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THE DEVELOPMENT OF ANOPHELES PUNCTIPENNIS SAY.¹

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Anopheles punctipennis Say, is a strictly American form of the so-called malaria mosquito. *A. punctipennis* and *A. maculipennis*, as well as *A. crucians*, have been constantly associated as malaria carriers, especially since Dr. Duprée's discovery of the parasites of malaria in the salivary glands of all three. But in the case of *A. punctipennis* there is growing up a reasonable doubt as to whether in the north it is really a malaria carrier, or at least whether the malaria carried by it is not a different form from that conveyed by *A. maculipennis*. In 1903 Dr. J. B. Smith of New Jersey stated that in that state only *A. maculipennis* had been actually demonstrated to be a malaria carrier; and so far as known this has not been proved to be otherwise. In 1903, also, Hirshberg of Johns Hopkins published the account of his noteworthy inoculations of fifty-eight females of *A. punctipennis*. He allowed them to bite patients afflicted with estivo-autumnal malaria, without finding the parasites in the walls of the stomach, or intestine, in the body cavity, or in the salivary glands. In the opinion of the experimenter himself, however, the fact that out of forty-eight similar inoculations of *A. maculipennis* only eight were infected detracts from the certainty of the results with *A. punctipennis*.

Breeding Places. Larvæ, pupæ, and eggs have been taken from seven different pools near Ithaca, at Forest Home, from October to the middle of August. The people living close by do not have malaria, and on the evidence of reliable citizens, have not had it

¹ This study was carried on in the entomological laboratories of Cornell University under the kindly supervision of Dr. J. G. Needham, to whom I am greatly indebted for constant advice and help.

for at least fifteen years. Whereas, down on the flats at the head of Cayuga Lake, and where *A. maculipennis* is common, there is more or less malaria all the time. Forest Home does not produce *A. maculipennis* at all, while *A. punctipennis* is there in comparatively great numbers. This is not unlike the situation at Baltimore where in 1902 Hirshberg and Dohme reported that "*A. punctipennis* breeds in the higher sections, while *A. maculipennis* is to be found in lower localities."

Local Occurrence. On October 21 a great many full-grown larvæ, also many pupæ, were found in pool 1, which is a drinking place for cattle, fed by a spring, and never dry. It was covered with Lemna, although not densely. A few masses of Spirogyra and Cladophora were there. Larvæ of may-flies, dragon-flies and damsel-flies, various beetles, mostly Dytiscidæ and Hyrophilidæ, great quantities of small crustacea and spring-tails, a great many chironomids of various species, and with some oligochaete worms, a few hydrachnids, and hydras—all these and some other forms, including Culex, were present in the pool. Water-striders and whirligig beetles were very few. The Lemna certainly furnished some food to the larvæ of *A. punctipennis*, for they were often seen to be brushing the leaves, after being brought into the laboratory. The larvæ were found in plentiful numbers, but always in separate groups, as if each had developed from a different laying of eggs.

Pool 2 was of an entirely different character, and about a quarter of a mile away. It was very small, not more than four feet in width at the widest place, and about a foot deep. The bottom was covered with dead leaves, and the water was clear, and there were no visible algæ or other plants in it. It was deeply shaded, and contained just one brood of larvæ, of less than fifty specimens, all of about the same size, and nearly ready to change to the pupa. They were almost black with a white mid-dorsal line. They were the largest larvæ that we have found. None found in the spring were so well developed as these October forms, from which came very large adults.

All the adults developing in autumn are exceedingly hardy. One female emerged from the pupa on November 3, and was left with no food or water. On December 2 it was still alive and eagerly drank water and fed on moistened dates. It lived for three weeks more with access to this food, never having tasted blood, having

spent its whole life in the cage. In the spring and summer they die within three days, under the same treatment of water and food.

A larva of *A. punctipennis* was frozen solid on December 3, was thawed out on December 4, and in an hour was apparently normal.

The full-grown larvæ found in the autumn differed more from one another than those found in the spring and summer, when they are mostly green or brown. In autumn there were many more of the striped and speckled forms. They all developed into *Anopheles punctipennis*, with some differences in size.

Pool 2 did not yield larvæ of *A. punctipennis* in the spring, having dried up completely, and pool 1 was much later in developing them than 3 which was near by—within a hundred feet.

Pool 3 on May 24 yielded larvæ of all sizes. Most of them, however, were ready to pupate. They represented the first laying of the spring. The water of this pool was several feet deep at one end, and clear and cool. No larvæ have been found at this spot, in the shade of an overhanging bank where *Spirogyra* grows deep, giving its clear green hue from below the surface. A few feet away, in shallow water with *Spirogyra* protruding from the surface, were plenty of larvæ. With the *Spirogyra*, and also in fruiting condition, was plenty of *Zygnema*. In and above these mats of fruiting algæ, many of them feeding down among the filaments, were larvæ of *A. punctipennis*, mostly full grown. And with them in great numbers were several species of Chironomid larvæ, weaving their houses of the delicate threads, and feeding there as well. This pool was visited at intervals, until on June 10 there were scarcely any larvæ, but a few pupæ and plenty of empty pupa cases. On June 17, however, great numbers of very small ones from 1 to 2 mm. and less were to be found again in pools 3 and 1. In 1 they were found only among the *Lemna*, and in 3 only in mats of *Spirogyra* and *Zygnema*. Meanwhile, multitudes of toad tadpoles had developed in pool 3, and along with their growth went the disappearance of the mats of algæ from the surface, and also of *Anopheles punctipennis* from that part of the pool. They were found a month later in almost the same place in great numbers among fruiting *Chara*, which had spread from the deeper part of the pool. There was protection and food among its filaments.

Pool 4 was a temporary pool beside Fall Creek, about six feet in length and three feet or less in width. It was sheltered by an overhanging stump, but was in the bright sunlight nearly all day. Pools 3 and 1 were sunny also. The only alga in it was *Mougeotia* which was in vigorous fruiting condition. Embedded within it were many masses of Chironomid eggs, as well as many full-sized Chironomid larvæ within their filmy homes. Many very small larvæ of *A. punctipennis* were at the surface of the water among the delicate filaments. A great many minnows were darting about below the algæ, also small Coleoptera. There were no water-striders or whirligig beetles, or other surface feeders. After two weeks the *Mougeotia* had sunken to the bottom and the larvæ of *A. punctipennis* had disappeared, excepting a few large ones.

Pool 5 was near 4 but in the deep shade, the larvæ being confined to a mat of *Spirogyra*. Here the water flowed slowly. Pool 6 was near, but in sunlight all day, and the larvæ were concentrated among the filaments of a mass of *Mougeotia*, and were to be found nowhere else in the pool. The water flowed quite rapidly by, but the *Mougeotia* was anchored.

All the larvæ found in the *Mougeotia* were exceptionally transparent, even in the older stages. Just after molting, they were almost as clear as glass, and were at all times the best ones to study. On the other hand, larvæ found in pool 7, a muddy dark pool in a very shady place, were always very dark and opaque. Here the larvæ were found among the large floating leaves of a *Polygonum*, and nowhere else in the pool.

Feeding Habits. A number of larvæ of all ages were put in a watch crystal with small masses of *Zygnema* and *Mougeotia*, all in fruiting condition. *Cladophora* was added also. A good deal of surface material was devoured by all the larvæ. The younger ones were more particular in the matter of particles, rejecting a good many. They moved around among the filaments, head bent downward, finding plenty to their liking on the *Cladophora*, as well as on the others. They merely brushed off the particles from the larger filaments. A larva of only 1.5 mm. was seen to swallow a filament of *Zygnema*, and to make an effort to swallow large ones of *Spirogyra*, of which it would merely chew the broken ends. Many small larvæ were seen eating the delicate *Mougeotia*. When the food is merely surface particles the head is turned a

half circle with mouth parts upward, next to the surface film; but when feeding on a filament, usually the mouth parts are underneath and the head is bent downward. When feeding in this position, the antennæ and maxillary palpi are spread wide apart, bristles and hairs all extended. The large lateral bristles, outside the antennæ are held out like a fan, and the six branched hairs on the top of the head are raised, making a complete hedge above the rotating brushes.

If particles of food are scarce in the surface film, the young larvæ bend and run their brushes over their own bodies as far as possible, the limit of their reach being apparent by the Vorticellæ and diatoms clinging to the anterior parts of some unlucky individuals. Naturally these are not so active in their movements as the others.

In eating the filaments they swing the brushes furiously until a filament is brought within reach. Or they dive in among the filaments, head down and brushes rotating. Sometimes they merely crush out the contents of the cells, at least in the case of large zygospores of *Spirogyra*, leaving the empty filaments. In that case they grasp the filament anywhere and run it through the mandibles, swallowing only the green parts. The delicate filaments of *Zygnema* and *Mougeotia* are swallowed entirely, also slender filaments of *Spirogyra*. They show considerable preference for the more delicate filaments. Usually they draw the filament into the mouth with the rotating brushes, bite it in two, and then rapidly draw in one broken end. They rarely leave a filament partly consumed, and often go back to the other broken end.

The food of the larva of *A. punctipennis*, is not necessarily entirely of an herbivorous nature. Once I saw two young water fleas (*Simocephalus*) almost dragged into the abyss. They struggled valiantly and escaped by setting up counter currents with their feet. The three currents could be seen close to one another. One went too near and lost an antenna which was broken and swept in by the brushes. It drew back but was temporarily incapacitated and unable at once to leave the vicinity. Finally, it escaped. A little *Chydorus* all but lost its life, being drawn in by the brushes, but escaped.

Fatalities. Dr. J. B. Smith in his report on the Mosquitoes of New Jersey (1904) gave a detailed account of the enemies of mosquitoes. He remarked that the larva actually faces greater dan-

gers than does the adult. He placed as enemies, first weather conditions; then diseases, of which we know little. We have seen several conditions which relate to this subject, however.

Four young larvæ that had passed their second molting were every one of them afflicted with a protruding intestine, which extended out from the body for fully half the length of the larva. This condition lasted for more than a day after they were taken from the pool. A scarcity of food,—for they were kept in clear water for twenty-four hours—evidently cured them. They were normal on the second day after.

Spirogyra furnishes food for the growing larva, but it also shelters enemies. It is the favorite habitat of several species of chironomids, and in just so far is it a check to mosquito development. The chironomid larvæ build their tubes of the algæ filaments, and seemingly of other things too.

In six different instances we have had evidence that some chironomid larvæ destroy the larva of *Anopheles punctipennis*, and feed upon its tissues, as well as using portions of its body to fill in the crevices of their houses. In all the cases, the *Anopheles* was in the quiescent state just preceding or following the molting process. In one case the chironomid built its tube close up beside the body of the dead *A. punctipennis*, and gradually transferred the tissues into the walls of its home. In another case a chironomid was seen to swallow a part of the dark mass of the dead mosquito larva, and the digestion in the chironomid was watched through the transparent organs. A healthy larva of *A. punctipennis* was supposedly alone in a dish of water with algæ for food. It was left over night. In the morning its head was torn from its body and was floating at a distance. The only living animal in the dish was a chironomid larva.

Development. Careful observations on the swarming of *Anopheles punctipennis* have been reported by Knab (1907). The males were seen to swarm a little before 5 o'clock of a sunny afternoon in October. They came from different directions to form the swarm which contained less than a hundred mosquitoes. They circled about above a projecting mass of foliage. Mating of a number of individuals occurred, and by 5.30 o'clock the swarm began to diminish.

In the opinion of Kulagin (1907), based upon at least six years

of observation of *Anopheles* in Russia, mating occurs in the autumn except in a few isolated cases, where it occurs after hibernation. The hibernating females deposit the eggs during the whole of the next spring and summer, and the September and October larvæ result from the isolated cases. Accordingly, he is of the opinion that there is but one generation in a season, the newly developed forms not depositing eggs until after hibernation. There has been insufficient study of this subject.

The eggs are laid singly and a number of times by each individual during a single breeding season. Dr. Duprée found that specimens of *A. punctipennis*, which were kept in the laboratory and supplied with blood, would lay at six or seven different periods, with a total number of more than 2,000 eggs. As many as nine layings were noted in one case. The eggs, from 100 to 300 at a time, are deposited separately, or sometimes in clusters of just a few. They are apt to float below the surface, although some of them are on the surface. Stirring the water will bring a good many eggs to the surface, from which they soon disappear again. They seem not to possess so perfect a means of keeping afloat as is recorded of the eggs of *Anopheles maculipennis*, which has the light clasping membrane about the whole rim of the egg. In *A. punctipennis* this clasping membrane is restricted to the sides. It is very delicate, apparently, for in several instances it was badly broken, or had perhaps been eaten away. A young crustacean was seen to devour the clasping membrane from one side of an egg.

The egg (fig. 1) is .55 mm. in length, mottled in appearance, and dark brown in color with blotches of silvery white. At the end are scattered light and dark spots, the former of which have been called "knobs" and which are arranged in a more or less regular pattern. Sometimes the eggs lose nearly all their dark color, and float. They float with the concave surface underneath. The larva breaks through the convex surface at the larger end, leaving a little fragment of the shell cut out on three sides, and bending over like a little canopy at one end of a little boat.

First Stage of the Larva (fig. 2). At first the young larva spends periods of time quietly. Suddenly it will start a strong current of water by means of the rotary brushes, which are large in proportion to the remainder of the body. These are fully developed

from the first. With these it sweeps in any small particles in the surface film. It takes but a short time to exhaust the immediate supply, and so the larva moves from place to place. Frequently it bends and brushes over its whole body as far as it can reach, removing anything like vorticellæ or diatoms. Sometimes these develop too rapidly and it is not at all uncommon to find larvæ less than three days old, thickly fringed with these sessile forms. Of course, such as attach themselves to the head and anterior part of the thorax remain there, being out of range of the jaws.

If food particles are scarce, the larva will brush over any algæ or other plants that may be at the surface. One very small larva was seen to remain down below the surface film for about half a minute in search of food. One of these very young ones was seen to eat a short filament of fine *Spirogyra*, when the larva was less than two days old. It refused a row of diatoms. It started back from an on-coming rotifer immediately in front of it. The same rotifer ran into and over the long anterior bristles from the thorax and the young larva gave no response. But when it happened again from the front, the larva darted away. The most conspicuous movement of an *Anopheles* larva of any age is its turning of the head through an angle of 180 degrees, when feeding. The young larva does this with energy whenever feeding on surface particles. It turns the head always in the same direction—counter-clock-wise, as was determined by the periodical disappearance of a *Vorticella* which was attached to the side of the head. This always moved downward and out of sight before appearing at the other side.

The young larva measures from .7 mm. to .8 mm. in length, not including the hairs at either end of the body. The second and third segments of the thorax have not yet united and the head is very prominent. The dorsal surface is dark brown in color, except where there are conspicuous yellow spots, which are caused by the œnocytes within. On the segments of the thorax and on five of the segments of the abdomen these yellow spots are very noticeable, especially on the third segment of the abdomen. The head is colored an even gray, with a dark spot in the center, and one small spot on each side. The single eyes and "collar" are reddish in color and remain so during the first day. The branched

hairs from the sides of the head, outside the antennæ, are relatively longer than in any succeeding stage. The six bristles across the dorsal surface of the head (a) are unbranched, as are the two hairs which lie between the brushes and the central terminal hairs, which remain unbranched throughout the larval life.

The most conspicuous structures of the head of the young larva are the long simple hairs on the top, and the long lateral hairs at the side of the head, which with hairs of the antennæ form a barricade. Four simple hairs and the absence of a rudder-like tuft on the ninth segment (a) of the abdomen, are characteristic of the first stage.

When about two days old the larva will have changed considerably in appearance. It will measure by this time about 1.5 mm. in length. The thorax will have become distinctly formed and the long hairs will appear somewhat shorter. The head will have quite a different shape from that of the larva of one day. It will have become very dark, especially the "collar" which will be almost black. The head is much narrower and deeper. The integument has a snug, tight-fitting look (fig. 3). This figure shows a larva in the act of molting for the first time. In this case it died in the process, the integument of the thorax having failed to split apart.

For some time previous to each of the molting processes in the larval life, the head shows this peculiar narrowing, with the increased width and deepening color of the "collar." The larva measures just the same after the molting process as before it. In this case it was 1.5 mm.

Second Stage of the Larva. The changes at the time of the first molting are conspicuous. Although the thorax is distinct and large, the head is even larger (fig. 4). The four dorsal hairs of the posterior end have become eight (a), and the ventral tuft (b) has appeared, consisting of two rows of long branched hairs with a fan-like arrangement. On the head the simple hair just inside of each rotary brush (d) has become much branched, and is used for combing out the brush. The six simple hairs lying back of the brushes, on the top of the head, have become very much branched. The eyes are still little developed.

The yellow spots on the dorsal surface have increased in number and density, being most conspicuous on the third, fifth, and eighth segments of the abdomen, and on the thorax.

Within the thorax a clear specimen shows a number of parts: the anterior lobes of the heart with a large valve, the contractions of which synchronize with the throbbing between the tracheæ of the abdomen; the beginning of the arch of the tracheæ, surrounding the food tube, and from which branches are later given to the organs of the thorax; the three pairs of imaginal buds.

Within a few hours after the first molt, one larva was eating vigorously of fine filaments of algæ, and preferably of surface particles. It swallowed the filaments from the end, just as does the full-grown larva.

By the time the larva is six days old, it begins to show signs of the approaching second molt, which occurs from the seventh to ninth day. As before, the head becomes narrow and the "collar" dark and broad. One measured 2.2. mm. at this time, making a growth of .7 mm. in about two days.

Third Stage of the Larva (fig. 5). The most important changes which are evident after the second molt are: the rapid increase in the size of the thorax accompanied by the enlarged internal organs; the appearance of the ommatidia of the adult eye, around the larval eye; the disappearance of two pairs of the long hairs and the general shortening of all the thoracic hairs; the darkening of the integument of the head, which shows an unexpected pattern in the maculation. Eight specimens examined at this age showed almost exactly the same arrangement of pigment on the head. It may not be constant, but it is certainly more so than that of any other stage.

Respiratory Siphons (figs. 17, 18, 19). At this time it is possible to see, along with the rapid growth of the wing buds, the formation of the pupal respiratory siphons in the prothorax. From the time of the first molt, a pair of straight tubes in each side of the thorax has been evident. Now they have become more conspicuous. They seem to change their position, sometimes being close up to the anterior wall of the thorax, and sometimes they lie with the end quite away from the wall. Developing near by, at an angle with the first tube, and penetrating also through the wall, is another structure, tube-like at first, but after a time changed to form a part of the now plainly visible respiratory siphon. The siphons, also, are frequently drawn away from the integument, at other times being pushed up close to it.

Fourth Stage of the Larva. By the twelfth or thirteenth day the larva has increased in length to 4–5 mm., and it then shows the signs of an approaching molt, the darkening of the head and collar and the widening of the latter. Molting is getting to be a critical process and a great many lose their lives in the act. They are conspicuously large and cannot protect themselves meanwhile. Sometimes the integument of the head does not separate off easily. One was seen in which the head had not molted for more than twelve hours after the rest of the integument had been shed. For a day or more before the third molt, the changes about to occur are evident. They consist of the appearance of the palmate, or stellar hairs, which are of use in clinging to the surface film (fig. 7). The body needs more support in its horizontal position. The palmate hairs (ph) appear on the third, fourth, fifth, sixth, and seventh segments of the abdomen, and may be seen projecting out from the segments for more than a day before the molting. They appear quite suddenly. At the same time the reddish adult eyes have become very conspicuous and are seen to be increasing in the number of ommatidia.

After the molt, the maculation of the head appears to be different from that just after the previous molt. The pattern of the spots is the same, but the increased amount of pigment in the surrounding integument prevents the spots from showing clearly. The buds within the thorax have become very prominent by this time and in addition to the respiratory siphons and the wings, we may see the projecting buds of the legs beneath, and of the halteres. In the head, which remains especially clear for several hours after each molt, we may see now the forming buds of the antennæ (fig. 20), the main portion of the brain with nerves branching to the eyes, to the antennæ, and to the digestive tube. Numerous muscles to the mouth parts, with their relations to the muscular lining of the integument, may be seen at this time also, as long as the integument remains transparent. Now, too, the clear, dark spots between the abdominal segments first become visible (fig. 7.).

From about the fifteenth day to the twenty-second, growth is rapid, if plenty of food is available. The record of one larva in the laboratory was as follows:

- July 2—first molt (about 3 days old).
 “ 13—third molt (second not seen).
 “ 21—transformed to pupa.
 “ 23—adult female.

This makes the complete cycle occupy about twenty-four days, in midsummer in Ithaca. This specimen was taken from a spring-fed pool, was transferred to the laboratory and raised in a porcelain dish in the water of the Cornell campus reservoir. Its food was *Spirogyra*, *Zygnema*, and *Mougeotia*, with *Cladophora* for browsing and to furnish surface particles. The adult which emerged was no smaller than many that have come from pupæ taken at the same pool. The only special care given was to prevent the afternoon sun from shining upon it, and to add fresh water frequently.

Some of the Records of Developing Larvæ.

- A. July 26—hatched .825 mm. long.
 27—10.30 A. M. 1. mm.
 27—3 P. M. No change, head broad.
 28—10.30 A. M. Collar wider, head dark, 1.55, mm.
 29—11.30 A. M. 1.55 mm., collar still larger, 3 days old, not molted. (Died.)
- B. July 26—hatched .825 mm. 4 hrs. old when measured.
 27—head large 1.05 mm. 18 hrs. old, 10.30 A. M.
 27—head large 1.17 mm. (About) 24 hrs. old, 2.45 P. M.
 28—head narrower, collar wide, thorax more distinct 10.30 A. M.
 “ 29— 1.5 mm., 3d day 10.30 A. M.
 “ 30— 1.5 mm., 4th day molted. (Died.) 10.30 A. M.
- C. July 25—had molted once 1.5 mm. 9 A. M.
 29—second molt, about $4\frac{1}{2}$ days between 1st and 2d molt.
 30—no palmate hairs, single eye spots, no antennal buds.
 31—large eye beginning to show, 10 days.

- Aug. 1—palmate hairs not started, respiratory siphon well developed 1 P. M.
 1—palmate hairs not visible yet, 10 P. M.
 3—palmate hairs visible, eyes distinctly double, collar wider, head narrower.
 6—molted for the 3d time, 15 days, 2 P. M.
- D. July 23—1.6 mm., had molted once, about 4 days old.
 30—4.5 mm., stellar hairs have appeared, 11–12 days.
 31—4.6 mm., 13th day (Apr.) 8 P. M. Just molted for 3d time; eyes double, palmate hairs; transparent areas of abdomen for first time.

Full-grown Larva (fig. 7). There is a period of about nine days between the third molting and the transformation to the pupa, during which there are few changes in appearance. The head usually grows darker, although in many specimens it never becomes really dark. The adult eyes show greater development of the ommatidia and the coming rounded eyes of the adult are being revealed. The "collar" becomes wider with age, and the thorax assumes a decidedly round appearance. Often the whole body is green, or an even brown, or with a white stripe, or spotted along the mid-dorsal line. These differences in color do not appear in the younger forms. Dr. Howard proved that in the case of *A. maculipennis* young larvæ fed on algæ will turn green.

The conspicuous structures of this last stage of the larva are the five pairs of palmate hairs of the abdomen, the transparent areas between the abdominal segments, and the little two-lobed projections of the prothorax, which are indications of the developing respiratory siphons of the pupa.

When not in use, the palmate hairs are folded against the body. They have been clearly described by Nuttall and Shipley (1900) for *A. maculipennis*. Like all the other forms of hairs of the larva's body, they are wonderfully fitted to their work of holding to the underside of the surface film. They are not found on the first and second segments of the abdomen. On about the last day before the change to the pupa, there suddenly appears on the first segment of the abdomen a large tuft of branched hairs, a certain indication of approaching pupation (fig. 7).

The transparent areas of the segments of the abdomen are like

windows into the interior. Here we may see the rhythmic pulsations of the long heart.

Notched Processes of the Thorax. On the prothorax are two notched projections just over the respiratory siphons. In one specimen, on the second day after the third molting, an opening was plainly visible at the base of both the first and the second hairs on each side of the thorax (fig. 19). The delicate two-lobed structure had developed between the hairs and the openings. After a time the two hairs fell off, the inner one falling several hours earlier than the outer. This left the third hair, which is branched, with the two delicate processes and the openings close to them. Each opening is rimmed with several little ridges placed parallel to the hairs. The transparent lobes would seem to protect the openings. Within, each leads into one of the two tubes before described, and which must lead into the tracheal system, although their connection with the tracheæ is not evident from the outside. The openings are situated just where the thorax is applied to the surface film, and are related to the respiratory siphons of the pupa.

The Change to the Pupa. After the tuft of branched hairs has appeared on the first segment of the abdomen, we may look for the change to the pupa before many hours. The thorax will soon show a series of transparent spots, suggesting the areas in the abdominal segments, and due, doubtless, to the segmented structure of the thorax. The throbbing heart becomes plainly visible along the mid-dorsal line. The following record of the time was kept as one specimen changed: (8.30 P. M.) The larva was perfectly quiet for a long time, with the antennæ and hairs of the head drawn up close. The body expands frequently and the head is drawn in somewhat. The hairs on the thorax lie limp, pointing downward. The sides of the metathorax become somewhat sunken, as if parts beneath had changed position, (9.40 P. M.) Tiny dots on the rims of the respiratory siphons show through the transparent integument. (These may be identified later on the edges of the siphons of the pupa.) There is a rowing motion of the hairs of the whole body, and a regular pushing with the posterior end against the sides of the watch glass. In the abdomen, fine ridges or wrinkles appear across the segments, between the palmate hairs. (10.15 P. M.) The segments of the abdomen begin

to draw up, with a strong pulling away from the posterior end. (10.20 P. M.) The integument of the anterior end splits all at once, very quickly, and the two siphons are drawn out instantly, the rest of the body being drawn out with a sudden twist. (10.22) It has shaken off the skin and lies quietly expanding once more. The actual change has taken place in a little less than two minutes. After ten minutes, with a final jerk, it is a fully developed pupa, darting here and there.

The Pupa. The color of the pupa is similar to that of the larva, showing either the stripe or the blotch of white, or else being green or brown. They all turn quite dark before emerging. The pupal period is about two days.

One specimen which had changed to the pupa at 7.30 P. M. on July 1, at 5 P. M. on July 3 had emerged as the adult. The thermometer was above 90 degrees during both days. By the beginning of the second day the pupa showed evidence of the coming change.

At the anterior end of the pupa three parallel grooves appeared, the central one of which lengthened as the hours passed. Two dark spots suddenly appeared near the anterior end. Across the thorax between the respiratory siphons came a transparent place in the integument, in the center of which the heart was seen. Above the heart is a mass of striations arranged in a V-shaped structure. Each of the striations shows a dark rounded base or origin, and when compared with the thorax of the adult is plainly a hair. They appear at the beginning of the second day, and grow more distinct.

The Emergence of the Adult. After the changes mentioned above, the whole pupa becomes very dark in color, and remains very quiet, except for frequent quiverings. The heart beats more rapidly than hitherto, as may be seen through the transparent integument. Suddenly the abdomen becomes straight and the body erect. Through the skin the stigmata within the thorax may be seen in pulsation close to the wings. Before we are aware of any break in the pupa case, the head and mouth parts are completely out. They remain bent downward for considerable time, the wings, abdomen and legs being gradually drawn out. The wings come first and are kept flat and folded over one another, the spots being clearly visible from the first, although the whole wing

is pale in color. The abdomen, greenish in color and very flexible, begins to move backward and forward with a pushing motion. Here seems to be the center of all the motion. Finally the abdomen is completely extricated and pushed backward, outside the case. Now comes the most critical part of the process. The two fore legs are drawn out together, being kept parallel with one another. Similarly the second and third legs of each side are kept parallel. The second pair separate from the third pair, and are removed from the cases at exactly the same instant. Then the first and second pairs are spread out firmly on the water, or other support if possible, and all the effort is concentrated on the removal of the third pair which are much longer than the others. Unless an accident occurs, they will be pulled out together and placed firmly out backwards. Then the insect will rest quietly during the hardening of all the parts. The antennæ of a male mosquito were kept folded tightly for several hours after emergence. During all the process the pupa case rested up against the side of the watch glass. In one instance we moved the pupa case away from the glass as the process was going on, which apparently caused failure in extricating the hind legs, and death of the mosquito. Within the cast-off pupa case may be seen, at first, a large air bubble which is of service in balancing the emerging insect. (Nuttall and Shipley, 1907.)

The colors of the parts of the insect as it was emerging under electric light were remarkable, the eyes being a deep iridescent green, the legs dark green, the abdomen light green with gray edges, and the yellowish spots of the wings becoming more and more distinct. Within ten minutes from the beginning of the process of emergence, our mosquito had spread its wings.

DIFFERENTIAL CHARACTERS APPEARING DURING DEVELOPMENT.

| | Growth (average). | Change. | Period. | Characteristics. |
|-----------------------|-------------------|-------------------------|----------|---------------------------------------------------------------------------------------------------------------------------------|
| Egg | .55-.57 mm. | 2d-3d day (hatching) | 2-3 days | Irregular spots; clasping membrane on sides only. |
| Larva 1st Stage | .8-1.5 mm. | 4th day (molt) | 3-4 days | Metathorax free at first; 6 simple hairs on head; 2 simple hairs inside brushes; rudder hairs absent; eyes single. |
| 2d Stage | 1.5-2.2 mm. | 8th day (molt) | 4-5 days | 6 branched hairs on top of head; 2 branched hairs inside brushes; rudder hairs present; eyes single. |
| 3d Stage | 2.2-4.5 mm. | 14th day (molt) | 6-7 days | Head smaller than thorax; head dark with pigment; eyes double; respiratory siphons forming. |
| 4th Stage | 4.5-8.5 mm. | 22d day (to pupa) | 8-9 days | Palmate hairs on abdomen; transparent areas on abdomen; notched processes on thorax; dorsal tuft on abdomen. (Late in forming.) |
| Pupa | | 24th day (to adult) | 2-3 days | |

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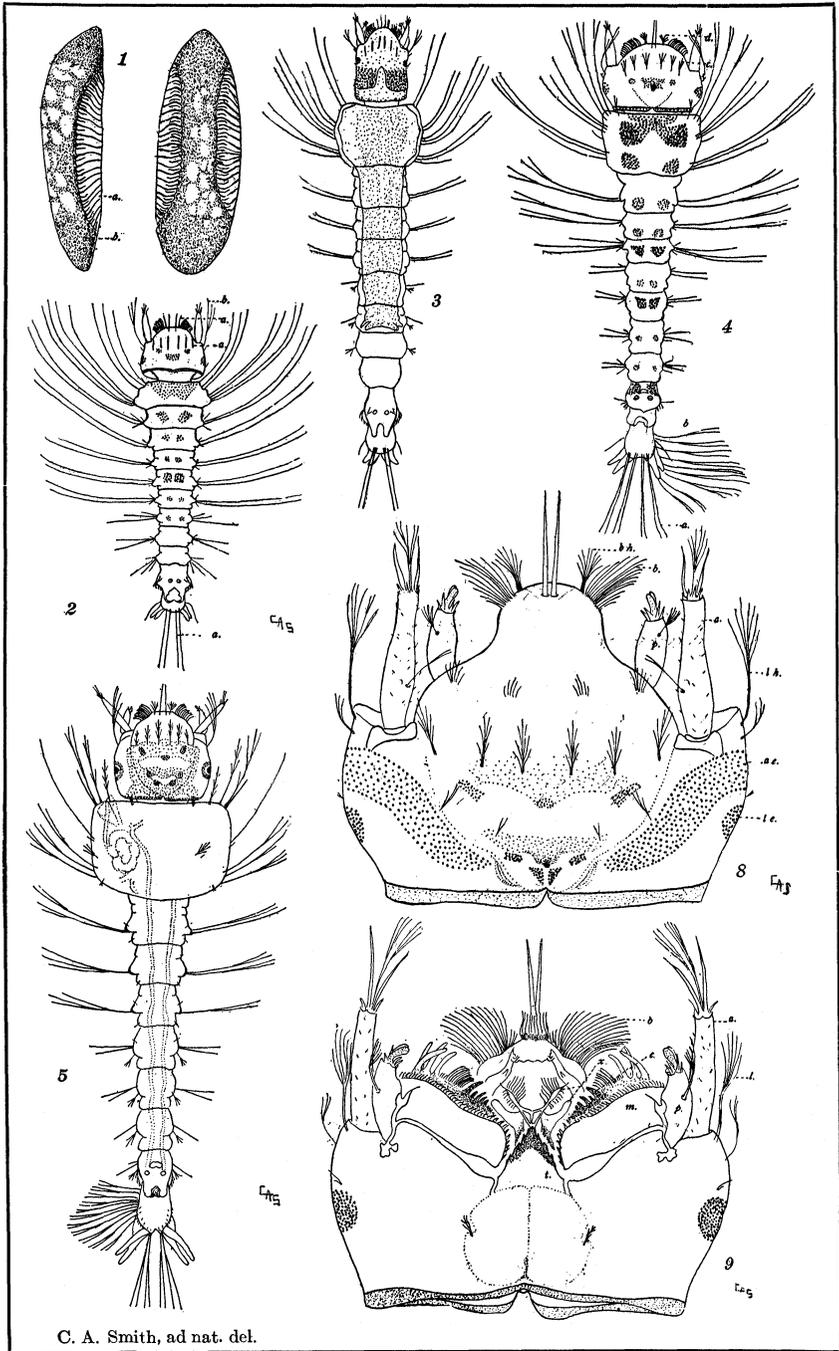
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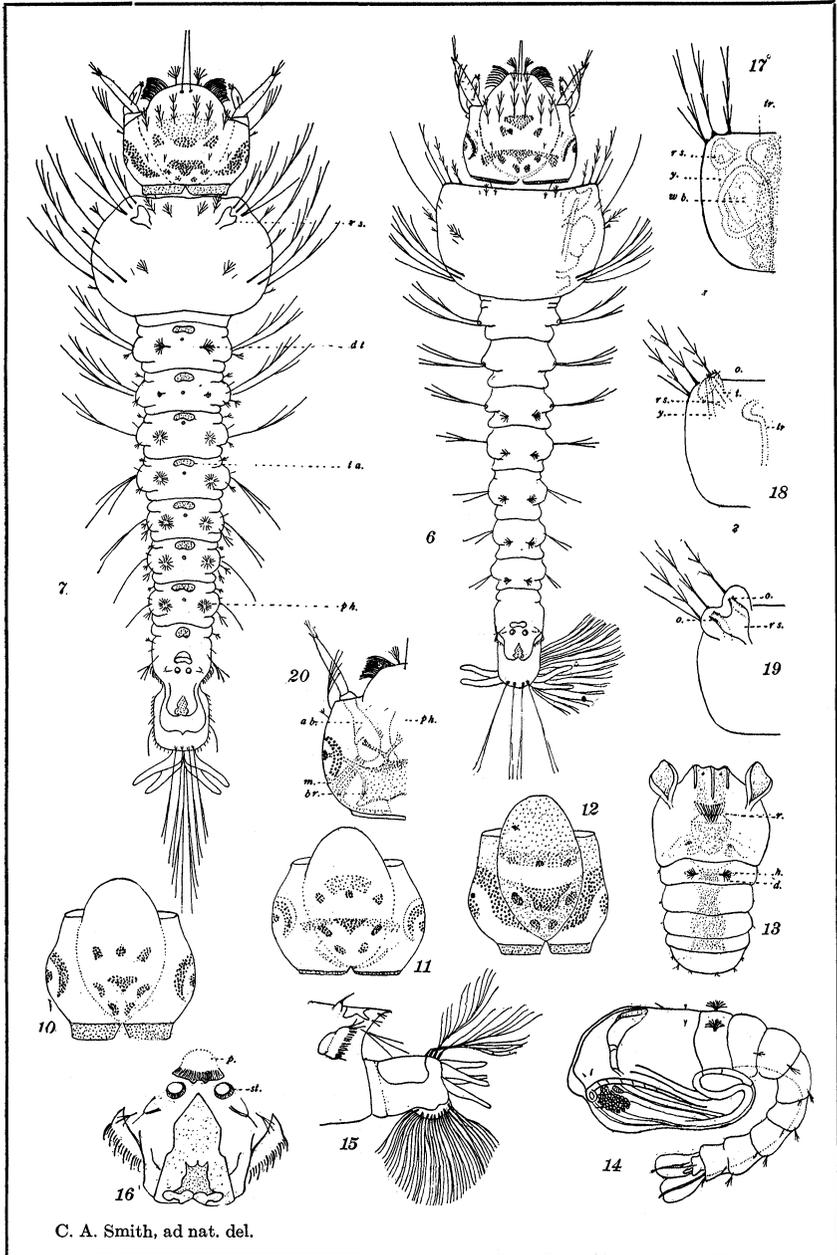
EXPLANATION OF FIGURES.

- Fig. 1. Eggs of *Anopheles punctipennis*. Actual measurement .55 mm.; (a) clasping membrane, (b) "knob."
- Fig. 2. Larva just out of the egg. Actual measurement .825 mm.; (a) hair that is branched later, (b) lateral hair.
- Fig. 3. Larva during the first molt, age 3-4 days.
- Fig. 4. Larva just after the first molt. Actual measurement 1.5 mm.; (a), (b), (c), (d) branched hairs.
- Fig. 5. Larva just after the second molt, age 8-10 days. Actual measurement 2.2 mm.
- Fig. 6. Larva just after the third molt, age 13-15 days. Actual measurement 4.5 mm.
- Fig. 7. Larva just before the change to the pupa, age 21-23 days. Actual measurement 8.5 mm.; (rs) respiratory siphon, (dt) dorsal tuft, (ta) transparent area, (ph) palmate hair.
- Fig. 8. Dorsal view of head and larva; (a) antenna, (p) maxillary palp, (b) brush, (bh) branched hair, (ae) adult eye, (e) larval eye, (lh) lateral hair.
- Fig. 9. Ventral view of head of full-grown larva; (b) brush, (c) teeth of mandible, (m) maxilla, (p) maxillary palp, (a) antenna, (l) lateral hair, (t) mentum, (x) chitinous structures within the mouth.
- Figs. 10, 11, and 12. Maculation of the head of the larva; (10) just before the third molt; (11) just after the third molt; (12) just before the change to the pupa. (Sometimes there is more pigment; often less.)
- Fig. 13. Dorsal view of pupa on the second day; (d) dorsal tuft, (h) outline of one of the halteres, (r) dorsal hairs of adult, showing through the pupa case.
- Fig. 14. Side view of pupa.
- Fig. 15. The ninth segment of the abdomen from the side.
- Fig. 16. Stigmata of eighth segment of the abdomen of a full-grown larva; (st) stigma, (p) plate which folds over the stigmata when the larva is below surface film.
- Fig. 17. Showing buds of wings and of respiratory siphons in the thorax of the larva just after the first molt; (tr) trachea, (rs) bud of respiratory siphon, (wb) wing bud, (y) movable tube.
- Fig. 18. Respiratory siphon just after the second molt; (y) movable tube, (t) tube, (rs) respiratory siphon, (tr) trachea, (o) opening.
- Fig. 19. Respiratory siphon (rs) of larva just before the change to the pupa, (o) openings.
- Fig. 20. Some of the organs of the head as seen just after the third molt; (ab) bud of antenna, (ph) pharynx, (br) brain with nerves to eye, antenna and pharynx, (m) muscles to mouth parts.



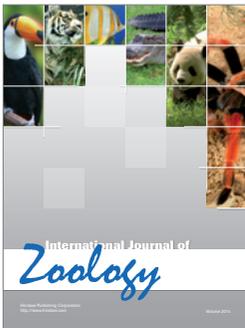
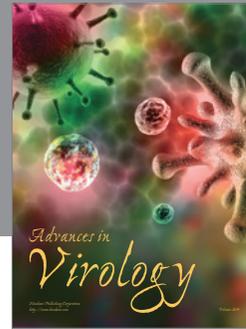
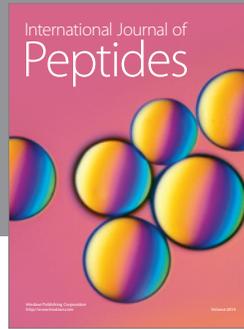
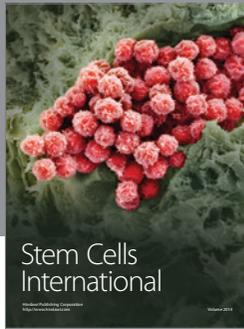
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