THE BIOLOGY AND BIONOMICS OF *AEDES AEGYPTI* IN THE LABORATORY

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The importance of *Aedes aegypti* in medical entomology and its ease of rearing have made this insect one of the most exhaustively studied species in the laboratory. The mass of published data concerning *A. aegypti* requires considerable abridgement and condensation to meet the limitations of the present paper.

EGG STAGE. The eggs are laid singly within 1 inch of the surface of water held in an oviposition container (Drake-Brockman, 1913). Deposition may be obtained on a variety of materials and wood appears to be a preferred surface. If the water is foul, eggs may be laid on the water surface itself (Roubaud, 1929).

At 77° to 86° F., the egg reaches the 4-cell stage in 1.5 hours; the blastoderm nuclei occur in the periphery in 3 hours; the serosal cuticle is secreted by 16 to 17 hours; the nervous system is formed in 48 hours and the mature embryo in 72 hours (Gander, 1951). At 70° to 73° F., the embryo requires approximately 90 to 100 hours to mature (Johnson, 1937). As the serosal cuticle is secreted, the eggs become resistant to dehydration by hypertonic saline (Harwood and Horsfall, 1959).

Within 1 or 2 hours after the egg is laid, the endochorion of the shell changes from a soft white layer to a hard dark one (Gander, 1951), becoming somewhat impermeable to water and after 17 hours becoming hydrophobic (Harwood and Horsfall, 1959). Despite these changes in the shell, eggs kept moist for only 25 hours often fail to hatch, and a 48-hour period of maintained moisture is needed to make the eggs resistant to subsequent air drying (MacGregor, 1916).

For the best hatch, a period of several days of air drying prior to wetting appears advantageous. Eggs dried at 75° F. for 12 hours and then placed in water required 6 to 15 days for complete hatch, whereas eggs air dried for 4 days required only a few minutes to hatch after being flooded (Shannon and Putnam, 1934; Atkin and Bacot, 1917). Eggs completely submerged on flooding hatched in less than 10 minutes but eggs floated on the water surface required 1 to 5 days to hatch (Shannon and Putnam, 1934). When submerged in sterile medium, the eggs may fail to hatch or show delayed hatch (Thomas, 1943). Eggs, air dried for 1 to 4 weeks, hatched in 30 minutes when submerged in water containing fresh food but required only 7 minutes in water containing 12-hour-old food (Shannon and Putnam, 1934).

The reduced oxygen content resulting from bacterial action on the food is considered to stimulate the hatching response (Geigy and Gander, 1949). Eggs may require redrying and reflooding for a complete hatch. Eggs flooded when 4 days old gave 95 percent hatch, reflooding at 16 and 25 days of age gave additional hatch of 3 percent and 0.4 percent, respectively (Shannon and Putnam, 1934). Eggs can be held for extended periods in 80 to 90 per cent relative humidity and will show good rates of hatch, e.g., 70 percent hatch was obtained from eggs held for 13 weeks (Woodhill, 1948; Morlan, *et al.*, 1963).

Properly conditioned eggs can withstand marked changes in temperature. With 5-minute exposures to 118° F., 115° F., and 113°F., survival was 10 to 25 percent, 50 to 90 percent, and 100 percent, respectively (Macfie, 1920; Farid, 1949). With longer exposures, however, the survival rate was reduced. Eggs held at 108° F. for 12 hours failed to hatch. Few eggs hatched

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after exposure to 102° F. for 24 hours, but normal hatch was obtained following 24- and 48-hour exposures to 95° F. (Bacot, 1916). The eggs can also survive low temperatures; eggs hatched after a 1-hour exposure to 2° F. but could not withstand a 48-hour exposure to 22° F. (Reed *et al.*, 1901; Bliss and Gill, 1933).

LARVAL STAGE. The time interval required for completion of the larval development is dependent upon several factors among which are water temperature and crowding, as well as quality and quantity of food. Larvae in water 41–46° F. become stiff and immobile and can survive only short exposure periods (Bacot, 1916; Bar-Zeev, 1957). Even at 50° F. continued exposure produces death, probably from drowning (Howard *et al.*, 1912; Bar-Zeev, 1958). From 53°–55° F. some minimal growth progress is made; and at 61°, 64°, and 68° F. complete growth requires 29, 24, and 19 days, respectively (Headlee, 1942).

The optimum water temperatures are in the 77° to 84° F. range with time to pupation of 5 to 7 days with the first three instars requiring one day each and the fourth instar taking 2 to 3 days (MacGregor, 1915; Shannon and Putnam, 1934; Christophers, 1960). Although more rapid growth may occur at 86° to 88° F., the adults that emerge are smaller than those from larvae reared within the optimum temperature range (MacGregor, 1915). Water temperatures above 90° F. have adverse effects, and larvae reared at 97° F. fail to complete development (Bar-Zeev., 1958).

Although continuous exposure to 106° F. is fatal (MacGregor, 1915), larvae can withstand shorter exposures. Five-minute exposures to 106° F. allowed survival and subsequent emergence of adults, but at 109° F., 50 percent mortality and no adult emergence occurred (Macfie, 1920). At 117° F. only a few larvae survived a 5-minute exposure (Farid, 1949). With 4th instar larvae 50 percent mortality was produced by 3.4-hour exposure at 106° F., by 1-hour exposure at 109° F., and by 9-minute exposure to 129° F. (Bar-Zeev, 1957). Within the optimal temperature range of 77°–84° F. a variable temperature is more stimulating to development than a constant temperature (Headlee, 1942). When given a choice of water temperatures, the 2nd instar larvae prefer 73°–90° F., and the 3rd and 4th instar larvae prefer 82°–90° F. (Omardeen, 1957).

Larvae can be conditioned to a degree to extreme temperatures. When held at 50°, 63°, and 93° F. for 24 hours, larvae showed chill coma at 38°, 42°, and 51° F., respectively, and alarm response at 48°, 52°, and 58° F., respectively (Mellanby, 1958). Larvae acclimatized at 86° and 99° F. had thermal death points at 108° and 111° F. respectively (Mellanby, 1954). By raising larvae and pupae at 11 different temperatures between 93° and 59° F., it has been found that the dry weight, the wing length, the leg length, and the thorax length of the emerging adults increased with the lowering of temperature (van den Heuvel, 1961).

The effects of crowding the larvae are reflected in mortality and delayed pupation. With 100 larvae/liter of water, pupation occurred in 5 to 7 days with only 5 percent mortality. With 400, 1000, and 4000 larvae per liter in similar containers the pupation values were 5 to 10, 7 to 16, and 10 to 60 days respectively, and the mortality values were 15, 21, and 48 percent (Shannon and Putnam, 1934). In mass rearing 1.4 larvae/ml. and 1.4 larvae/sq. cm. of surface gives very favorable pupation and survival (Morlan *et al.*, 1963).

The larvae eat suspended or detachable organic material and thrive on living bacteria and yeast but fail to survive on dead bacteria alone (Atkin and Bacot, 1917). A suspension of autoclaved yeast and liver extract provided normal growth (Trager, 1935) but yeast alone was insufficient and the addition of an ammonium salt of folic acid or vitamin B complex was necessary (Goldberg *et al.*, 1944). The essential parts of the vitamin B complex were shown to be thiamin, riboflavin, pyridoxin, nicotinic acid and pantothenic acid (Gold-

berg *et al.*, 1945). Diets lacking protein or amino acids prevent larvae from reaching the second instar (Goldberg and de Meillon, 1948). In mass production, complete larval growth can be obtained in 6 days by feeding 0.2, 0.3, 0.4, and 0.6 mg. of laboratory chow per larva on days 1, 2, 3, and 4 to 6 respectively. Generally, best results are obtained with powdered food which passes a 40-mesh screen (Morlan *et al.*, 1963).

Larvae can survive adverse food conditions, and in complete absence of food 1st and 3rd instar larvae have lived for 14 and 13 days, respectively. With very small amounts of food present some growth is possible. Under these conditions, 30 percent of test larvae were one-third grown and alive at 36 days, 22 percent were one-half grown in 41 days, and 2 percent reached pupation in 114 days but died at that time (Shannon and Putnam, 1934).

The larvae can withstand limited desiccation and have been observed to survive 13 days on moist earth (Howard *et al.*, 1912) and 10 days on moist filter paper (Bacot, 1916). Light had little or no effect on the time necessary for larval growth (Jobling, 1937). However, movement over a rearing container will cause the larvae to descend (Mellanby, 1958). Unrestrained larvae may remain submerged for 1 or 2 hours (Mitchell, 1907). If the rearing container is tapped, the larvae show an alarm response but resurface within 4 minutes (Mellanby, 1958).

Larvae are not readily affected by water pressure; they will voluntarily descend 5 and 6 feet and have been driven to 8 feet of depth with no adverse effects (Macfie, 1923). They can be held continually submerged up to the last instar and will develop in aerated water (Hopkins, 1936). However, the 4th instar larvae showed only 25 percent survival when submerged for 20 hours (Macfie, 1917). The larvae can withstand submergence until they lose the power to absorb liquid from the tracheae. The anal gills are the only surface freely permeable to water (Wigglesworth, 1938).

Hypertonic salt solutions with mono- and bivalent ions cause depletion of water from the hemolymph (Wigglesworth, 1933). When placed in solutions containing radioactive sodium, 90 percent of the material was taken up by the anal papillae and 10 percent by gut absorption (Treherne, 1954). The larvae can concentrate sodium, potassium, and chlorides from very dilute solution into the hemolymph (Wigglesworth, 1938; Ramsey, 1953). Low concentrations of radioactive phosphorus and strontium, absorbed by the larvae, produce no effects on growth. Higher concentrations cause failure in the development of the ovaries, and still higher concentrations cause failure to achieve pupation (Bugher and Taylor, 1949).

PUPAL STAGE. Pupation has been observed to occur occasionally at temperatures as low as 23° F. (Hatchett, 1946), but the best water temperatures are in the range of 82° to 90° F. (Omardeen, 1957). The length of time spent in the pupal stage varies from 1 to 5 days depending upon water temperature (Mitchell, 1907). Within the 82° to 90° F. range a difference in development time is evident for each sex; the males require an average of 1.9 days, the females 2.5 days (Shannon and Putnam, 1934).

The younger pupae are less susceptible to extremes of water temperature; pupae less than 36 hours old showed 50 percent mortality following 65-minute exposure to 109° F., whereas the older pupae, with only a 40-minute exposure, showed the same mortality rate (Bar-Zeev, 1957). With 5-minute exposures to 115° F. survival values have ranged from 20 to 84 percent probably as a function of age of the pupae (Macfie, 1920; Farid, 1949). Even at 117° F. for 5-minute exposures some pupae survive (Farid, 1949). The ability of pupae to withstand cold water is demonstrated by 82 to 100 percent survival following 24-hour exposure to 40° F., but only 15 to 22 percent survival when the exposure was extended to 72 hours (Woodhill, 1948). Pupae will show normal development when held at 40° F. during the entire period (Bacot, 1916).

Pupation is not influenced by the periodicity of light and dark intervals (Haddow *et al.*, 1959). At the time of ecdysis from the 4th instar larval skin, the respiratory trumpets spring up and attach the pupa to the surface film. The appendages of the head and thorax, initially free, become cemented to one another (possibly by the ecdysial fluid), form a shield over the front and sides of the body, and retain a ventral bubble of air which causes the pupa to float (Christophers, 1960). When alarmed, pupae descend in the water. Forced to a depth of 90 to 100 cm. pupae show distress, and those forced to a depth of 210 cm. were unable to ascend (Macfie, 1923). Pupae withstand drying with fair success. Fully developed pupae held on wet filter paper for 24 hours were able to develop into adults after being replaced in water. Half-developed pupae were able to complete development under similar conditions (Shannon and Putnam, 1934). When exposed to radioactive phosphorus or strontium solutions the pupal cuticle proved impermeable to concentrations readily absorbed by the larvae (Bugher and Taylor, 1949).

For certain purposes separation of the pupae from the larvae and of the male and female pupae is desired. If magnetic iron oxide dust is added to the rearing containers, the larvae ingest the particles and become magnetic. About 1 to 1.5 hours before pupating, the larvae evacuate the iron oxide dust from the gut and become nonmagnetic. By taking advantage of photo-negative responses of larvae and pupae they can be directed under a magnet. The nonmagnetic pupae and prepupae pass freely under the magnet while the magnetic larvae are trapped (Fay *et al.*, 1963). It is also possible to separate larvae and pupae by pouring the contents of a rearing container into a mechanical separator, which by means of an adjustable slit permits the free flow of larvae but traps the pupae (Fay and Morlan, 1959). Separation of the male and female pupae may be obtained with other equipment by the same principle since the male pupae are smaller than the female pupae (McCray, 1961).

When exposed to irradiation with cobalt-60 for sterilization purposes, the female pupae are more sensitive than the males. The young male pupae, less than 24 hours old, are more easily killed by irradiation than are the older pupae (Fay *et al.*, 1963).

ADULT STAGE. Just prior to adult emergence, air appears between the pupal and adult cuticles and also in the alimentary canal. The increased pressure splits the pupal cuticle along the middorsum of the thorax. The adult swallows air distending the stomach, and this forces the body of the adult up from the pupal case. There is an increase in blood pressure that maintains the legs rigid until they are dry (Marshall and Staley, 1932). The course of adult emergence is not affected by light (Haddow *et al.*, 1959). The average weight of newly emerged adult males and females ranged from 1.18 to 1.40 mg. and from 2.4 to 2.9 mg., respectively, depending on degree of crowding during larval development. Delayed emergence resulted in lighter weight adults (Morlan *et al.*, 1963).

At emergence the antennal fibrillae of the male cling to the shaft of the antennae but become erect and functional as hearing organs after 15 to 24 hours. The terminal segment of the abdomen also undergoes a rotation of 180° during the first 1 or 2 days after emergence, and copulation then becomes possible (Roth, 1948).

The wing beat frequency of both sexes increases during the first 3 days of adult life. The male frequency is 100 to 200 counts per second higher than that of the females (Tischner and Schief, 1955).

With regard to temperature, the adults are killed in 24 hours at 43° F. and by prolonged exposures at 45° to 48° F. The best temperature appears to be approximately 79° F. (MacGregor, 1915). Colony maintenance at 97° F. has not been possible (Davis, 1932), and 106° F. proved fatal with relatively short exposures (MacGregor, 1915).

At 100 percent relative humidity and 50° F., unfed adults of both sexes lived about 30 days. At 73° F. and less than 70 percent relative humidity, the average

life of the males was 4 days and the females 5.5 days; with 80 to 90 percent relative humidity, the averages increased to 6.5 for males and 8.5 to 10 days for females; and at 100 percent relative humidity the values were 12.5 days and 15.5 days, respectively. At 86° F. and 100 percent relative humidity all adults died in 10.5 days (Lewis, 1933).

When water alone is available for imbibing, the mosquitoes will survive at least 10 days, and the females will be inseminated (Macfie, 1915). The longevity of both sexes is increased by feeding on honey, and non-blood-fed females have been held for 102 days (Goeldi, 1905). The non-blood-fed females showed a mean age of 82.2 days, whereas blood-fed females averaged only 62.2 days (Putnam and Shannon, 1934). With increased blood feedings the mortality rate is apparently a function of oviposition since virgin females did not experience a shortened life span (Lavoipierre, 1961). The type of host is another factor entering into the life span. Females fed on gecko showed significantly lower longevity than females receiving human blood (Toumanoff, 1949). Blood from one type host may have a more acceptable ratio of amino acids than from a second species of host (Dimond *et al.*, 1956).

Adults from larvae that required over 30 days for growth seldom fed on blood (Hatchett, 1946). Normal adults will feed on the second or third day after emergence when offered a blood host (Macfie, 1915). Immediately after emergence the adult excretes a highly nitrogenous material, mainly uric acid. Then, if maintained on a sugar diet, low nitrogenous excretion occurs. Within 1 to 2 hours following a blood meal, a watery fluid is excreted, and thereafter excretory products are dehydrated and solid. (Terzian *et al.*, 1957).

When the food source is nectar or sugar solution, the food is stored in the oesophageal diverticula; however, blood is channeled to the stomach. Mixtures may go to either site depending on relative concentrations of nectar and blood (Hosoi, 1954). At a single feeding, 3-day-old females took 1.06 to 3.16 mg. of blood or an average of 2.07 mg. (Roy, 1936). Using a blood source having cerium-144, the volume was 4.2 $mm.^3$ representing a weight gain of 2.5 to 2.7 $mm.^3$ of blood and excretion of 1.5 $mm.^3$ (Boorman, 1960).

The adult females fed on erythrocytes through a membrane but not on plasma (Hosoi, 1954). When heparinized chick blood was at air temperature, only 20 percent of the females would feed, but when air temperature was 75° F. and the blood temperature was 107° F., 51 percent fed and at air temperature of 82° F. and blood temperature of 107° F., 71 percent fed (Bishop and Gilchrist, 1946).

The adults feed anytime between sunrise and sunset, mainly in the late afternoon; some feed in the early morning but never at night (Goeldi, 1905). The females may take 17 to 40 blood meals on humans in a 25- to 50-day period (Toumanoff, 1949). Adult females have been observed to feed at 57° to 59° F. in the presence of high humidity (Davis, 1932; Lumsden, 1947). Even at 77° to 86° F. the females bite less readily at 60 to 70 percent relative humidity than they do at 95 percent relative humidity (Lewis, 1933).

Adult mosquitoes respond to external stimuli of various types (Laarman, 1958). The flight activity is stimulated by changes in atmospheric pressure of 7 mm. Hg. or more. Adults can be acclimatized to static pressures ranging from 550 to 800 mm. Hg. but require from 3 to 6 hours at a given pressure (Haufe, 1954).

The direction and speed of flight is conditioned to a large extent by visual stimuli, since the mosquitoes compensate flight to reduce movement of the background lateral to their eyes or from back to front. In daylight, 82 to 85 percent of test mosquitoes flew upwind but in the dark only 55 to 63 percent moved upwind. With a moving background and no wind the adults flew in the direction of the background at a forward movement at 17 cm./second. In a 33 cm./second wind their

speed was increased to 49 cm./second to give a 16 cm./second forward movement. Air speeds were increased to 150 cm./second to compensate for increased winds (Kennedy, 1939). Since the mosquitoes cannot orient by tactile responses in flight, they must rely on visual stimuli reinforced by odor, humidity, and temperature stimuli (Kalmus and Hocking, 1960). Amputation of flagellar segments of the females progressively lessens response to the various stimuli (Bassler, 1958).

Mosquitoes are attracted to tyrosine, lysine, alanine, mixtures of aspartic and glutamic acids, serine and threonine, proline and histodine, as well as to resorcinol and to catechol (Roessler, 1960; Brown and Carmichael, 1961). In addition, certain dilutions of human urine have attracted mosquitoes (Roessler, 1961). The presence of carbon dioxide causes activation, orientation, and alighting, but temperature appears to be a more powerful factor (Burgess, 1959). Addition of 10 percent carbon dioxide to warm dry air increased its attractiveness for mosquitoes but not when added to warm moist air (Brown *et al.*, 1951). Rats cooled below room temperature were less attractive than rats at or above room temperature (Kingscote and Francis, 1954). When given a choice of a warm or cold hand of a given individual, the females chose the warm hand. When both hands were the same temperature, the choice was the drier hand (Smart and Brown, 1956).

No difference is evident in the biting avidity of virgin and mated females for the first few days (Seaton and Lumsden, 1941), but the biting activity of the older virgin females is less intense than that of mated individuals (Lang, 1956). Both virgin and inseminated females show a 4- to 6-day cyclic pattern of probing when exposed to warm moist air and activated by carbon dioxide (Burgess, 1959).

The adult males are not ready to mate until 24 hours after emergence during which time the genitalia rotate through 180° (Seaton and Lumsden, 1941). The terminalia of newly emerged females are too retracted for successful copulation (Wheeler and Jones, 1960); and, furthermore, the females are not attractive to the males prior to attaining a higher wing beat pitch about 2 to 2½ hours after emergence. Wing beats in the range of 320 to 512 vibrations per second are attractive to males with the highest attractiveness at 480 vibrations per second (Roth, 1948).

Copulation normally requires less than one minute and takes place in flight (Roth, 1948). Partially engorged females mate most readily; fully engorged females mate rarely. Experiments have indicated that 12, 50, and 82 percent of the females are mated at 24, 48, and 72 hours, respectively, after emergence (Seaton and Lumsden, 1941). With a series of females, one male was observed to copulate 17 times but transferred sperm only 6 times (Roth, 1948). In another test, one male mated with 8 females. The first three females laid fertile eggs; the fourth and fifth females laid non-fertile eggs, while the sixth to eighth females did not oviposit. It was concluded that the fourth and fifth females were stimulated to oviposit by seminal fluid that lacked motile sperm (Gillett, 1956). Decapitation of males of this species has not enhanced mating activity (Schwartz, 1961).

A single mating may suffice for all egg production since one female produced over 700 eggs in 15 batches during 62 days following a single mating (Bacot, 1916). Blood meals are essential for egg development and oviposition. A non-blood-fed, inseminated female did not oviposit during a 102-day period; then, following a blood meal, fertile eggs were produced. The test further indicated that sperm within the female remain active for long periods (Goeldi, 1905).

With chemically known diets, ten amino acids seem essential for egg production. There appear to be favorable proportions of the various amino acids that may make one species of blood host preferable to the next. Sodium and potassium salts also appear necessary for good egg production since in their absence egg production is reduced by one half (Dimond *et al.*, 1956).

Females from undernourished larvae require two blood meals to mature the first batch of eggs. Females from larvae reared in "rich" medium laid twice as many eggs in the first batch as did females from larvae reared in "poor" medium (Mathis, 1938).

About 0.82 mg. of blood represents the minimum necessary for production of the first egg batch, and about 0.5 mg. is necessary for the second egg batch. In producing the second egg batch, females that obtained 0.5 and 0.8 mg. of blood produced 15 and 33 eggs, respectively. Those receiving 1.3 and 2.0 mg. of blood produced 40 and 58 eggs as averages, and those receiving 2.8 mg. of blood produced 71 eggs (Roy, 1936).

The minimum ovulation period is about 3.4 days and is regulated by temperature. The length of the period is shortest at 81° F. in tests over a range of 73° to 84° F. (Putnam and Shannon, 1934).

In seeking an oviposition site the females explore all heights in flight, frequently descending to the ground. If the female lights on the surface of salty water, she leaves in a few seconds; if the water is fresh, she walks or flies to a suitable edge (Kennedy, 1942). For oviposition *A. aegypti* females prefer 0.021 M sodium chloride to either distilled water or 0.043 M sodium chloride. In the absence of 0.021 M NaCl the 0.043 M solution was preferred over the distilled water (Wallis, 1954).

The influence of light on the choice of oviposition sites is quite variable. One strain laid 68 percent of the eggs at a site having 0.02 lumens/sq. ft.; a second strain laid only 14 percent of its eggs at the same site. The competing site had light intensity of 3.5 to 6.5 lumens/sq. ft. (Wood, 1961). There is apparent choice of a rough surface over a smooth surface (O'Gower, 1957). In one study, when the water was clear, all eggs were laid on floating wood chips. With slightly foul water, eggs were laid both on the chips and the water surface, and with very foul water all the eggs were deposited on the water surface (Roubaud, 1929).

Oviposition begins on the 6th or 7th day after emergence and on the 3rd or 4th day after a blood meal. Subsequent batches are deposited at 3- to 4-day intervals with as many as 15 batches in a 50-day period (Macfie, 1915). Oviposition is rhythmical and is induced by a light period followed by dark. Oviposition peaks commence about 24 hours following a single light period. Without this stimulus, oviposition is highly irregular and non-periodic (Haddow *et al.*, 1961).

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