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LARVAE OF ANOPHELINE MOSQUITOES, WITH FULL DESCRIPTION OF THOSE OF THE INDIAN SPECIES

BY

I. M. PURI

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PART I.
GENERAL.

BY
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INTRODUCTION.

Although, since the publication of the important paper of Nuttall and Shipley (1901) dealing with the detailed external morphology and biology of the larva of A. maculipennis Meigen, a great deal of work has been done on the larvae of anopheline mosquitoes of the various countries, yet our knowledge of the larvae of the different species is far from adequate. Martini (1923) and Root (1924) are, besides Nuttall and Shipley, about the only other authors who have described the external morphology of the larvae in detail, Martini dealing with the European, and Root with some of the American species. Most of the other workers in their descriptions of the larvae have dealt with only such characters as are useful mainly in differentiating the various species, usually only those occurring in the particular locality with which the author happened to be connected. Consequently, their descriptions are of little or no help in making a general comparative study of the larvae of different species in any group or subgenus.

During recent years a number of papers have been published redescribing briefly larvae of some of the Indian species, again giving only the main differentiating characters. Moreover, these papers deal with only a few species at a time and no comprehensive or comparative study has so far been made. Where larvae of all the Indian anopheline mosquitoes have been dealt with in literature, the authors have mainly made use of descriptions and figures already published, in many cases vague enough to apply equally well to larvae of more than one species. It was to meet this deficiency and to make a comprehensive study of every detail of external larval structure of the large number of species occurring in India, and especially to work out the affinities as shown by larval characters between those, that an inquiry on the larvae of all the Indian anophelines was started about three years ago at the suggestion of Brevet-Colonel S.R. Christophers, F.R.S., I.M.S. (Director, Central Research Institute, Kasauli), and financed by the Indian Research Fund Association, Delhi. As a result of this exhaustive study it has now been possible to give detailed descriptions of the larvae of all the anopheline mosquitoes so far recorded from India and to compare with them larvae of some of the species occurring in other countries.

For the study of the morphological characters of the larvae of various species mainly the last larval moults were in the first place used, the larvae themselves being examined only after the moults had been thoroughly worked out. The special features of this work have been, firstly, to use as far as possible only last larval moults of which the corresponding adults were available for study, secondly, to make a minute examination of all the characters of the larvae and, thirdly, of corroborating them by examining, when available, a large number of specimens of each species and if possible collected from different parts of India. In the case of three species (A. ludlowii, A. tessellatus and A. multicolor), however very few specimens of each were available to me for study.

To avoid any confusion, most of the large number of specimens used were collected, reared and numbered by myself, nobody else being allowed either to number or to handle any of the numbered specimens; whether larval mounts or adults. The larvae of some of the species, however, were collected and bred out, at my request, by a number of other research workers to whom I wish to express my thanks for their very generous help. I am indebted to Major G. Covell, I.M.S. (Assistant Director, Malaria Survey of India), for specimens of ludlowii (from Andamans), to Dr. G.C. Ramsay (Labac Central Hospital, Cachar, Assam) for some specimens of aconitus, philippinensis and kochi, to Mr. R. Senior-White (Malariologist, B.N. Railway) for specimens of aconitus and to Dr. K. Raghuvindra Rao (Special Malaria Officer, Madras) for specimens of culicifórmis, sintoni, majidi and tessellatus, to the last named also for his great help to me during my tour to parts of Madras Presidency. To Mr. F.W. Edwards, M.A. (Natural History Museum, South Kensington, England), I am grateful for his kindness in lending me mounted last larval moults of A. multicolor and to Captain P.J. Barraud, Entomologist to the Malaria Survey of India, I am indebted for some specimens of vagus and hyrcanus collected by him in Assam.

To Dr. D.P. Curry, Assistant Chief Health Officer, Panama Canal Zone, I am grateful for the large collection of bred out isolated specimens along with the corresponding last larval moults of a number of species from the Panama Canal Zone and to Dr. C. Manalang for the large number of A. minimus adults along with their isolated corresponding last larval moults from the Philippine Islands. My thanks are also due to the Director, Zoological Survey of India, and to Dr. B. Prashad, Superintendent, India Museum, Calcutta, for giving me every facility for work at the Museum and for dending me books out of their valuable library whenever requested to do so. To Major J.A. Sinton, Director, Malaria Survey of India, I am deeply indebted for his kind help by way of material as well as literature and for allowing me the use of their very valuable collection of adult anopheline mosquitoes from different parts of the world. In conclusion I wish to express my sincere gratitude to Colonel S.R. Christophers, C.I.E., O.B.E., F.R.S., I.M.S. for the keen interest he has taken in the work and for his many valuable suggestions in connection with it, and also for giving for study his very useful collections of adult anophelines and the corresponding last larval moults from Canary Islands and Sierra Leone.

HISTORICAL

Nuttall and Shipley (1901) have traced the first mention of the larva of anopheles in literature to Joblot (1754) of Paris and have enumerated the very scanty references to these insects up to 1900. Since the publication of their classical work, however, a large number of papers have been published dealing with anopheline larvae and during the last twenty years the literature on this subject has increased to such a degree that even to give a list of the papers dealing with their external morphology and biology is a considerable task. Mention may, however, be made of the important works of Howard, Dyer and Knab (1912), Root (1922, 1924 and 1926), Martini (1923), Swellengrebel (1919) and of Christophers (1916, 1922), who, among a large number of other workers, have added greatly to our knowledge of anopheline larvae.

BIONOMICAL

Habits and habitat.

Anopheline larvae are purely aquatic, breeding in collections of water of almost every description. Some species breed exclusively in running water with plenty of sunlight, while others prefer somewhat shady streams and still others breed in streams which are in very heavy shade. Of those species which are found in standing water, some breed in wells, some in shallow temporary collections, and larvae of some are found exclusively in water contained in tree-holes and leaf-bases of epiphytic plants. Some species breed only in saline waters while some appear to be indifferent in the choice of their breeding-place. In fact there are one or more species of anopheline mosquitoes connected with almost every kind of temporary or permanent water collection, each species more or less restricted to its own particular type of breeding-place.

Larvae live freely in water and are very active swimmers. They are apodous, not being provided with any limbs, swimming about with their tail-end foremost, by a rapid wriggling movement of the whole body. When undisturbed they lie flat against the surface film of water, usually at right angle to any foreign object or to the edge of the water container. On being disturbed they swim rapidly, tail first, to the bottom of the container where they lie motionless as if dead, often floating about slowly with the currents of water. After a few moments they again swim tail foremost to the surface of water and coming to lie nearly flat against the surface film, wriggle rapidly along it to the edge of the pool or to a floating debris. They are provided with a number of strctures (e.g., palmate hairs, spiracular apparatus, etc.), which are unwettable, owing probably to the secretion on them of some waxy substance. When a larva swims to the surface these structures break the surface film of water and the larva remains floating without any effort on its part, the force of cohesion of the film supporting the weight of the larva which would otherwise sink to the bottom, being heavier than water. That these structures on the larva break the surface film of water when the former is lying at the surface is obvious from the fact that a slight wind disturbing the surface of water wafts the larva about with it.

As has been mentioned already, larvae when lying flat against the surface film of the water usually rest at right angles to a foreign object, with their tail-end touching it. This happens only if the foreign object against which they are resting is 'wet', as on the other hand, if it be 'dry', the larvae lie parallel to it, with nearly the whole of their body touching it. Hacker (1925) is of opinion that the characteristic horizontal position of the Larvae is due to the phenomena of 'differential wetting' or in other words to the action of the surface tension of water, by which Watson (1920, p.188) has explained the apparent attractions or repulsions exhibited by small floating bodies on the surface of a liquid. Two floating objects moistened by the liquid (on which they are floating) are 'attracted' to each other, as also are two floating objects when neither of them is moistened, while a moistened and a non-moistened floating object are repelled from each other. According to Hacker the anal segment of the larva bearing the spiracles and the caudal hairs is 'wet' and therefore attracted to the edge (when that is also 'wet') while the spiracles are 'dry' and therefore repelled, forcing the larva out into this characteristic horizontal position. This explanation does not seem to be correct as the phenomena of 'attraction' and 'repulsion' owing to the action of surface tension of water is applicatble only to such objects as show a concave or convex meniscus (Atkinson and Reinold, 1902, p.116) when lying at the surface. The anal segment of a larva is completely submerged below the surface of water and is not 'wet' in the same sense of the word as is a floating piece of wood. It could have been attracted towards the 'wet' edge only if it had been somewhat above the surface level of water and thus had a concave mensicus surrounding it. The majority of anopheline larvae, when they swim to the surface of water, wriggle tail first towards any foreign object probably by instinct and do not remain free in the open, a prey to all their enemies. They wriggle actively with their tail-end foremost and consequently it will be this end which will come in contact with any foreign object first. Moreover, as the larvae float flat against the surface film of water, if they were to lie with their head-end touching a wet foreign object, this, the heavier end of the larvae would be at a higher level than the tail-end or the lighter end. This would be applicable also in the case of a larva touching a 'dry' foreign object were it to lie with only its tail-end touching the latter. Both these positions are obviously highly improbable. Whether the horizontal position of the larva is due to a number of physical forces acting together in conjunction with the action of the surface tension of water or whether it is simply explained by the method of wriggling of the larva with its tail-end foremost or probably to both is, rather, difficult to say definitely. The fact, however, remains that a larva coming within the range of the curve of a meniscus (concave) in the vicinity of any foreign floating object is attracted towards it if most of its body is supported by the cohesion of the surface film of water. This can be easily shown by the following simple experiment: Kill a few larvae by formalin vapour while the former are floating fat against the surface film of water. Very gently disconnect the palmate hairs of some of them from the surface film. It will be noticed that these larvae when brought near the wet edge of the dish will not touch the latter though they will float up along the concave meniscus. The distance of the tail-end of these larvae seems somehow to depend on the angle the body of a larva makes with the surface film and appears to be directly proportional to it. This is also seem in nature as anopheline larvae not possessing any palmate hairs or having very weak ones (such as A. turkhudi) when supported against the surface film only by their spiracular apparatus, the rest of their body not touching it, are not attracted to the edge. That the attraction of a larva to the edge is not due to the so-called 'wetness' of the tail-end can very easily be demonstrated. The anal segment is undoubtedly completely submerged, the surface tension thus having no effect on its being attracted or repulsed. The dorsal caudal hairs, however, may be supposed to come up slightly above the surface film and to produce a concave meniscus, they being wettable, but the larva coming in the range of the curve of the meniscus is attracted to the edge even when the caudal hairs are cut off completely. From the fact that culicine larvae and such anopheline larvae as hang down from the surface film of water in a like manner, although they possess a so-called wettable analsegment, are not attracted to the wet edge, it appears that the suspension of the whole length of the larva from the surface film, by means of a number of other structures besides the posterior spiracular apparatus, is an important factor in determining the position taken up by the larva against a foreign object. The dorsal surface of the spiracular apparatus is 'dry,' except for a narrow area along the posterior border of the scoop (vide infra). This narrow area along the posterior end of the spiracular apparatus is wettable and probably holds the explanation for the apparent attraction of the posterior end of the larva towards a 'wet' object, the rest of the non-wettable spiracular apparatus, and the suspension of the larva from the surface film by means of the other 'dry' structures, accounting for the right-angled position and the distance of the larva from a 'wet' object respectively.

Method of feeding.

Anopheline larvae, although at times they are seen browsing at the bottom and the sides of the container in which they happen to be breeding, are pre-eminently adapted for feeding at the surface of water. This is shown by the presence on them of a number of leaf-like structures which help them to maintain their position at the surface without any exertion and by their ability to rotate their head through an angle of 180°. Nuttall and Shipley have already described in detail the method of feeding of the larave at the surface. An anopheline larva coming to the surface of water attaches itself to the surface film by the help of its spiracular apparatus, palmate hairs and a pair of flat, bilobed, striated organs* situated at the anterior end of the thorax. Almost at the same time it turns its head suddenly on its neck as an axis, through an angle of 180° and raises it slightly with a jerk, breaking the surface film of water with the leaf-like appendages at the tip of the maxillary palps and with the submentum (Plate VI, fig.6). The mouth-brushes or the cephalic fans then commence a rhythmical movement setting up currents which bring food particles toward the mouth.

Christophers and Puri (1927), while explaining as to why anopheline larvae feed at the surface, have described two methods of feeding, 'film-feeding' and 'free-feeding,' and have also described the various food currents produced in water at the time. The mechanism of feeding is more or less the same in both the cases except that in the former the larva feeds on the bacterial film, formed at the surface, dragging it by a slow stroking movement of the mouth-brushes. In the case of free-feeding, however, 'the fans are worked with a rapid rhythmical almost vibratile movement and extremely active currents are set up near the surface. All particles lying just beneath the surface film, as they come within the range of the currents, are swept towards the mouth, passing beneath the actual surface film which is little if at all disturbed,' 'Particles can be seen commencing to move towards the larva from a distance of at least the larval length' (at times even three to four larval lengths). This is the incoming current (Plate II, fig.1). The depth of this current was found by them to be not more than about the thickness of the larval head and is clearly a very shallow and superficial disturbance of water (Plate II, fig.2). The outgoing currents are normally freed from particles and so are practically invisible, going out at right angles to the head on either side (Plate II, figs.1 and 3). 'They are powerful rapid circumscribed currents like the gulf-stream leaving the Caribbean Sea. They are caused by the main incoming current being deflected by the smooth outer surface of mandibles which are kept closed,' when the fans are working.

As described already, the maxillae and submentum make contact and even protrude from the surface film. They thus block all backward exit for incoming currents, i.e., all passage except laterally under the maxillae. The maxillae are kept in constant vibratile movement and comb the cur-

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* Nuttall and Shipley's Organ described later on.

rent as it passes beneath them. They are provided with a large number of fine setae on their dorsal surface and these can remove even such small particles as Bacillus coli. The water thence striking the mandibles is shot out at right angles as described.

A culex larva while feeding (Plate II, fig.4), hanging down from the surface film, moves forward all the time when its mouth-brushes are working. Anopheline larvae on the other, in spite of the fact that the currents set up by the movement of their mouth-brushes are comparatively much stronger than those set up by a culex larva, remain stationary whether resting against a foreign object or not. Iyengar (1928) erroneously thinks that this difference in the behaviour of the two types of larvae is due to the presence of palmate hairs and the bilobed organ on the prothorax of the larvae of Anophelini. He says 'it is believed that the thoracic appendages by holding on to the surface tension act as anchoring-organs to maintain the larva in a constant position and withstand forward creep while working the mouth-brushes and setting up a current in water.' The surface film of water is not a tangible object and no structure on the body of any aquatic animal can hold on to it in such a way as to prevent movement taking place along the film. When any structures on the body ofthe larva are holding on to the surface film, surface tension saves the larva only from sinking and does not have any effect on the movement of the larvae in a horizontal direction unless the larva happens to be near a dry or a wet floating foreign object. The real reason of the difference in the behaviour of a culex and an anopheles larva lies in the fact that while feeding at the surface different types of water movements are set up by the two. In culex larvae feeding at the surface by the movement of the mouth-brushes, water is drawn up from below (Plate II, fig.4) as an incoming current and passing through the maxillae is shot out parallel to the surface behind the larvae. It is this backwardly directed outgoing current which propels the larva forwards when its mouth-brushes are working. In anopheline larvae, on the other hand, the incurrent comes from below only through a depth of the larval head (Plate II, fig.2) and striking the surface film runs parallel to it towards the head of the larva. This incurrent is in the form of two eddies (Plate II, fig.1 and 3) one on each side. The outgoing current is shot out at right angles to the head of the larva on each side and has for this reason no propelling effect on the larva which remains stationary while it is feeding. When a fairly full-grown larva comes in the direction of the ingoing current of even a comparatively much smaller larva, the former is carried away with the currents produced by the latter. This also shows that the various structures of the larva holding on to the surface film are not able to prevent any movement along the surface film.

Food.

According to Purdy (1924) and to Christophers and Puri (1927) the surface layer of water contains abundance of minute organisms and it is on these that the anopheline larvae feed. The food consists mainly of fresh water algae of various kinds, diatoms, small ciliates and flagellates, small rotifers and other minute organisms which do not break the surface film. Only those organisms in the subsurface layer of water, that have a considerable power of swimming and are able to resist the incoming current set up by the mouth-brushes escape while all the rest are brought to the mouth with the food currents and are swallowed. As has already been observed by many workers, these larvae are omnivorous. They take in anything that is brought to the mouth-opening and do not seem to possess any power of selection of food other than to reject such objects as are too large. It may be said that a sufficiently minute freely floating object of any type (organic or inorganic, living or dead) or a weakly swimming organism that is carried to the mouth-opening with the incoming current will be included in the larval diet. Crushed lycopodium spores, particles of carmine and of silica put on the surface of water have been readily swallowed by the larvae and passed out in due course. Whenever an object too large to be swallowed is brought with the currents the larva bites it with its mandibles and keeps on nibbling it till either it is eaten up or it is carried away by other currents. The cast off skins of anopheline larvae keep on floating at the surface and when carried by the incoming current to any larva, they are swallowed in bits.

Anopheline larvae can easily be reared in the laboratory by feeding them on crushed spirogyra, a filamentous green alga, or on crushed chara adding a small quantity of the fresh alga every day or every other day. Some workers (James and Liston) are of opinion that the food of anopheline larvae consists mainly of minute aquatic animals and that a purely vegetable diet is not enough for them to grow on. Barber (1927) on the other hand, dealing with larvae hatched out from sterilized eggs, has shown that 'Algae alone, bacteria alone, or infusoria alone may constitute a sufficient source of food for anopheles larvae.' He used pure cultures of a motile unicellular green alga, possibly chlamydomonas, a large and a small variety of spirillum and colpidium, for algal, bacterial and infusorial diet respectively. I have also successfully reared from eggs a number of species of anopheles in the laboratory, feeding most of them on green algae and some of them on pure cultures alone. It was found that the larvae did not thrive on pure bacterial cultures (B. coli) and fared better when the cultures got contaminated with amoebae. Even with pure algal cultures* they did not thrive so well as with a mixed bacterial and algal emulsion. Larvae, which breed in tree-holes, in water contained in bamboos (and probably also those breeding in leaf-bases of epiphytic Bromelids), however, appear to be predaceous in habit and develop much better on animal food, such as crushed flies, etc., than on algal food.

Metz (1919), and according to him, Smith (1914) found larvae of crucians and punctipennis respectively breeding in clear water pools, the bottom of which was covered with dead leaves. They conclude from it that larvae may develop prolifically on dead, disintegrated plant tissue, but as has been pointed out already by Purdy (1920), one cannot judge by the apparent clearness of water alone, whether living larval food is abundant or not. It has been often noticed that water which appeared practically clear to the naked eye contained large number of microscopic organisms. Purdy has suggested that to demonstrate this fact, anopheles larvae should be placed in a watch glass of apparently clear but unfiltered water (as from a ditch or puddle) and their feeding process observed under a low magnification. It will be seen that visible masses of food accumulate in the semi-transparent 'throat' of the larva, and are swallowed at the rate of 7 to 10 time per minute. In his experiments of rearing anopheline larvae on disintegrated plant tissue, Metz introduces small larvae to sterilized water to which dried and ground leaves had been added. Various organisms, both animal and plant, often stick to the body of the larva and spores of a number of organisms are very often present on dried leaves. All these are bound to develop quickly in a culture medium produced by adding ground dried leaves to sterilized water. Barber, using larvae reared from sterilized eggs and dealing with pure cultures, came to the conclusion that dead organic material, in culture at least is far less suitable than living organic material as a source of food.

A number of writers, such as Williamson (1926) and Boyd (1926), have successfully reared anopheline larvae on yeast. According to the latter who used Fleishman's yeast, this diet is much better than algal food (spirogyra). In my

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*Pure cultures of a motile, unicellular green alga closely related to chlamydomonas.

rearing experiments I, however, found that these are some drawbacks in using yeast instead of green algae or chopped up small insects. When yeast is being given as food the water in the culture pans has to be changed every day or every two or three days depending on temperature, this being unnecessary while using green algae or animal food. Moreover, it larvae are being reared for the purpose of collecting larval moults for the study of larval characters, yeast is certainly harmful as the yeast cells settle down on the larvae and obscure the various structures present on its body.

Colour.

Many writers are of opinion that the colour of the larvae is not constant and that it varies with the food. My observations, however, do not support this conclusion. There is no doubt that the gut contents showing through the more or less transparent body-wall do modify the colour of the larvae but the actual general coloration is due to a layer of pigmented fat-body cells lying beneath the epithelial lining of the body-wall. In some species, e.g., barianensis, the pigment in these cells is so dark that the seeing of gut through this layer is out of the question. That the nature of the food has no appreciable effect on the colour of larvae is clearly shown by the fact that larvae of a number of species, although reared on more or less the same food and under the same conditions, yet developed the different colours they have in nature. Larvae of barianensis were very dark grey while those of stephensi developed a pale greenish yellow pigment and culicifacies were pale grey. Similarly lindesaii were dark greyish green while turkhudi were pale yellow. Among the other species reared were superpictus, sergentii, pulcherrimus and maculatus, all of which developed a different coloration from one another.

The various silvery spots found on the dorsal surface of larvae of many species are due to the deposition of some crystalline substance, probably excretory in nature, in the epithelial and sub-epithelial layers of the body-wall in localized areas. These spots, however, fail to appear in some species when reared in the laboratory. That these spots too are not effected primarily by the nature of the food is seen from the circumstance that A. dthali and A. superpictus larvae although reared on the same kind of food and under the same conditions developed spots which differed in size and distribution in the two species and in A. sergentii, reared along with these two species under the same conditions they were altogether absent.

Duration of larval life and moults.

Except in certain specialized species, the duration of larval life varies from seven days to seven weeks directly depending on temperature and the amount of food available. During the summer months in most of the species in India it lasts from 7 to about 18 days, while in winter it extends to about two weeks in South India but between three to seven weeks in the colder regions. The type of food-supply also seems to have some effect on the duration of larval life as, according to Boyd, two batches of larvae hatched out together from the same batch of eggs, one fed solely on spirogyra and the other solely on Fleishman's yeast, took over a month and 9 to 13 days respectively to reach the pupal stage. In specialized species, i.e., those breeding in three-holes, the duration of larval life is very variable, often extending to over three months (especially during winter) and is comparatively longer than in species breeding in open pools. This seems to depend mainly on the peculiar habits of the larvae and does not appear to be governed by temperature or the amount of food-supply available. This is obvious from the fact that the larvae of maculatus var. willmori and stephensi took 3 to 6 weeks to reach the pupal stage while of those of **A.** barianensis, hatched out of eggs at about the same time and reared under the same conditions, had not pupated even after 9 weeks.

From the egg to the pupal stage a larva grows in length from about 0.8 mm. to 5-8 mm. varying in the different species and with temperature and food-supply. Temperature seems to have only an indirect effect on the size of a larva. During the summer months larva undergo development quickly and hatch into comparatively smaller adults than those hatched out of larvae developing slowly during the winter months but feeding voraciously as usual. That the amount of food available has an appreciable effect on the size of growing larvae can easily be shown by rearing separately two batches of larvae hatched out more or less from the same lot of eggs simultaneously. One batch is supplied with abundance of food while the other fed very sparingly and the full-grow larvae will be seen to vary greatly in their size, those in the latter batch being much smaller than those inathe former.

During its whole life a larva passes through four instars, ecdysis taking place at the end of each and pupation occurring after the fourth moult. At the time of ecdysis a larva comes to lie at the surface, a slit appears on the head along the lateral and posterior borders of the frontoclypeus, extending anteriorly to the region of the antennae, and another slit on the thorax along the mild-dorsal line reaching backwards to about the region of the meta-thorax. The head and thorax are then withdrawn and the body is gradually drawn out by slow peristaltic movements. In most of the species living in the open, healthy larvae moult every 2 to 5 days depending on temperature but the duration of any of the instars may be lengthened. In species breeding in streams, pools, wells or swamps larvae hatched out of a batch of eggs about the same time reach the pupal stage more or less together, the duration of the various instars being about the same in all the larvae. In specialized species breeding in tree-holes, i.e., barianensis, on the other hand, larvae from the same batch of eggs reach the pupal stage at varying intervals, so that only few at a time reach the adult stage. In a brood of larvae hatched out of the same batch of eggs, more or less simultaneously some larvae may reach the pupal stage while the rest may still be in the first or second larval instar. The duration of the larval life is thus very variable in the larvae breeding in tree-holes, some reaching the pupal stage in about three weeks while others may be in the larval stage even after three months. This retarding of the growth of some larvae in the same batch, growing under the same conditions and in the same receptacle is obviously due to the peculiar habits of the larvae and not to the effects of temperature and food-supply, both of which are the same for all larvae in that particular batch. Owing to the scarcity of their peculiar breeding-;laces, this hatching of only a few adults at a time from the same brood of larvae ensures the propagation of the species.

TECHNIQUE.

Preserving and mounting larval moults.

As the whole of this study on the larvae was primarily carried out mainly on the last larval moults of the various species it will not be out of place to describe the procedure used for collecting, preserving and mounting larval moults. The following method was employed throughout:--

Collecting and preserving.-- From a batch of larvae collected from any particular locality full-grown larvae just about to pupate are picked out and separated into sets of larvae having the same general naked-eye appearance, which is noted down. Larvae of each of the sets are then placed separately in some clean water in specimen tubes (3" X 1"), each tube having a single larva, with a letter written on the outside with a grease pencil, indicating the set to which any particular larva belongs. Soon after a larva pupates, the larval moult is transferred to a small specimen tube ($1\frac{1}{2}$" X $\frac{1}{4}$") by means of a medium-sized pipette; the tube filled with alcohol 70 per cent, a printed number,* kept ready beforehand for the purpose, placed inside the tube which is then plugged with a small pith-cork† and put in a large bottle or jar containing alcohol 70 per cent. A number, corresponding to that placed inside the small tube in which the larval moult is preserved, is also written with a grease pencil on the large specimen tube in which the corresponding pupa is left to hatch out, and the tube closed with a clean dry cotton-wood plug. When the mosquito emerges out of the pupa it is transferred to a dry specimen tube and the pupal exuvia put into the small specimen tube containing its larval moult placing with it another printed number corresponding to that for the larval moult but of a different colour. After the adult has been in the dry tube for about an hour or so it is mounted dry in the usual way with a printed number, corresponding to that for the larval and pupal moults but of a different colour, pinned under it. This system of using different coloured sheets of similar numbers for the three stages in the life history reduced the chances of error to the minimum.

Each of the adults is then examined carefully and its identification entered in the register against the number corresponding to it, and where particulars about locality, dates of pupation and of emergence of imago, and the general naked eye appearance of the larvae had already been noted. The small specimen tubes containing the larval and the pupal moults are all stocked in alcohol 70 per cent in

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* Numbers used were 'Watkins' sheets of numbers from 1-10,000 sold in various colours. Different colours were used for the larval and pupal exuvia and for the adults--pink for larval moults, blue for pupal moults and yellow for the adults.

† Pith plugs are easily made with a cork borer, selecting the borer of a size a little larger than the diameter of the small specimen tube. Cores are taken out by this borer, cut into small pieces, and kept soaked in alcohol 70 per cent ready for use. Pith is much better than a cotton plug because there is no likelihood of the various hairs on the body of the larva getting entangled in it as they do in the case of cotton plugs.