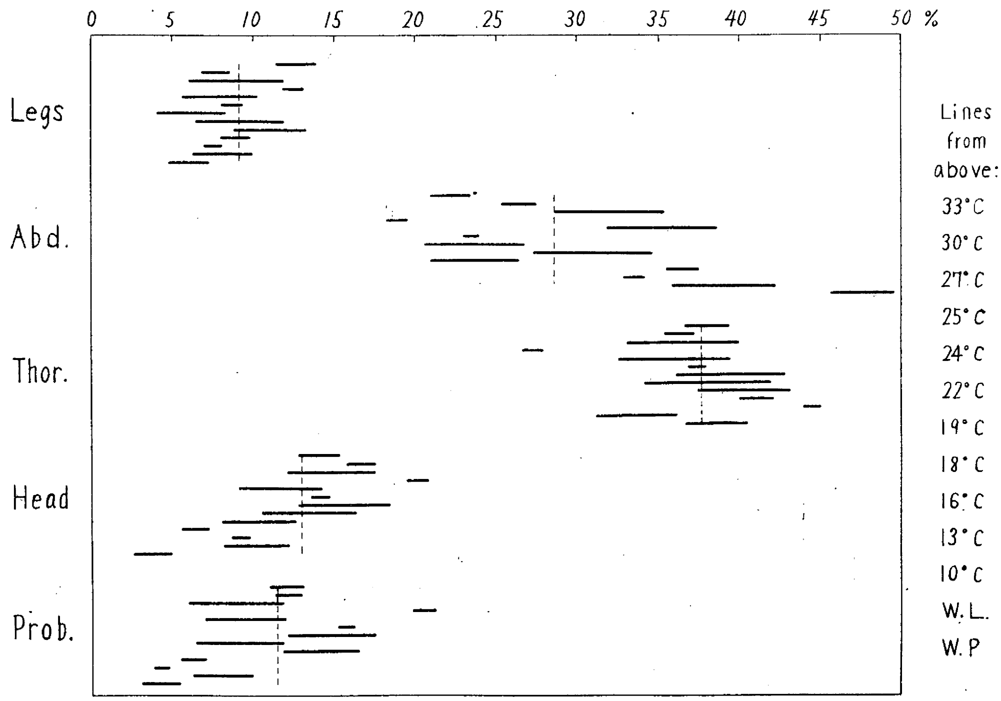


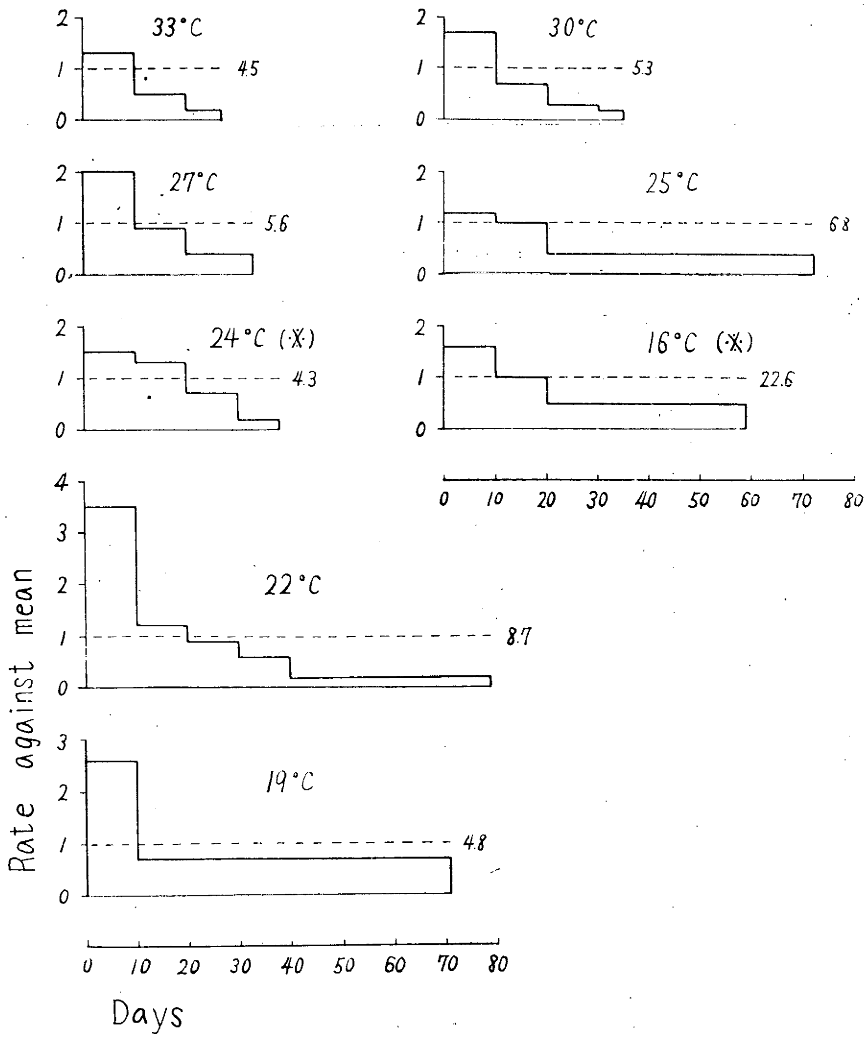
Fig. 6 60% confidence intervals for population pecentage number of infective larvae in five parts of the body of mosquitoes exposed to various constant and variable temperatures shown in Table 3



Remarks : 1) W.L. = Winter variable temperatures in our laboratory in 1953–54
W.P. = Winter variable temperatures at the north side passage of the laboratory in 1953–54
2) Vertical dotted line shows the percentage number of infective larvae in each part throughout the all experiments

Finally, the reduction in rate of the number of infective larvae per female mosquito which died naturally in each successive ten days, against the general mean number of those in each experiment is illustrated in Fig. 7, where the height in each figure shows the multiple value against the general mean number of infective larvae in each experiment which is given in figure on each broken line, and the length shows the longest longevity of mosquitoes.

Fig. 7 Reduction in rate of the number of infective larvae per famale mosquito which died naurally in each successive ten days, against the general mean number of those in an experiment which is given on the broken line. (* see text)



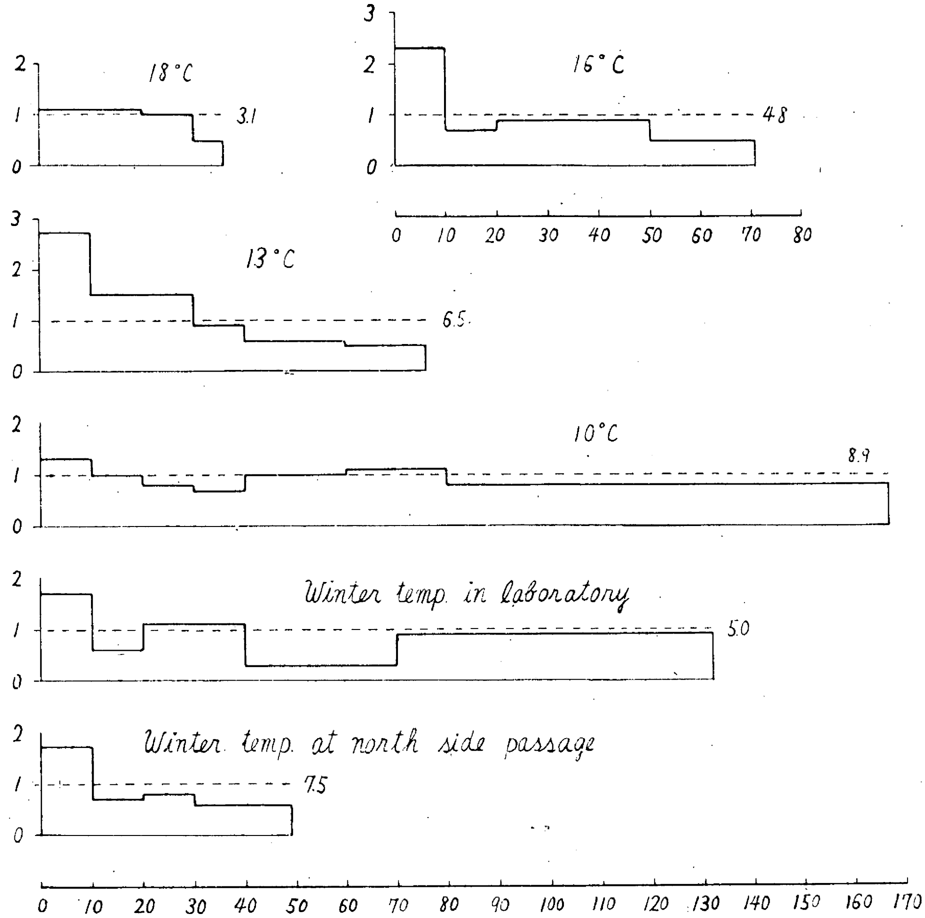


Table 3 Total number of infective larvae in five parts of the body of mosquitoes in each experiment

Experiment (or Lot) No.	Temp. under which mosq. were kept	Period of examination		No. of mosq. dissected	Number of infective larvae							Expected No. Mf Per ♀
		Beginning	Days		Legs	Abd.	Thor.	Head	Prob.	Total	Per ♀	
87.1,2,3	33°C	Nov., 18.'55	27	90	52	91	154	58	50	405	4.5	10.7
90.1,2	30°C	Nov., 19.'55	35	99	41	138	191	88	65	523	5.3	7.3
71	27°C	Dec., 11.'53	33	24	11	43	49	20	11	134	5.6	40.8
94.3,4	25°C	Nov., 20.'55	72	110	94	142	204	151	154	745	6.8	10.3
72	24°C	Dec., 11.'53	38	30	10	46	47	15	12	130	4.3	30.6
91.2,3,4	22°C	Nov., 19.'55	79	104	81	214	340	130	144	909	8.7	19.7
72.5	19°C	Dec., 14.'53	71	28	8	32	53	21	20	134	4.8	30.6
72.4	18°C	Dec., 14.'53	36	36	10	35	43	15	10	113	3.1	30.6
72.3	16°C	Dec., 12.'53	71	32	17	37	63	16	22	155	4.8	30.6
75.5,6	13°C	May, 15.'54	76	77	45	183	206	33	32	499	6.5	21.7
79.1,2	10°C	Nov., 22.'54	167	96	66	289	383	81	38	857	8.9	51.4
72.7	Winter, laboratory	Dec., 14.'53	132	37	15	74	63	19	15	186	5.0	30.6
72.6	Winter, passage	Dec., 14.'53	49	36	16	128	104	10	11	269	7.5	30.7
	Total			799	466	1452	1900	657	584	5059	6.3	

RESULTS OF EXPERIMENTS

The effect of parasitism of immature larvae

Table 1 shows that the number of immature larvae per naturally dead female is always not large when compared with that of filariae per female in group 2. The filariae in group 2 are, though self-evident it is, in advanced stage in development and composed of many mature larvae and some number of older II stage larvae. Therefore, the above shows that many females even when infected heavily with immature larvae usually can survive the incubation period of filariae to reach maturity.

While, two female mosquitoes, No. 12 in group 1 of Exp. 1 and No. 21 in group 1 of Exp. IV in Table 1 displayed abnormal behavior and were found on dissection infected heavily with immature larvae as mentioned above. The mosquitoes displaying such a behavior are usually fated to die within one or two days probably owing to the harmful effect of extremely heavy infection.

Thus, the influence due to the parasitism of immature larvae seems not necessarily harmful unless the infection is extremely heavy.

The migrating and penetrating habits of mature larvae

On reaching IIIa stage the filariae leave thoracic muscle of mosquito and enter into the body cavity i. e. thoracic or abdominal cavity or head and on reaching infective form one or two days thereafter some of them begin to penetrate into tissue interstices or various organs. They enter into proboscis; they do very often into femora and often tibiae and rarely tarsi; they thrust their cephalic end into the inner pits or basal segments of maxillary palpi very often, antennae in some cases and wing base in rare cases; they sometimes occupy compound eyes. They seem to move about freely from one place to another but those which enter into the second or third tarsal segments or thrust their cephalic end into the inner pits of appendages appear to die in length of time because some are found dead in such a state and others are found alive but incapable of coming back from the segments or pits.

Such migrating and penetrating actions of infective larvae seem to cause temporary or fatal functional disorders or abnormal behavior of their insect host.

The abnormal behavior of mosquitoes is easily detectable by the behaviors resting on the bottom of rearing cage, walking about or jumping suddenly at haphazard or being unable to fly even if they are incited. Mosquitoes showing such behaviors are observed more frequently on those days when filariae reached maturity and during some ten days thereafter, but occasionally on further after days and rarely on those days when filariae are still in immature stages. These mosquitoes are found on dissection, in many cases, infected heavily with infective larvae together with some number of older II stage larvae but in some cases, infected only several infective ones and in rare cases with many younger filariae. The number of mosquitoes becoming abnormal in behavior appears to be greater when they are allowed to feed on a patient harboring greater number of microfilariae.

It is noteworthy fact that the mosquitoes which display abnormal behavior usually die within one or two days but on occasion some are observed recovering from abnormality probably because of removal of infective larvae from an affected part. The recovery from abnormal states is also observed when such mosquitoes are exposed to low temperature. For example, 19 females in Exp. III of Table 1 which displayed abnormal behavior on the day of reaching maturity of filariae at 27°C and seemed to die within several days if continued to rear at that temperature, were subjected to 16°C where they could live for long period inspite of their being infected heavily with infective larvae. This is probably due to the becoming inactive in filariae at the low temperature.

Distribution of infective larvae in mosquito

Fig. 1 and 2 show that even though the infective larvae are fairly numerous in thorax and abdomen, they are distributed in head, proboscis and legs in not so small number and that the percentage number of them in each part of the body not necessarily vary with the advance in group number or with the lapse of time showing that they migrate or penetrate into not only proboscis but also into every parts of the body freely. The states of distribution are well demonstrated when reared at favorable temperature and especially on those days when filariae reached maturity and some ten days thereafter as shown in Fig. 3. It seems therefore that the entrance into the proboscis is merely due to the action controlled by the penetrating habit of infective larvae in the manner entirely similar to that used when they enter into tissue interstices or thrust their cephalic end into the pits of various appendages.

While, during subjection to low temperatures of 10°C (Fig. 4) and those at the north side passage of our laboratory in winter (Fig. 5), the infective larvae are more numerous in abdomen and thorax and the number of them seems to increase in abdomen with advance in group number and rather decrease in proboscis, head and legs. The above is probably due to the infective larvae becoming inactive or dormant and unable to migrate from the place where they were distributed on the day of exposure to the low temperatures.

The states of distribution are again well demonstrated in Fig. 6 in which the distribution of the total number of infective larvae in an experiment at a certain temperature is compared with each of those at a series of 13 temperature conditions. The infective larvae are found numerous in an average (shown in vertical line) in the order of thorax, abdomen, head, proboscis and legs. Strictly speaking, however, the percentage number of infective larvae in each part of the body appears to vary with the contact temperatures, increasing in percentage very faintly in thorax and remarkably in abdomen, while on the contrary, gradually decreasing in proboscis, head and legs with decrease in contact temperatures. This shows that the infective larvae become inactive and dormant and consequently unable to migrate from the place where they were distributed before the exposure to low temperatures, on the contrary, at favorable or higher temperatures they are moving about into every parts of the body freely.

Reduction in number of infective larvae in mosquitoes with the lapse of time

In Fig. 7 the rate of the mean number of infective larvae in a mosquito in each successive ten days, against the general mean of those in an experiment is illustrated.

The figure shows that the rate is the highest in the first ten days in every experiments and decrease in height with the lapse of time; the reduction in rate is rather steep at higher temperatures and is gentle at lower ones; and that mosquitoes can live, in general, longer at lower temperatures. The above means that the mosquitoes which are infected heavily with infective larvae die, as a rule, earlier than those lightly infected ones and it is remarkable at higher temperatures and is not so at lower ones.

Here, the trend will be seen more concretely in the result of an experiment marked with * (see also Exp. III in Table 1) in which 19 females which displayed abnormal behavior on the 12th day of rearing at 27°C and seemed to die within one or two days, were subjected to 16°C, and the remaining 30 females were transferred from 27°C to 24°C on the same day. Although the females exposed to 24°C harbored only 4.3 larvae per female, those exposed to 16°C did as much as 22.4. The reduction in number of infective larvae occurred in nearly the same way in both cases but the mosquitoes in the latter could live for much longer period in spite of the heavy infections.

From the above, we can say that the mosquitoes heavily infected with infective larvae die, as a rule, earlier than those lightly infected, in other words, the infective larvae have harmful effect on the life of the host animal. The effect is revealed distinctly at favorable or higher temperatures but is suppressed at lower ones.

SUMMARY

1) This is the fifth report of the theses and deals with the effect of parasitism of immature larvae on the host insect; the movement, distribution, and migrating and penetrating habits of infective larvae; and the effect of parasitism of infective larvae upon the life of the host.

2) The influence due to the parasitism of immature larvae seem not necessarily harmful unless the infection is extremely heavy.

3) On reaching IIIa stage the filariae leave thoracic muscle and enter into the body cavity of mosquitoes and on reaching infective form some of them begin to penetrate into tissue interstices or various organs and seem to move about place to place. These actions in infective larvae seem to cause temporary or fatal functional disorders or abnormal behavior of their insect host and, hence, the earlier death of heavily infected individuals. This is remarkable at favorable or higher temperatures, while, is not so at lower (than about 16°C) ones.

4) Although the infective larvae are fairly numerous in thorax and abdomen, they are distributed in not necessarily small number in head, proboscis and legs and their entrance into proboscis seems to be due merely to the action controlled by the penetrating habit in the manner entirely similar to that used when they enter into the tissue interstices or organs or thrust their cephalic end into inner pits of various appendages.

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