

SEXUAL DIMORPHISM OF LARVAE AND PUPAE OF *Aedes Aegypti* (Linn.)

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For certain specific research problems it is often necessary to distinguish the sex of immature insects. Yet only a few references are to be found in the literature on the sex determination of the immature stages. Several workers have been able to sex both the larval and pupal stages of mosquitoes. The need for recognition of sex in immature forms of mosquitoes was pointed out by Hayes (1953) who required virgin females for insemination studies, and by Jones (1957) who discussed the application of sex determination in the immature stages to various aspects of biology and control. The experimental physiologist, likewise, needs to know the sex of the immature forms, especially for hormonal studies.

The observations on sex determination in this paper are based on the larval and pupal characters of *Aedes aegypti* (Linn.) which is the mosquito that has been most commonly used for laboratory studies.

LARVAE

METHODS. The contents of the digestive tube of the larvae often obscured the internal structures. To overcome this difficulty the following procedures were found satisfactory for permanent slide preparations:

1. Place live larvae in 2 percent sodium citrate for 6 hours to evacuate the fecal materials.
2. Transfer larvae to water for 10 minutes to wash off the citrate.

3. Transfer larvae to KAAD preservative solution (Peterson, 1943), for 12 hours.
4. Make small incisions in thorax with fine micro-needles (Carpenter and La Casse, 1955).
5. Transfer larvae to 80 percent ethyl alcohol for 5 minutes.
6. Place larvae in a 1:1 solution of cellosolve and beechwood creosote for 30 minutes.
7. Transfer larvae to a 1:1 solution of xylol and Canada balsam for 5 minutes.
8. With a dissection needle make an incision between the seventh and eighth segments so the air tube and anal segment will be in a lateral position.
9. Mount in Canada balsam.

Living larvae can be examined and sexed in Gillet's (1942) larvascope. This device is a cell easily made either by grinding a notch in a microscope slide about one and a half centimeters square or by cutting out a piece with a glass cutter. When the notched slide is cemented between two slides, there results a chamber as large as the cut notch and as thick as the thickness of the notched slide. The chamber is filled with water and the larvae are introduced with a pipette. Observations can be made with the microscope tilted slightly to prevent the spillage of water from the cell.

To reduce the normal movements of the larvae it is necessary to inactivate them by placing in a small vial completely filled with water and covered with a cover slip. The larvae are left in this tube for a period of about 40 minutes and then placed in the observation cell. After a detailed study of the larval structures in permanent preparations, the characters can be recognized in the Gillet's larvascope.

DISTINGUISHING CHARACTERS. The larval characters that have been used to separate the sexes of mosquitoes are color, and the

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size and shape of the imaginal discs. These imaginal discs, as pointed out by Bodenstein (1953), are destined to become organs of the adult. Sexual differences have been noted in the gonads and in the imaginal discs of the antenna and the external genitalia.

The color pattern as used by Jones (1957) for *Anopheles* does not seem useful for *A. aegypti* since the pigmentation is alike in both sexes.

Few authors have used the developing gonads in mosquito larvae to separate the sexes. Adic (1912) observed in the sixth abdominal segment of *Anopheles* swellings which he thought to be gonads. Langeron, 1926 (cited in Boyd, 1930) confirmed these observations. Warren and Breland (1963) were able to sex the larvae of two culicines by observing the gonadal testes through the body wall employing a dissecting microscope. The descriptions of the gonads that follow are from balsam mounts.

The testicular gonads (Figs. 1-2) in mature fourth stage larvae are located ventrally to the dorsal tracheal trunks in the sixth abdominal segment. Each gonad is pyriform and measures 270μ in length and 115μ in width. Each gonad appears to be divided into transverse pseudo-chambers. Typically, there are seventeen of these chambers which represent the spermatid tubes. In these chambers can be observed the germ cells which are irregularly arranged and which practically fill the organ. It is possible to distinguish only a small zone of spermatid differentiation at the posterior extremity of the gonad. At the anterior end is a short and slender filament which is a suspensorial ligament. From the posterior end originates the vasa deferentia which is sinuous and difficult to follow beyond the eighth abdominal segment. Each testicular gonad is covered with a sheath of connective tissue which must be removed by dissection to make detailed observations on the internal structures.

The ovarian gonads (Figs. 3-4) are located in a position latero-ventral to the

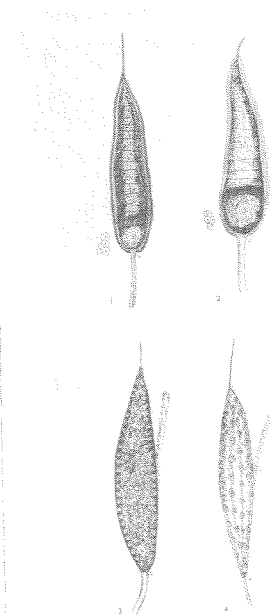


Plate I.—Larval Gonads. Two different stages of development of gonads of male and female fourth instar larvae. Figs. 1-2, testicular gonads. Figs. 3-4 ovarian gonads.

dorsal tracheal trunks in the sixth abdominal segment. Each gonad is fusiform, 550μ long and 160μ wide in late fourth stage larvae. The germ cells which are distributed irregularly in the gonads are rounded, 20μ in diameter, with a double membraneous wall. Each germ cell contains seven smaller cells. The ovarian gonads are held in position by a suspensorial ligament which extends in an antero-lateral direction to an attachment point on the dorsal wall of the fourth abdominal segment. The oviductus lateralis extends posteriorly along the median line where the two fuse, possibly in the seventh segment.

The ovarian gonads can be identified in the second and third stage larvae with great difficulty. Thus, they are not useful for sexual determination in young larvae. In the fourth stage they are covered sometimes with pigmented tissues and are,

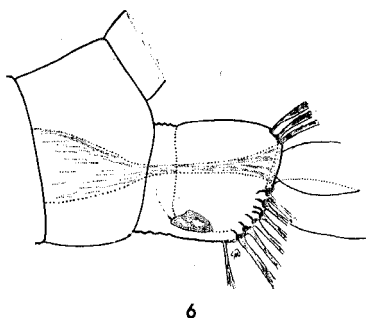
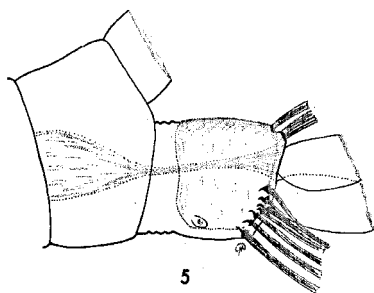


Plate II.—External genitalia imaginal discs present only in males of fourth stage larvae. Fig. 5, early stage of development. Fig. 6, late stage of development.

therefore, more difficult to locate than the testicular gonads.

The sexes can be distinguished both by the gonads and the external genitalia imaginal discs. Rempel (1937) noted that in Chironomidae the external genitalia of the future adult are noted primarily in the pupae although the ducts of the reproductive organs, antennae and legs are visible also in the last larval instar. As pointed out by Jones (1957) the imaginal discs of the external genitalia of *Anopheles* larvae can be used as a distinguishing character because it appears only in the male. The same holds true for *A. aegypti* larvae as determined in the present study.

These external genitalia discs are located in a proximo-latero-ventral position in the anal segment. It is presumed that these discs are homologous with the tenth abdominal segment or genital pouch of

pupae and the basistyle of the adult. The discs can be quickly located as they lie under the proximo-ventral angle of the anal saddle. In *Anopheles* Jones (1957) states that the discs are more medially located. The typical shapes of these discs are ovoidal to subtriangular as shown in Figs. 5-6.

According to Jones (1957) living fourth stage larvae of *Anopheles quadrimaculatus* have well developed antennal imaginal discs so that identification of the sexes is relatively easy. The author indicates that, at first, recognition of the sexual differences may be possible only with larvae held between a coverslip and a slide but, after some practice, the structures can be observed without holding the specimens. Young fourth stage larvae of *A. aegypti* also have well developed imaginal antennal discs which are easily distinguishable, but in the earlier stages of development it is impossible to differentiate between sexes without holding the larvae still.

The development of the antennal imaginal discs in *A. aegypti* fourth stage male and female larvae are shown in Figures 7-16. The differences in size and the relationship with other structures in the larval head may be correlated with the age of the larvae.

The terminology of Jones (1957) is modified as follows to fit the structures as seen in *A. aegypti* (Fig. 7):

A. Pedicel.—Includes the stalk and antenno-genous tube.

B. Body.—Distal enlargement of the imaginal disc.

C. Lateral lobes.—Lateral lobes of the body.

Two structures, the imaginal eye and the imaginal disc of the posterior legs, are helpful in establishing the position relationship with the imaginal disc of the antennae and the age of the larvae.

PUPAE

Moorefield (1951) discussed in some detail the sexual dimorphism of pupae of *Aedes stimulans* (Walk.), *Ae. trivittatus* (Coq.), *Ae. vexans* (Meig.) *Anopheles*

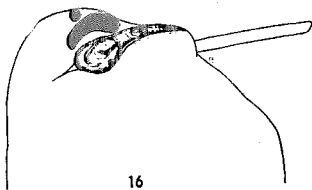
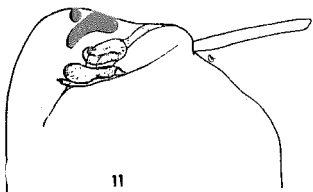
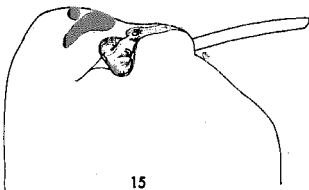
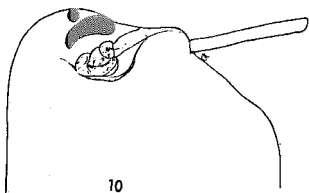
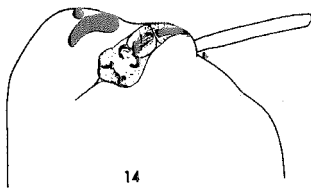
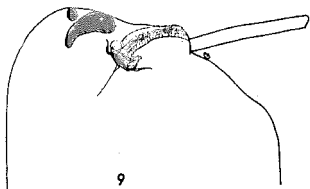
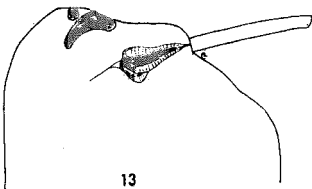
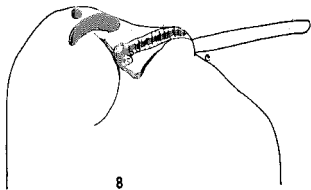
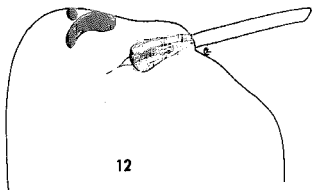
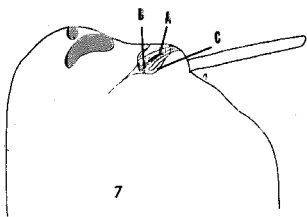


Plate III.—Antennal imaginal discs. Dorsal view of the principal stages of development of the imaginal antennal discs of male and female fourth stage larvae. Figs. 7-11, male discs. Figs. 12-16, female discs. Fig. 7, A=pedicel, B=body, C=lateral lobes (see text).

punctipennis (Say.), *Culex apicalis* Adams, *C. restuans* Theob., *Culiseta inornata* (Will.) and *Psorophora ferox* (Hub.). He cited only Cantrell (1939) and Penn (1949a, b) as having also noted dimorphism. He was able to identify the sex of live pupae by suspending a droplet of water at the tip of an eye dropper examining with a hand lens. Following the same technique Carpenter (1952) studied 32 species of mosquitoes from North and Central America representing 30 genera. Carpenter indicated that the size of the tenth segment corresponded to the size of the genitalia of the adult male and female. He also noted a relationship between the length of the tenth segment and the abdominal paddles. He considered the ability to distinguish the sex of pupae to be important in taxonomic studies.

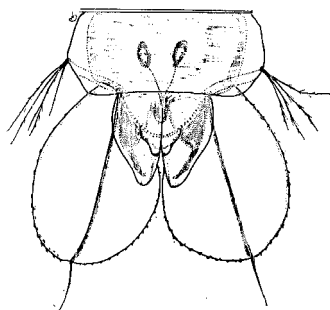
Adie (1912) correlated the size of the pupae with sex. Hayes (1953) used size for the differentiation of *A. aegypti* pupae with satisfactory results.

In this study it was found that, just before emergence of the adult, pupae show appreciable differences in size; however the female is normally larger than the male. Since the range in size overlaps considerably it is deemed necessary to select more definitive characters such as the comparative sizes and shapes of the ninth and tenth abdominal segments.

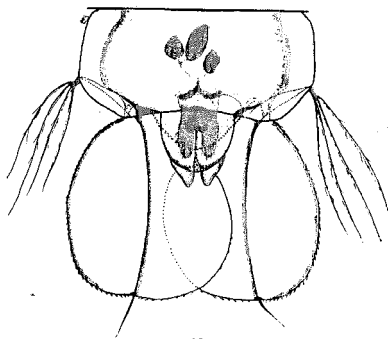
MALES (Fig. 17). The ninth segment which fuses to the tergite of the eighth segment has a crescent-shaped posterior margin with a length of approximately half that of the eighth segment.

Ventrally the tenth abdominal segment is united to the sternite of the eighth segment. In this segment are located the genital pouches which exhibit considerable sexual dimorphism. The sexual differences are easily noted in the central and secondary lobes of the tenth segment.

The central lobe is divided from near the base to the apex into two lateral pouches that are nearly twice the length of the eighth segment; these pouches are bluntly rounded at the posterior tip. They



17



18

Plate IV.—Anal segments of pupae. Ventral view, showing differences in morphological characters between sexes. Fig. 17, male. Fig. 18, female.

are sinuously attached to the base, with the most pronounced curvature in the posterior third of their length. The central lobe is about a fourth as wide as the length of the paddles and it is wider at the base than the corresponding width of the ninth abdominal segment. In the central lobe are formed the genitalia of the future adult. In a median position and dorsal to the central lobe there is on each side a secondary lobe that extends slightly beyond the posterior border of the crescent-shaped ninth segment.

FEMALES (Fig. 18). The ninth segment is fused with the tergite of the eighth segment. It has a posterior margin more acutely curved than in the male and with

concavities near the basal third. Around the posterior margin is a band of folds or corrugations. The length of the ninth segment is approximately equal to the eighth segment.

As in the male pupae the sexual dimorphism is easily seen in the central and lateral lobes. Also as in the male, the central lobe of the female has a median cavity which appears to be connected, in some way with the anal opening of the adult.

Neither the lateral nor the central lobes reach beyond the basal fourth of the longitudinal bar of the paddles; also this lobe (10th segment) is not wider at the base than the corresponding width of the ninth segment.

SUMMARY

The ability to distinguish between sexes of mosquito larvae and pupae is much needed for taxonomic, biological and toxicological purposes. In larvae of *Aedes aegypti* (Linn.) the gonads and the imaginal discs of the antennae and the external genitalia show sufficient sexual dimorphism to permit separation of the sexes. In pupae, the ninth and tenth abdominal segments differ with respect to sex. The contents of the digestive tube which obscure some of the internal structures in the larvae can be evacuated with 2 percent sodium citrate.

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