

## A MORPHOLOGICAL STUDY ON THE GROWTH OF THE MOSQUITO LARVAE

HIROYOSHI OKA

*Department of Clinical Pathology, Yamaguchi Medical School, Ube*

(Received December 10, 1954)

The larvae of mosquito which have hatched from eggs, undergo four moultings before they reach the stage of pupae and then grow into a winged imago. As is well known the life cycle of the mosquito is divided into the periods of the eggs, the larvae, the pupa, and the imago, and the larval period is further subdivided into four stages<sup>1)</sup>. Although egg, larva, pupa and imago are easily distinguishable on morphological characters, the larval periods are not so simply discriminated. Inasmuch as few attempts have hitherto been made to disclose the criteria of the growth stages of larvae, body length has been usually employed for their arbitrary determination<sup>2)</sup>. Needless to say, this is not entirely reliable, because the size of the larval body varies greatly depending on the environment (temperature, foods, etc)<sup>3,4)</sup>. Only one report<sup>5)</sup> has been published on this subject with *Anopheles hyrcanus* var. *sinensis* as material, in which its larvae were determined for their growth stages by the breadth of the head and the number of ramification of hair No. 6 on the third abdominal segment.

A study on the morphological changes during the larval development was therefore undertaken with respect to *Culex pipiens* var. *pallens*, *Culex tritaeniorhynchus* and *Anopheles hyrcanus* var. *sinensis*, in the hope that a practical method for the accurate determination of larval growth stages which could be applied for any species might be discovered. Studies of this kind were also supposed to furnish a basic knowledge required for the right evaluation of the theory of "the periodic and concurrent oviposition"<sup>6)</sup> which has recently been advanced in connection with the procreation of the mosquitoes and aroused general interest<sup>7-11)</sup>.

### MATERIALS AND METHODS

The larvae of *Culex pipiens* var. *pallens* Coquillett 1898, *Culex tritaeniorhynchus* Giles 1901, and *Anopheles hyrcanus* var. *sinensis* Wiedemann 1828, amounting 302, 300 and 240 in number, respectively, were collected in the fields at Ozuki-machi, Shimonoseki City, during the

period from April to October, 1953. They were killed in hot water, placed on a slide glass, treated with a drop of Gater's solution<sup>12)</sup> and covered with a cover glass to prepare permanent specimens. Clusters of eggs looking like those of *Culex pipiens* var. *pallens* were gathered from a water jar placed outdoors for fire protection in the author's house on five occasions during that period, they were kept in well-water in a vessel for incubation, and as soon as the larvae were hatched they were introduced separately, to glass cups containing 70 ml. of well-water with an ear-pickful of fish meal, one larva to each cup. The number of moulting was counted for every larva by daily observation to classify four groups of larvae according to their times of moultings and thus the larvae of the first to the fourth growth stages were obtained. They were made to permanent specimens with Gater's solution as described above. One hundred and forty-four specimens were prepared in this way. The remainder of the larvae which were not dealt with were fed until they developed into imagines to ascertain that the egg clusters really belonged to *Culex pipiens* var. *pallens* by the morphological study.

All the larvae collected in nature as well as fed in the laboratory were measured for the maximum breadth of the head diameter (Fig. 1),

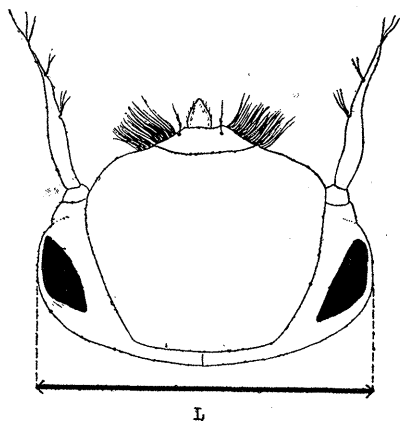


Fig. 1. Head diameter (L).

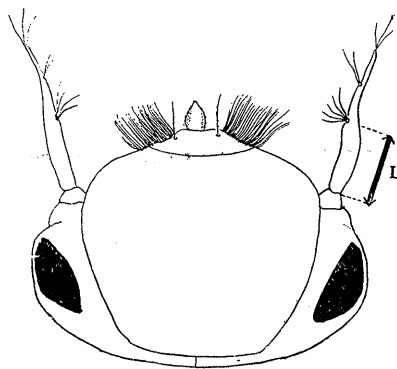


Fig. 2. Base-to-hair distance of antenna (L).

the distance between the base of the antenna and the antennal hair (Fig. 1) and the length of the siphon (Fig. 3) under a microscope (100x magnification). In the larvae of *Anopheles hyrcanus* var. *sinensis* the ramification of hair No. 6 on the third abdominal segment was counted on either the right or the left side.

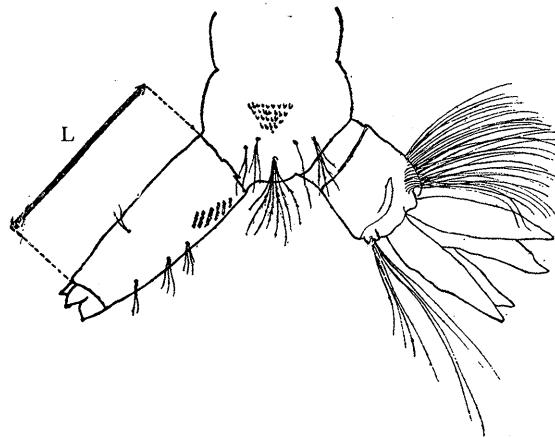


Fig. 3. Siphonal length (L).

RESULTS

The head diameter of the larvae of *Culex pipiens* var. *pallens* which were collected from the fields formed a distribution curve which had four peaks as shown in Figure 4, while those fed in the laboratory produced

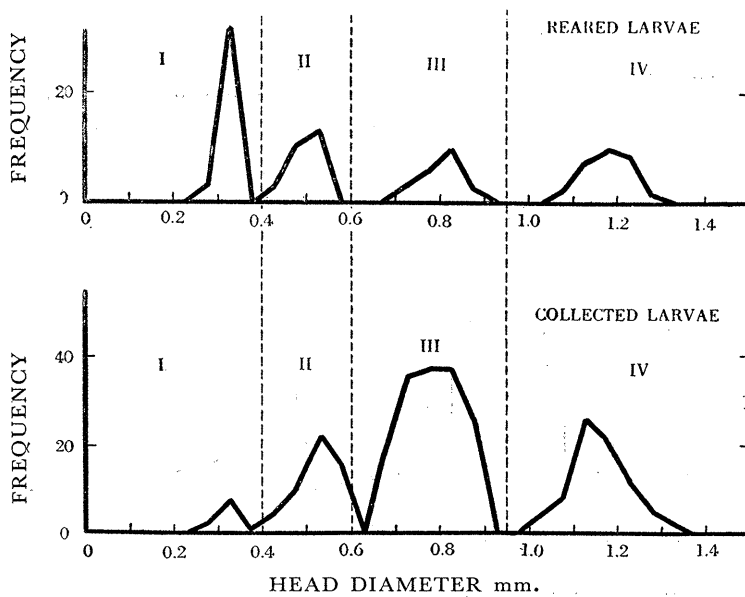


Fig. 4. Distribution of the larval head diameter. *Culex pipiens* var. *pallens*.

four groups which were readily separable from each other according to the difference in their growth stages. The diameters distributed around the mean values 0.32, 0.50, 0.80 and 1.18 mm. in the first, second, third and fourth instars, respectively, and the instars were divided clearly when three border lines which passed through 0.40, 0.60 and 0.95 mm. were assumed. The head diameters of the collected larvae were also allocated by these lines into four groups, the mean values of which coincided with 0.34, 0.52, 0.78 and 1.16 mm., respectively, without any appreciable overlapping. These are practically identical with the mean values of the head breadth of the larvae reared artificially. It is therefore justified to presume that the larvae of *Culex pipiens* var. *pallens*, both natural and reared, fall into the first, second, third or fourth instars according to the size of head diameters being less than 0.4 mm., 0.4 to 0.6 mm., 0.6 to 0.95 mm. or more than 0.95 mm.

The distance between the base of antenna to the antennal hair presented, in the reared larvae of this culicine mosquito, a distribution which was separated into four independent groups by the vertical lines that passed through 0.075, 0.16 and 0.27 mm., respectively, as clearly depicted in figure 5, and these also divided the collected larvae into

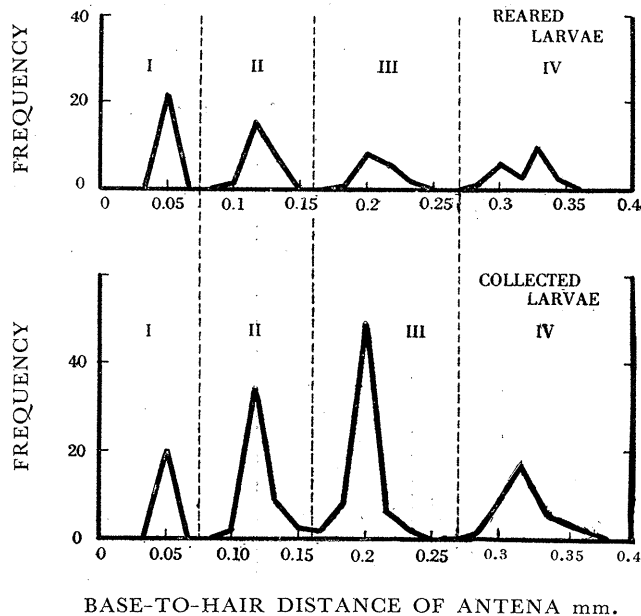


Fig. 5. Distribution of the base-to-hair distance of antenna. *Culex pipiens* var. *pallens*.

four groups which were apparently independent of each other. In addition the base-to-hair distances of the collected larvae were in good accordance with those of the reared as listed below.

	I	II	III	IV
Reared (Instar)	0.053	0.128	0.217	0.333 mm.
Collected (Group)	0.058	0.126	0.212	0.330 mm.

The groups in the collected larvae can therefore be regarded to be equivalent to the instars in the reared; and the larvae, both reared and collected, will be able to be determined for the first to fourth growth stages on the basis of the base-to-hair distance of antenna by means of the demarcations: short of 0.075 mm., 0.075 to 0.16 mm., 0.16 to 0.27 mm. and over 0.27 mm.

Figure 6 reveals the distribution curve of the length of siphon in *Culex pipiens* var. *pallens*. From this figure it is obvious that the

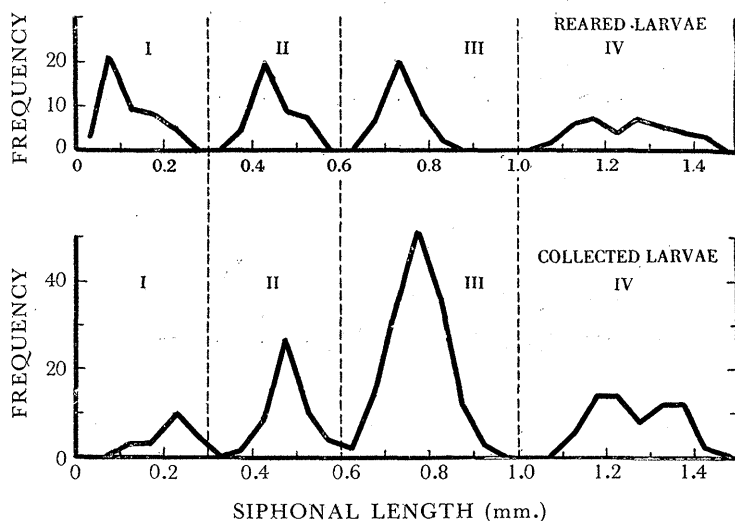


Fig. 6. Distribution of the siphonal length. *Culex pipiens* var. *pallens*

measurement of siphonal length is as useful for the determination of larval instars as are the head diameter and the base-to-hair distance of antenna, when the demarcation, less than 0.30 mm., 0.30 to 0.60 mm., 0.60 to 1.02 mm., and more than 1.02 mm., are established for the first, second, third and fourth growth stages, respectively.

These observations also hold good with *Culex tritaeniorhynchus*. The distributions of the head diameter, base-to-hair distance of antenna

and siphonal length, in the collected larvae of this species, are presented in Figure 7. The criteria of the first to fourth growth stages will be

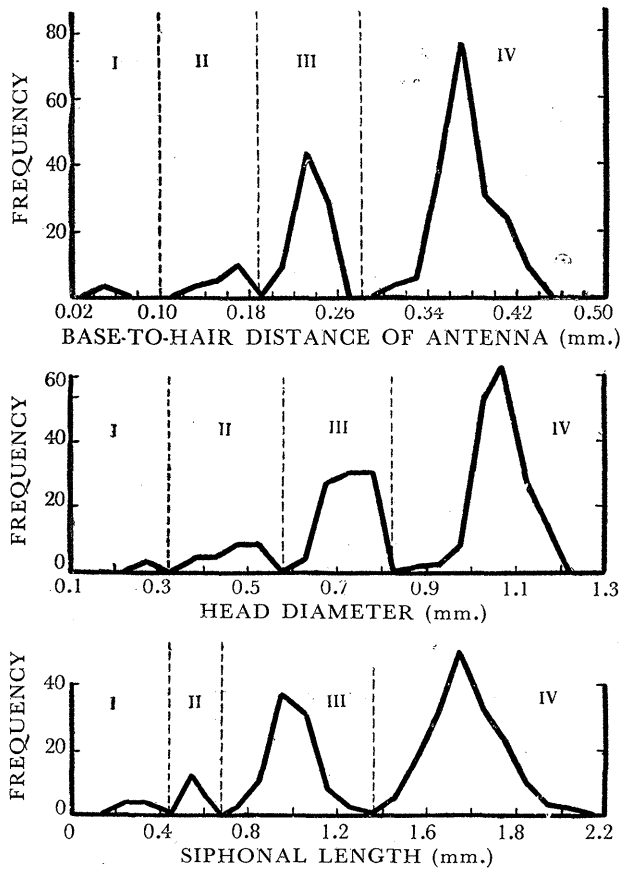


Fig. 7. Distributions of the base-to-hair distance of antenna, head diameter and siphonal length. *Culex tritaeniorhynchus*.

given from the scrutiny of this figure as follows.

Growth stage	First	Second	Third	Fourth
Head diameter	Short of 0.35	0.35-0.58	0.58-0.85	Over 0.85 mm.
Base-to-hair distance of antenna	Less than 0.10	0.10-0.19	0.19-0.28	more than 0.28 mm.
Siphonal length	Less than 0.45	0.45-0.65	0.65-1.35	more than 1.35 mm.

The larvae of *Anopheles hyrcanus* var. *sinensis* collected from the

field gave a head diameter distribution which were divided into four groups, I to IV from left to right in Figure 8, by the border lines

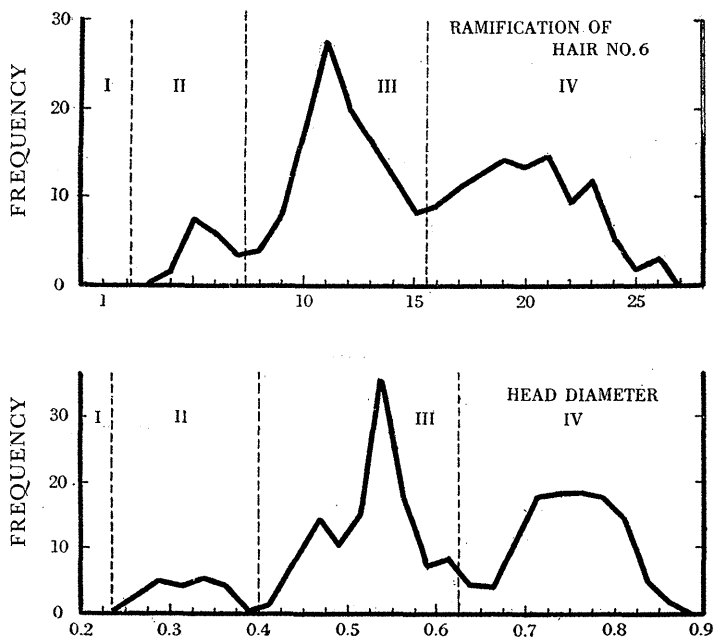


Fig 8. Distributions of the head diameter and the number of ramification of hair No. 6 on the third abdominal segment. *Anopheles hyrcanus* var. *sinensis*.

passing through 0.24, 0.40 and 0.62 mm., although group I was zero in number. They were also classified into four groups by the ramification of hair No. 6, when demarcations were instituted at the numbers between 2 and 3, 7 and 8, and 15 and 16, respectively. However, the larvae which belong to each group are practically the same in number, whichever classification is employed, as described below.

Group	Number of larvae in each group			
	I	II	III	IV
by head diameter	0	20	114	106
by ramification of hair No. 6	0	19	110	111

The above mentioned border lines afford, accordingly, the criteria for the determination of larval growth stages in *Anopheles hyrcanus* var. *sinensis*.

## DISCUSSION

As stated at the beginning of this paper, the larvae of mosquitoes make four moultings before they reach the pupal stage, and the larval period is divided into four stages according to the number of the past moultings. The morphological changes occurring during the larval growth have for a long time been little studied, and the attention has only been centered around the fourth stage larvae which are fully grown.

Although it has been known that the larvae of Genus *Culex* can be assorted for growth stages by the measurement of head diameter, it is of quite recent years that the criteria for the determination of larval instars has been established in detail by Shibata<sup>5)</sup>, who had been engaged in the study of the procreation of this species<sup>6-8)</sup>.

The species selected for the author's study are among the commonest mosquitoes which are found in this country and they are supposed to be useful as materials for disclosing the general principle of the morphological criteria for the determination of larval instars which are applicable to other species as well.

The larval head diameter of *Culex pipiens* var. *pallens* averages 0.34, 0.52, 0.78 and 1.16 mm. in the first, second, third and fourth instars, respectively, increasing in size with the advance of growth stage in geometrical progression at a common ratio of 1.5 (1.54 for the reared and 1.54 for the collected larvae). Larval heads enlarge therefore 1.5 times in diameter on every ecdysis. This attitude of the larval head growth was already described with respect to *A. hyrcanus* var. *sinensis* and Genus *Lepidoptera*, and it has been known as Dyar's law<sup>13)</sup>.

In *Culex tritaeniorhynchus* values of 0.28, 0.47, 0.71 and 1.06 mm. are computed for the mean head diameters of the first to the fourth instars, respectively, from the data depicted in Figure 7. These are also in a geometrical progression whose common ratio is nearly equal to 1.5. The same holds good for *Anopheles hyrcanus* var. *sinensis*, in which larval head diameters average 0.33, 0.51 and 0.81 mm. in the second, third and fourth instars, respectively, increasing progressively at the common ratio of about 1.6.

The base-to-hair distance of antenna and the siphonal length grow in a way somewhat different from what has been described. Table I reveals that the base-to-hair distance increases, in *Culex pipiens* var. *pallens*, at a rate dropping gradually with the advance in instars, namely it undergoes 2.17 to 2.40 times elongation on the first larval moulting, whereas as little as 1.68 to 1.70 and 1.55 to 1.56 fold enlargements are attained on the second and the third moultings, respectively. The same



TABLE I.  
Head diameter, base-to-hair distance of antenna, siphonal length and  
body size of mosquito larvae (mean values)

Instar	First	Second	Third	Fourth
1. Head diameter (mm.)				
<i>Culex pipiens</i> var. <i>pallens</i> reared in laboratory	0.32	0.50	0.80	1.18
collected from fields	0.34	0.52	0.78	1.16
<i>Culex tritaeniorhynchus</i> reared in laboratory	0.28	0.47	0.71	1.06
collected from fields		0.33	0.51	0.81
2. Base-to-hair distance of antenna (mm.)				
<i>Culex pipiens</i> var. <i>pallens</i> reared in laboratory	0.053	0.128	0.217	0.333
collected from fields	0.058	0.126	0.212	0.331
<i>Culex tritaeniorhynchus</i> collected from fields	0.047	0.149	0.244	0.365
3. Siphonal length (mm.)				
<i>Culex pipiens</i> var. <i>pallens</i> reared in laboratory	0.11	0.45	0.72	1.25
collected from fields	0.21	0.49	0.78	1.26
<i>Culex tritaeniorhynchus</i> collected from fields	0.33	0.57	0.96	1.80
4. Body length (mm.)				
<i>Culex pipiens</i> var. <i>pallens</i> collected from fields	1.32 (0.98-2.0)	2.54 (1.8-3.5)	4.12 (2.5-6.5)	6.25 (3.0-8.5)
<i>Anopheles hyrcanus</i> var. <i>sinensis</i> collected from fields	1.01 (0.87-2.0)	2.37 (1.8-3.0)	4.15 (2.5-5.0)	6.02 (3.5-8.0)

All the figures in this table indicate the mean values except for those in the bracket which represent the range of variation.

is nearly true with the growth of the siphonal length, which exhibits 2.2 to 4.1, 1.58 to 1.60 and 1.60 to 1.70 times elongation on the first, second and third moultings. The larvae of *Culex tritaeniorhynchus* undergo also a growth which is quite similar to that of *Culex pipiens* var. *pallens*. The average increase rate in the length of the larval body is given below for the sake of comparison.

	1st→2nd stage	2nd→3rd stage	3rd→4th stage
<i>Culex pipiens</i> var. <i>pallens</i>	1.92	1.65	1.52
<i>Anopheles hyrcanus</i> var. <i>sinensis</i>	2.3	1.75	1.45

These figures show us that the gradual decline in the rate of increase which was found for the base-to-hair distance of antenna and the length of the siphon is again encountered in the growth of the body size,

probably because the larval body, except for the head, may enlarge at a speed which drops with the advance in instar.

In any event, the body length is not helpful for the determination of larval growth stages, since, as was pointed out by Shibata<sup>4)</sup>, it distributes in a curve which has such a distinct overlapping between the adjacent instars that the peaks can hardly be discriminated with accuracy.

Inasmuch as the larvae of Genus *Culex* have no or few feathered or branched bristles on the body, the ramification of bristles fails to give a criterion for the determination of growth stages. This constitutes a character of *Culex* which is quite different from Genus *Anopheles*. The number of ramification of hair No. 6 on the third abdominal segment is therefore not available, whereas this provides an excellent criterion in *Anopheles hyrcanus* var. *sinensis* as has been stated above.

It is concluded therefore that the head diameter gives a universal criterion for the determination of growth stages irrespective of the genera of mosquitoes, while the base-to-hair distance of antenna and siphonal length are useful in Genus *Culex*, and the number of the ramification of hair No. 6 on the third abdominal segment is helpful as a criterion of the instars only in Genus *Anopheles*. The fact that these criteria exhibit little discrepancy in the mean values for each instars between the collected and the reared larvae provides an assurance for their reliability, since it is supposed that they grow with the progression of instars quite independently of the environmental condition which exerts a considerable effect upon the size of larval body.

#### SUMMARY AND CONCLUSION

Morphological study was attempted on the growth of the mosquito larvae with *Culex pipiens* var. *pallens*, *Culex tritaeniorhynchus* and *Anopheles hyrcanus* var. *sinensis* as material, and the following conclusions were derived.

1) The morphological characters which are useful as the criteria for the determination of larval instars (growth stages) are the head diameter, the base-to-hair distance of antenna, the siphonal length and the ramification of hair No. 6 on the third abdominal segment. Head diameter gives universal criterion. Base-to-hair distance of antenna and siphonal length are available for Genus *Culex*, whereas ramification of hair No. 6 is limited for Genus *Anopheles*.

2) The diameter of larval head grows in geometrical progression with the advance in the instars, but quite independently of the environment. The instars are therefore readily determined, if numerical data

have been collected for each instar beforehand.

3) The base-to-hair distance of antenna and the siphonal length elongate like the size of the body at a rate which declines gradually with the advance in instars. They seem to be liable to the influence of environment, as for instance, when foods are so poor in amount that the larvae cannot grow well in size. However, in the author's experience, they used to afford useful criteria, when demarcation for each instar had been correctly established beforehand with respect to the larvae collected from the field.

*Grateful acknowledgement is made to Prof. Shigeki Mori for his kind advice in preparing the manuscript as well as to Prof. Susumu Shibata for his kind direction during the course of the study.*

#### REFERENCES

- 1) CHRISTOPHERS S. R.: *Fauna of British India*. Diptera vol. 4 Anophelini, Taylor and Francis (London), 1933
- 2) KOMIYA Y., AND OHUCHI, M.: On the larvicidal effect of paris green upon the anopheline larvae. *Shanghai Shizenkagaku-Kenkyusho Iho*, **12**: 98-126, 1942
- 3) HOSOI, T.: Relationships between size, weight and the number of ovarian follicles in *Culex pipiens pallens* Coquillet bred under various larval conditions. *Eiseidobutsu (Jap. J. Sanit. Zool.)* **3**: 118-119, 1952
- 4) SHIBATA, S.: Is it possible to determine the growth stages of anopheline larvae by the measurement of their size? *Igaku to Seibutsugaku (Medicine and Biology)*, **10**: 166-169, 1947
- 5) SHIBATA, S.: Criteria of the growth stages of anopheline larvae. A morphological study on *Anopheles hyrcanus* var. *sinensis* *Bull. Yamaguchi Med. School*, **1**: 37-48, 1953
- 6) SHIBATA, S.: On the periodical and concurrent oviposition of mosquitoes. and a way to presume its time in nature. *Igaku to Seibutsugaku*, **13**: 145-148 1948
- 7) SHIBATA, S.: On the life span of *Anopheles hyrcanus* var. *sinensis* in nature. *Igaku to Seibutsugaku*, **13**: 263-267, 1948
- 8) SHIBATA S.: On a certain overlooked aspect of the mosquito breeding. *Seiri Seitai*, **2**: 23-33, 1948
- 9) MIZUKAWA K.: On the periodical and concurrent oviposition of *Anopheles hyrcanus* var. *sinensis* (Observation in Kurashiki). *Igaku to Seibutsugaku*, **15**: 31-34, 1949
- 10) KITAOKA M. MIURA, T., AND OGATA, T.: Periodicity of the emergence of *Culex pipiens* var. *pallens* assumed by the observation in Tōkyō in 1949. *Igaku to Seibutsugaku*, **17**: 68-71 1950
- 11) NOMURA K., AND FUTATSUCI, H.: Some comments on Shibata's theory regarding to the periodical occurrence of mosquitoes. *Eiseidobutsu*, **4** (Special number: Jubilee Publication in the Commemoration of Dr. Harujiro Kobayashi's Seventieth Birthday): 309-315, 1954
- 12) SASA, M., AND ASANUMA, Y.: *How to study mosquitoes (Ka o shiraberu hito no tameni)*. Tokyo Shuppan kabushikikaisha (Tokyo), 1949 (pp. 96-98, 189-190)
- 13) IMMS A. D.: *A general textbook of entomology including the anatomy*, Methuen (London), 1936