

**STUDIES ON THE DIFFERENT TYPES OF ANOPHELES HYRCANUS  
SINENSIS WITHIN THE ANOPHELES HYRCANUS GROUP. II.  
COMPARATIVE MORPHOLOGICAL STUDY OF  
THE PUPAE**

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In this paper the result of a comparative morphological study on the pupae of the different types of Anopheles (A.) hyrcanus sinensis in China is reported. According to a previous paper of the author there are 4 different types in this group on the basis of characteristics of the eggs. But the morphological study of the pupae included only 3 types because of lack of pupal material of the large narrow-decked egg type. The characters of the 3 types of pupae are easily distinguishable; some of the characters are given in the following table.

The distinguishing characters of the pupae of 3 types of Anopheles  
hyrcanus sinensis

Type	The small narrow-decked egg type	The large wide-decked egg type	The small wide-decked egg type
Pupa size	small	larger	largest
Body color	light	dark	—
Wing case	small, spots along veins indistinct	larger, spots along veins distinct	largest, spots along veins usually distinct
Trumpets	dark especially the basal part, edge with large spines, meatal cleft narrow and deep	lighter throughout, edge with small spines, meatal cleft narrow but shallow	dark, spines intermediate, meatal cleft shallow and wide
♂ Genital pouch ventral lobe	distal 1/2 distinctly darker	uniform color	similar to that of large wide decked type

A comparison of the pupae of our various types with those reported from Japan and Malaya was made and it was found that the number of branches of hairs of abdominal segments of our specimens is less than those of the above two countries.

The presence of different types of pupae within the Anopheles hyrcanus group has been reported from Malaya (Crawford, 1938; Reid, 1953), Japan (Otsura and Ohmori, 1960; Ohmori, 1959; Mizukawa, 1951; Kohita, 1951), and the Philippines (Baisas, 1936). The author studied the morphology of the eggs of the Chinese anopheles, Anopheles

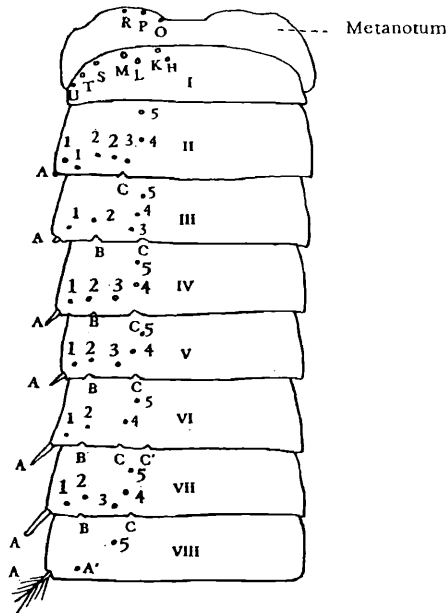


Fig. 1. Position of setae on the metanotum and tergites of the abdomen of an Anopheles pupa.

hyrcanus sinensis, and reported 4 different egg types: The small narrow-decked egg type, the large narrow-decked egg type, the large wide-decked egg type, and the small wide-decked egg type (Ma, 1963). The author attempted to determine if the pupae that were reared from the eggs of the various egg types were also different. The results of this study are reported in this paper.

## I. MATERIALS AND METHODS

Larvae hatching from eggs of the various egg types were reared to adults, and specimens of pupae and pupal exuviae were collected and preserved in 70% alcohol. The pupae were mounted on slides and examined under the microscope.

Three pupal types were examined: One came from small narrow-decked eggs, one from large wide-decked eggs, and the third from small wide-decked eggs. Because of the small number of pupae reared from large narrow-decked eggs, such pupae were not included in the present study. Pupae from small wide-decked eggs were taken from Moo-tan-kiang; pupae from small narrow-decked eggs from Hangchow, and pupae from large wide-decked eggs from Hangchow and Nanking.

The general forms of pupae were first described, and the numbers of branches of the setae on the dorsal plates of the metathorax and abdomen were analyzed for possible use in separating the pupae into types. The system of naming the setae used in this work is that of Christophers (1933): Setae on metanotum: O, P, R; setae on I (the first abdominal segment): H, K, L, M, S, T, U; setae on II: A, C, 1, 1, 2, 2, 3, 4, 5; setae on III, IV, V and VII: A, B, C, 1, 2, 3, 4, 5; setae on VI: A, B, C, C', 1, 2, 4, 5; setae on VIII: A, A', 5. Paddle hair, accessory paddle hair, and paddle index were also used in describing the pupae (Fig. 1).

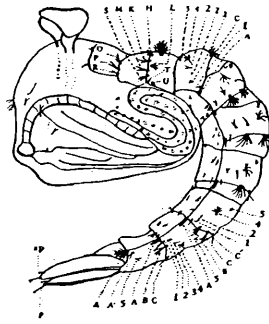


Fig. 2. Lateral view of the Chinese anopheles pupa.

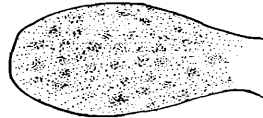


Fig. 3. Distribution of spots on the wing case of pupa of the small narrow-decked egg type.

## II. RESULTS

### (1) General Morphology

Some of the characters used by Crawford (1938) in describing Anopheles pupae are used in this paper.

A comma-shaped pupa is divided into cephalothorax and abdomen or tail. The cephalothorax contains the head and the thorax and their appendages of the future adult; all appendages, such as mouth parts, antennae, pulpi, legs, and wings, are covered with sheaths. The eyes of the pupa and the compound eyes of the future adult are located on the anterior lateral parts of the cephalothorax; on the dorsal part there is a pair of trumpets, which are narrow at the base and broad at the distal end. The general shape of the trumpets, the sizes of teeth and setae along the edges, and the meatal cleft are important characters in classifying the pupae. The appendages of the head and thorax, as well as the first eight abdominal segments of the adult can be clearly seen through the pupal skin. The genital pouch of the pupa contains the external genitalia of the adult; it is attached to the base of the 8th abdominal segment of the pupa and extends beyond the 9th segment. The pouch is larger in the male than in the female. On each side of the pouch there is a pear-shaped paddle; these two paddles overlap, and their lateral margins are thick and somewhat transparent, and bear teeth and setae. There is a seta on each of the dorsal and ventral surfaces near the distal end of a paddle, and a longitudinal midline or rib on each paddle. There are setae of various shapes on both dorsal and ventral sides of the abdominal segments; the shapes of the setae are important characters in classifying the pupae.

### (2) Characteristics of Pupal Types

1. Pupae of the small narrow-decked egg type. Based on 13 specimens obtained from Hangchow, the pupae of the small narrow-decked egg type are described as follows.

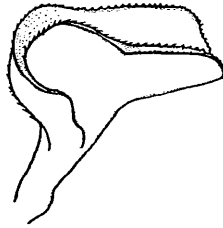


Fig. 4. Trumpet and meatal cleft of pupa of the small narrow-decked egg type.

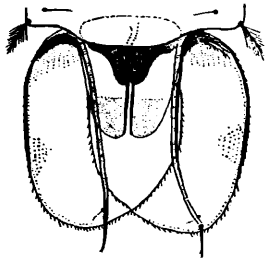


Fig. 5. Ventral lobe of the male genital pouch and paddles of pupa of the small narrow-decked egg type.

Body small, lightly colored; wing case small, with indistinct spots along longitudinal veins and arranged in horizontal lines (Fig. 3). Sheaths covering antennae, palpi, and legs with dark bands. Each side of the posterior margin of the mesonotum with a triangular-shaped dark spot. Metanotum with many dark spots; the lateral portions with dark longitudinal lines. Trumpets dark, especially in the basal parts; edges of trumpets with large spines; meatal cleft narrow and deep (Fig. 4). Distal half of the ventral lobe of male genital pouch darker than the basal half (Fig. 5). Paddles with length to width ratios of 1.51-1.09, averaging 1.30. On cephalothorax and abdomen, bases of setae surrounded with dark spots; tergites of III and IV with dark, elliptical spots. The above characteristics can be used to differentiate the pupae of the small narrow-decked egg type from the pupae of the other two egg types described here.

The branching of setae in this pupal type also differs from that of pupae of the other egg types, including setae 4 and 5 on II, seta B on III-VII, seta C on VI, setae A and 4 on VII, setae A and 5 on VIII, and the setae on the paddles.

There are also differences in the branching of setae in the male and female pupae. Seta B on III: 9-20 branches in the male (average: 12.6, commonly 12), and 12-30 branches in the female (average: 18.0, commonly 13-15); seta C: 6-21 branches in the male (average: 12.6, commonly 12), and 10-18 branches in the female (average: 14.4, commonly 12-18). Seta B on V: 8-16 branches in the male (average: 11.5, commonly 11-12), and 8-22 branches in the female (average: 14.0, commonly 14-15). Seta 4 on VII: 3-5 branches in the male (average: 4.1, commonly 5), and 3-5 in the female (average: 3.4, commonly 3).

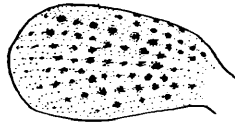


Fig. 6. Distribution of spots on the wing case of pupa of the large wide-decked egg type.



Fig. 7. Trumpet and meatal cleft of pupa of the large wide-decked egg type.

2. Pupae of the large wide-decked egg type. Specimens which were obtained from Hangchow and Nanking are larger and darker than the pupae of the above type. Also, the wings are larger than in the other form; there are distinct dark spots along the longitudinal veins on the wing cases (Fig. 7). Sheaths covering antennae, palpi and legs with dark bands. Trumpets lightly colored, with short setae along the edges; meatal cleft narrow and shallow (Fig. 6). Ratios of length to width of paddles: 1.51-1.10, averaging 1.31. Ventral lobe of the male genital pouch of uniform color (Fig. 8).

The setae on the various parts of pupae of this type differ distinctly from those of the other two pupal types, especially setae 4 and 5 on II, seta B on III and VII, seta C on VI, setae A and 4 on VII, setae A and 5 on VIII, and the paddle hairs.

There are differences in the branching of the various setae between the two sexes: Seta H on I: 2-5 branches in the female (average: 3.1, commonly 3), and 1-4 branches in the male (average: 2.2, commonly 2). Seta K on I: 3-5 branches in the female (average: 4.1, commonly 4), and 1-4 branches in the male (average: 2.5, commonly 3). Seta 4 on II: 5-9 branches in the female (average: 6.4, commonly 6), and 4-8 branches in the male (average: 5.2, commonly 4). Seta B on III: 7-15 branches in the female (average: 11.0, commonly 10-13), and 5-13 branches in the male (average: 9.8, commonly 10). Seta B on IV: 9-18 branches in the female (average: 12.5, commonly 10), and 5-8 branches in the male (average: 9.5, commonly 7). Seta B on V: 5-14 branches in the female (average: 8.6, commonly 6-10), and 3-11 branches in the male (average: 5.4, commonly 4-5). Seta B on VI: 3-10 branches in the female (average: 6.2, commonly 6) and 3-6 branches in the male (average: 3.9, commonly 4). Paddle hair: 2-3 branches in the female (average: 2.3, commonly 2), and 1-2 branches in the male (average: 1.3, commonly 1).

3. Pupae of the small wide-decked egg type. Two male pupae and 5 female pupae collected at Moo-tan-kiang were examined. Body and wings larger than those of the other 2 types; spots on wing case usually distinct, but not as distinct as those on pupae of the large wide-decked egg type; these two types cannot be easily differentiated by this character. Sheaths covering antennae and legs with bands. Trumpets large and dark, spines along the edges intermediate in size; meatal cleft shallow and wide (Fig. 9). Ratios of length and width of paddles: 1.16-1.56, averaging 1.36. Setae on paddles heavy and long,

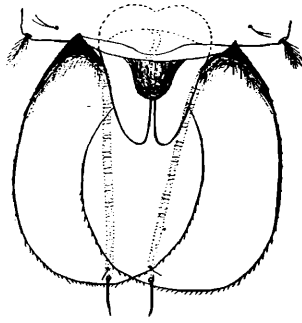


Fig. 8. Ventral lobe of the male genital pouch and paddles of pupa of the large wide-decked egg type.

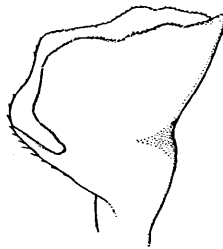


Fig. 9. Trumpet and meatal cleft of pupa of the small wide-decked egg type.

midline or rib thick and prominent. Ventral lobe of male genital pouch similar to that of the large wide-decked egg type pupae.

The branching of setae in this type differs from that in the other two types. Seta C on II of the male with 7-10 branches, averaging 8.5, and having 2 more branches than that in the other two types; seta 4: 5-7 branches, averaging 6; seta 5: 2-3 branches, averaging 2.5. Seta B on III: 15 branches, having more branches than in the large wide-decked egg type; seta C: 12-28 branches, averaging 20; this average is greater than the averages for the other two types. Seta B on IV: 13-19 branches, averaging 16; this average is also greater than the averages for the other two types. Seta B on V: 5-12 branches, averaging 8.5; this average is greater than that for the large wide-decked egg type, but smaller than that for the small narrow-decked egg type; seta C: 4-5 branches, averaging 6; this figure falls between the averages for the other two egg types. Seta B on VI: 4-6 branches, averaging 5. Seta A on VII: 77.4-86.0  $\mu$  long, averaging 81.2  $\mu$ . This seta is significantly longer than the averages for the other two types.

The few specimens examined showed some sexual differences. Seta 2 on II: 3-4 branches in the male, averaging 3.5, and 1-3 branches in the female, averaging 2; seta 3: 1-2 branches in the male, averaging 1.5, and 1-7 branches in the female, averaging 2.6. Seta C on III: 12-28 branches in the male, averaging 20, and 10-17 branches in the female, averaging 13; seta 3: 4-5 branches in the male, averaging 4.5, and 3-4 branches in the female, averaging 3.4. Seta B on IV: 13-19 branches in the male, averaging 16,

Table 1

Distinguishing characters of the pupae of 3 types of *Anopheles hyrcanus sinensis*

Type	Small narrow-decked egg type	Large wide-decked egg type	Small wide-decked egg type
Body size	Small	Larger	Largest
Body color	Light	Dark	—
Wing case	Small; spots along veins indistinct, may be arranged in indistinct lines	Larger; spots along veins distinct	Largest; spots along veins usually distinct; not easily distinguishable from the large wide-decked egg type
Trumpets	Dark, especially the basal part, edge with large spines; meatal cleft narrow and deep	Light throughout; edge with small spines; meatal cleft narrow and shallow	Dark and large; edge with intermediate spines; meatal cleft shallow and wide
Ventral lobe of male genital pouch	Distal half distinctly darker	Uniform color	Uniform color
Male pupae: abdominal tergites:	Branches:	Branches:	Branches:
II: seta 4	9	4	5-7
seta 5	4	2	2-3
III: seta B	12	10	15
IV: seta B	14	7	13-19
V: seta B	11-12	4-5	5-12
VI: seta B	11	4	4-6
seta C	3-5	2	2
VII: seta A	51.6 $\mu$	60.2 $\mu$	81.2 $\mu$
seta B	3	2	2
VIII: seta A	15-18	16	11
seta 5	2	1	1-2
Paddle hair	2	1	1

and 7-17 branches in the female, averaging 12.2; seta C: 7 branches in the male, 9-15 in the female (average: 11.4); seta 4: 4-5 branches in the male, averaging 4.5, and 4-14 branches in the female, averaging 6. Seta B on V: 5-12 branches in the male, averaging 8.5, and 8-14 branches in the female, averaging 11; seta C: 4-8 branches in the male, averaging 6, and 4-10 branches in the female, averaging 7.8. Seta B on VI: 4-6 branches in the male, averaging 5, and 3-10 branches in the female, averaging 7.5. Seta A on VII: 77.4-86.0  $\mu$  in length in the male, averaging 81.2  $\mu$ , and 60.2-86.0  $\mu$  in length in the female, averaging 75.2  $\mu$ . Seta A on VIII: 11 branches in the male, and 14-18 branches in the female, averaging 16. Paddle hair: without branching in the male, 1-4 branches in the female, averaging 2.2.

The characteristics of the three pupal types are summarized in Table 1.

### III. DISCUSSION

The results of this study show that pupae of the small narrow-decked egg type differ significantly from the pupae of the other two egg types; there are smaller differences between pupae of the large wide-decked and the pupae of the small wide-decked egg types. Therefore, I believe that the two wide-decked egg types are closely related, and that both are more distantly related to the narrow-decked egg type.

Pupal characters have been used to classify mosquitoes to species or subspecies (Reid, 1953; Reid and Knight, 1961; Otsuru and Ohmori, 1960; Crawford, 1938). Reid and Knight (1961) revised Edwards' classification of the subgenus Anopheles. According to the shapes of trumpets in the pupae and other characters, they divided this subgenus into two sections: Laticorn and Angusticorn. Each section was subdivided into three series: Laticorn section: Christya, Arribalzagia and Myzorhynchus; Angusticorn: Cycloleppter, Lophoscelomyia and Anopheles. The larger series were further divided into species groups and species. They placed the Anopheles hyrcanus group under the Laticorn section, series Myzorhynchus. The author agrees with their classification, which not only reveals the phylogenetic relationships, but also aids in the identification of the mosquitoes.

Otsuru and Ohmori (1960) and Reid (1953) described Anopheles hyrcanus sinensis and A. h. lesteri from Japan and Malaya. Comparison of pupae described from these localities with the pupae of sinensis from China showed that there was less branching of setae on the pupae from China than of those on the pupae from Japan and Malaya. This difference may be geographical variation.

Macfie (1919) asserted that there was no sexual difference in pupal chaetotaxy. However, Ohmori (1959) showed that sexual differences of setae A, B, C and 5 on the abdominal segments occurred in 6 Anopheles species. The present author found that certain setae not only differed between sexes, but also varied among the various pupal types.

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