

## THE BIONOMICS OF SOME MALAYAN ANOPHELINES.

By W. A. LAMBORN,

*Medical Entomologist, Malaria Bureau, Federated Malay States.*

A long series of breeding experiments were conducted by the writer at the Malaria Bureau, F.M.S., between April 1920 and April 1921 with a view to obtaining in bred families a sufficiency of Anophelines for the purpose of study which might perhaps set at rest, once and for all, various long-vexed questions as to the validity of certain species; and for the purpose of obtaining further data than were previously available as to the bionomics of the insects.

With regard to the former object it has long been recognised, for instance, that *A. hyrcanus* exhibits a very considerable degree of variation as to leg banding, colour of scaling on the wings and size of ventral tuft, which may even be absent. A not uncommon form of this mosquito occurs with leg banding so broad that a difference of opinion has arisen as to whether an insect so characterised should not be accorded specific rank, or should be treated possibly as a geographical race or a variation. The case is the more interesting by reason of the polymorphism of the larvae of this species. A similar difficulty has arisen with regard to the relationship, if any, between *A. subpictus* var. *malayensis* (until recently called *A. rossi*, Giles, in the F.M.S.), and *A. vagus*, which until quite recently was considered as being the var. *indefinitus* of the latter species. The females of *A. aconitus* are often found to be lacking the first black palpal band, a character more pronounced than some of those which have determined the bestowal of specific rank. *A. maculatus* is recorded as showing variations; and more recently attention has been directed to the variations of *A. umbrosus*.

Such variations, apart from their great interest to the student of heredity and variation, may well have considerable practical importance; for the indeterminate status of such Anophelines may serve to explain the discrepancies in the results obtained by various workers seeking to determine the relative malaria-carrying powers of the different species, and their apparent harmlessness in one country, or in one part of it, and their notoriety in another. *A. hyrcanus*, for instance, credited in the F.M.S. (where it is so variable) with being a harmless species, is considered to be an active malaria carrier in Japan and China, in which countries, so far as the writer was able to ascertain by the examination of some hundreds of larvae and adults, far less variation exists. It may well be that the forms are distinct species, that of the north, extending sparingly to the south, being a more active carrier of malaria than the southern forms. Such breeding work, the only true test of species, is therefore essential as a preliminary to any settlement of the question of the relative malaria-carrying properties of such species.

It is hoped that the questions of taxonomy will be dealt with elsewhere by an authority on the matter, the writer feeling strongly that such points are best left to systematists to deal with, attempts by those inexperienced in this special direction, and away from ample literature, tending to make confusion worse confounded.

In the following pages it is proposed to set forth only the facts of bionomic interest ascertained, with which will be included descriptions of Anopheline ova by Dr. A. T. Stanton, who has most kindly allowed his data to be added for the purpose of making the notes as full as possible. The drawings were executed for him by Mr. R. W. Blair. It will be noted that many of the lines of investigation are sketched out rather than fully developed, and that the data given make no pretence to be exhaustive; their presentation at this time is due to the unexpected transfer of the writer to another sphere of work at the end of the year.

### Methods of Breeding.

At the commencement of the work there was considerable difficulty such as has been experienced by other workers, which largely explains the absence of precise data on the life-history of Anophelines. The insects oviposited in any water provided, but though all sorts of water were tried in the laboratory for rearing the larvae, indifferent success was met with, even though water in which particular larvae occurred in abundance in nature was used and changed daily. Nor were better results obtained when the bowls containing the larvae of open country species were placed in situations comparable as regards sun, wind and temperature to those in which they are found in nature. For the small-pool breeders a hay infusion, in which a fairly luxuriant culture of small round green algae from such breeding-places had been obtained, yielded rather better results than were previously obtained for *A. vagus* and *A. subpictus*, and a few imagos of *A. maculatus* and *A. karwari* were obtained from the egg, the larvae feeding up on a species of *Spirogyra*. For certain species—*A. aconitus* and *A. maculatus*—gently-running water containing filamentous algae, such as are not readily washed away, was tried, and, at Dr. Hacker's suggestion, aeration of the alga-containing water was effected by means of a siphon. These devices were all to little purpose, so far as getting families of Anophelines sufficiently numerous for generalisation was concerned.

Stagnant and polluted water, containing a rich and almost pure culture of a *Euglena* (determined by Dr. Stanton as probably *E. viridis*) was then tried, and, as has been described elsewhere,\* on this unpromising medium were bred from egg to imago considerable families of all the open-country Anophelines: *A. hyrcanus*, Pall., *A. barbirostris*, Wulp., *A. maculatus*, Theo., *A. karwari*, James, *A. vagus*, Dön., *A. subpictus* var. *malayensis*, Hacker, *A. ludlowi*, Theo., *A. aconitus*, Dön., *A. kochi*, Dön., *A. fuliginosus*, Giles, and *A. tessellatus*, Theo. No success at all was met with in regard to *A. umbrosus*, and ova of *A. leucosphyrus* and *A. albotaeniatus* var. *montanus*, Stanton & Hacker, were not obtained, though young larvae of the former were readily bred to maturity on the medium.

The interpretation of the success met with in the use of this medium is possibly provided by the following paragraphs (from "Comparative Anatomy of Animals," i, 1909, by Dr. G. C. Bourne), though it is difficult to reconcile the apparent welfare of the "clean breeders" with their entire absence from such polluted water in nature, unless it is that the oxygen set free in abundance renders the products of decomposition harmless by oxidation immediately they are liberated.

"One of the most striking characteristics of *Euglena* is its green colour. . . . The green tint is due to the number of circular or oval discs known as chromatophors. . . . The green colour of the chromatophors is due to chlorophyll. . . . Just as green plants are able, through the agency of their chlorophyll corpuscles, to decompose the carbonic acid in the air, setting free oxygen and combining the carbon with water to form starch, so *Euglena* is able to decompose carbonic acid. . . . When *Euglenae* are kept in a vessel in bright sunlight bubbles are abundantly formed in the water in which they are contained, and these bubbles, if collected, can be shown to consist of oxygen." (p. 190.)

"It has been observed that *Euglena* will live and apparently flourish in complete darkness for as long as 39 days."

### The Reproductive Capacity of Anophelines.

The question is one of no little importance in connection with antimosquito measures. The ova laid by captive females were counted under a low magnification, and though very small errors in enumeration may have occurred by reason of the difficulty of the task, the data afforded as to each species are certainly correct within small limits. But the reproductive capacity of the insects, ample though it appears by the data, has probably been far underestimated, partly owing to the uncertainty

\* Bull. Ent. Res., xiii, pt. 1, p. 11, May 1922.

as to previous acts of oviposition on the part of the captured females, and partly by reason of their battered condition towards the end of long confinement, whereby the oviposition of more batches of ova may well have been prejudiced. The first of these difficulties, at all events, the writer saw no means of overcoming, since pairing between the insects in captivity will not take place, probably because the males are unable to execute the complicated dances which precede coupling. The following are the data :—

*Anopheles hyrcanus.*

The months of oviposition and the number of ova secured were :—

April 1920.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1921.	Feb.	March.
49	108	29	21	—	135	—	36	15	67	—	105
—	21	3	188	—	21	124	48	75	—	—	70
—	45	5	—	—	—	118	—	117	—	—	—
—	—	—	—	—	—	—	—	107	—	—	—
—	—	—	—	—	—	—	—	36	—	—	—
—	—	—	—	—	—	—	—	52	—	—	—

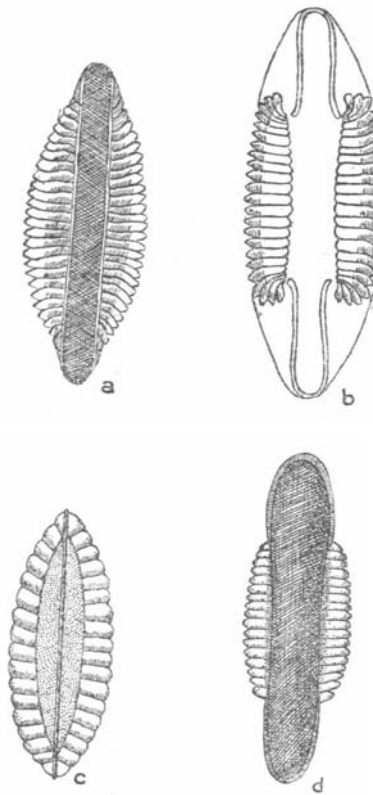


Fig. 1. Eggs of *Anopheles*: a, *A. hyrcanus*, Pall.; b, *A. maculatus*, Theo.; c, *A. aconitus*, Dön.; d, *A. fuliginosus*, Giles.

The maximum number of ova obtained from a single parent was therefore 188, and the average number from each parent, based on the data from 24, works out at 66. The following is a description of the ovum (fig. 1, a) :—" Length 0.56 mm., breadth 0.20 mm.; the upper surface is broad, 0.06 mm.; the floats are broad and cover three-quarters of the length of the ovum. Each float has thirty-two corrugations and these nearly touch the frill. The membrane on the lower surface has a reticulated pattern " (Stanton).

*A. hyrcanus* var. *paeditaeniatus*.

The months of oviposition and the number of ova secured were :—

April 1920.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1921.	Feb.	March.
—	90	—	—	—	—	—	55	—	—	147	40
—	10	—	—	—	—	—	—	—	—	22	—
—	13	—	—	—	—	—	—	—	—	—	—

The average number of ova for each parent, based on the data from 7, works out at 53, the maximum obtained from a single parent being 147.

*Anopheles barbirostris*.

The months of oviposition and the number of ova secured were :—

April 1920.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1921.	Feb.	March.
—	10	85	—	—	—	31	50	159	—	121	72
—	16	86	—	—	—	20	293	—	—	98	173
—	10	125	—	—	—	—	56	—	—	115	—
—	24	—	—	—	—	—	56	—	—	—	—
—	88	—	—	—	—	—	—	—	—	—	—

The maximum number of ova obtained from a single parent was 293, the average, based on the data from 20, working out at 69.

Dr. Stanton states that the ovum does not differ from that of *A. hyrcanus*.

*Anopheles maculatus*.

The months of oviposition and the number of ova secured were :—

April 1920.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1921.	Feb.	March.
—	18	143	34	42	—	56	100	80	20	—	—
—	—	—	37	30	—	128	123	20	22	—	—
—	—	—	95	—	—	71	16	72	56	—	—
—	—	—	—	—	—	64	—	72	—	—	—
—	—	—	—	—	—	30	—	300	—	—	—
—	—	—	—	—	—	12	—	24	—	—	—
—	—	—	—	—	—	81	—	70	—	—	—
—	—	—	—	—	—	81	—	17	—	—	—
—	—	—	—	—	—	36	—	15	—	—	—
—	—	—	—	—	—	—	—	68	—	—	—

The maximum number of ova obtained from a single parent was 300, and the average, based on the data from 32 parents, works out at 63. The following is a description of the ovum (fig. 1, b) :—“ The ovum is 0·46 mm. long and 0·14 mm. broad. The upper surface is broad, and the floats touch the margin ; the narrow striated frill around the margin of the upper surface is interrupted by the floats ” (Stanton).

*Anopheles karwari.*

The months of oviposition and the number of ova secured were :—

April 1920.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1921.	Feb.	March.
—	10	54	—	—	—	51	—	97	—	—	—

The maximum number of ova obtained from a single parent, therefore, was 97, and the average, based on the data from 4, works out at 53.

The ovum is precisely similar to that of *A. maculatus*.

*Anopheles vagus.*

The months of oviposition and the number of ova secured were :—

April 1920.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1921.	Feb.	March.
80	26	26	75	5	211	96	5	146	131	163	100
63	42	69	66	5	86	56	35	98	115	109	—
—	45	9	67	62	14	135	72	78	—	20	—
—	30	138	172	31	127	121	114	35	—	176	—
—	29	106	248	109	188	170	—	59	—	45	—
—	25	20	32	112	50	—	—	—	—	60	—
—	126	138	86	132	51	—	—	—	—	95	—
—	56	110	28	87	196	—	—	—	—	126	—
—	63	20	21	51	73	—	—	—	—	48	—
—	152	8	170	58	155	—	—	—	—	—	—
—	—	69	144	34	134	—	—	—	—	—	—
—	—	92	34	184	149	—	—	—	—	—	—
—	—	—	142	98	75	—	—	—	—	—	—
—	—	—	61	172	25	—	—	—	—	—	—
—	—	—	143	151	171	—	—	—	—	—	—
—	—	—	13	42	167	—	—	—	—	—	—
—	—	—	36	223	146	—	—	—	—	—	—
—	—	—	27	56	148	—	—	—	—	—	—
—	—	—	156	82	—	—	—	—	—	—	—
—	—	—	66	166	—	—	—	—	—	—	—
—	—	—	5	211	—	—	—	—	—	—	—
—	—	—	143	90	—	—	—	—	—	—	—
—	—	—	156	173	—	—	—	—	—	—	—

The maximum number of ova obtained from a single parent was 273, and the average number, based on the data obtained from 114, works out at 89.

Dr. Stanton thus describes the ovum of this species :—“ Length 0·54 mm., breadth 0·20 mm. The upper surface is broad and the frill continuous around the whole of its margin ; the floats, which are oblong, do not quite touch the margin and occupy three-fifths of the length of the ovum ; the corrugations number 25 ; the membrane covering the lower surface is reticulated.”

*Anopheles subpictus* var. *malayensis*.

The months of oviposition and the number of ova secured were :—

April 1920.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1921.	Feb.	March.
—	—	—	—	—	—	—	88	87	—	—	—
—	—	—	—	—	—	—	62	79	—	—	—
—	—	—	—	—	—	—	10	114	—	—	—
—	—	—	—	—	—	—	34	82	—	—	—
—	—	—	—	—	—	—	114	52	—	—	—
—	—	—	—	—	—	—	68	—	—	—	—
—	—	—	—	—	—	—	32	—	—	—	—

The average number of ova for each parent, based on the data from 12, works out at 68, the maximum number from a single parent having been 114.

*Anopheles ludlowi*.

The months of oviposition and the number of ova secured were :—

April 1920.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1921.	Feb.	March.
—	—	—	—	—	—	120	63	28	—	—	—
—	—	—	—	—	—	50	—	—	—	—	—

The average number of ova for each parent, based on data from 4, works out at 65, the maximum number from a single parent having been 120.

*Anopheles aconitus*.

The months of oviposition and the number of ova secured were :—

April 1920.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1921.	Feb.	March.
60	61	23	79	72	67	65	56	80	81	—	36
79	74	104	—	—	56	51	52	91	48	—	60
70	37	43	—	—	30	6	81	62	—	—	76
—	40	3	—	—	—	93	—	31	—	—	22
—	—	8	—	—	—	59	—	47	—	—	72
—	—	64	—	—	—	90	—	101	—	—	59
—	—	—	—	—	—	92	—	50	—	—	47
—	—	—	—	—	—	24	—	—	—	—	70
—	—	—	—	—	—	7	—	—	—	—	83
—	—	—	—	—	—	79	—	—	—	—	—
—	—	—	—	—	—	3	—	—	—	—	—
—	—	—	—	—	—	4	—	—	—	—	—
—	—	—	—	—	—	46	—	—	—	—	—
—	—	—	—	—	—	58	—	—	—	—	—
—	—	—	—	—	—	75	—	—	—	—	—
—	—	—	—	—	—	117	—	—	—	—	—
—	—	—	—	—	—	72	—	—	—	—	—
—	—	—	—	—	—	61	—	—	—	—	—
—	—	—	—	—	—	5	—	—	—	—	—
—	—	—	—	—	—	50	—	—	—	—	—
—	—	—	—	—	—	98	—	—	—	—	—
—	—	—	—	—	—	73	—	—	—	—	—
—	—	—	—	—	—	106	—	—	—	—	—

The average number of ova for each parent, based on data from 62, works out at 38, the maximum from a single parent having been 117.

Description of the ova of this species (fig. 1, c) :—" Length 0.40 mm., breadth 0.16 mm.; the upper surface is extremely narrow and the frill is continuous around its margin " (Stanton).

*Anopheles kochi*.

The months of oviposition and the number of ova secured were :—

April 1920.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1921.	Feb.	March.
—	30	—	—	—	—	110	—	38	41	—	85

The average number of ova, based on data from 5 parents, works out at 40, the maximum number from 1 parent having been 110.

Dr. Stanton thus describes the egg :—" The ovum is 0.45 mm. long and 0.16 mm. broad. The upper surface is narrow, and the floats do not touch its margin; the narrow striated frill is continuous around the whole of the margin of the upper surface. The floats are oblong in shape and extend over the middle two-thirds of the length of the ovum; each float has about 20 corrugations. The thin membrane which covers the whole of the lower surface of the ovum and part of its upper surface has a reticulated pattern."

*Anopheles fuliginosus*.

The months of oviposition and the number of ova secured were :—

April 1920.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1921.	Feb.	March.
—	73	61	—	—	95	—	—	—	31	66	75
—	28	—	—	—	79	—	—	—	53	—	—
—	—	—	—	—	—	—	—	—	38	—	—
—	—	—	—	—	—	—	—	—	46	—	—

The average number of ova, based on data from 11 parents, works out at 58, the maximum from a single parent having been 95.

Dr. Stanton thus describes the ovum (fig. 1, d) :—" Length 0.56 mm., breadth 0.20 mm. Frill not continuous; it is broad until it meets the floats, where it becomes narrow and is interrupted. Each float has 20 corrugations and covers the middle half of the length of the ovum. The upper surface is broad; the lower surface is covered by a membrane which is without pattern."

*Anopheles tessellatus*.

The months of oviposition and the number of ova secured were :—

April 1920.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1921.	Feb.	March.
—	33	—	—	—	—	49	20	25	—	—	—
—	—	—	—	—	—	28	38	—	—	—	—
—	—	—	—	—	—	28	—	—	—	—	—
—	—	—	—	—	—	15	—	—	—	—	—

The average number of ova, based on data from 8 parents, works out at 29, the maximum number having been 49.

Dr. Stanton finds that the ovum does not differ from that of *A. kochi*.

The ova referred to were laid in one batch, and though several bowls of similar water were often provided, almost always in one only. Exceptions were noted occasionally with *A. hyrcanus* and repeatedly with *A. tessellatus*, which usually laid a few ova day by day for five or six days. In only two instances were second large batches of ova obtained: one *A. hyrcanus* laid a batch of 45 ova during the night of 21st October and a second batch of 79 on the following night; and one *A. vagus* laid a batch of 93 ova during the night of 3rd August and a second batch of 180 ova in the course of the following afternoon.

Infection of Anophelines with the malaria parasite seemed to have no adverse influence on oviposition. An *A. maculatus*, which on dissection later showed eight zygotes in its stomach and sporozoites in all the lobes of the salivary glands, afforded a large batch of ova from which as many as 78 offspring were bred up. This observation was confirmed by experiment with 21 other females of this species, all of which, though infected, afforded ova that duly hatched out.

The tendency of the ova to drift together by capillary attraction as described for British species, and to form triangles, diamonds and other geometrical figures, was marked in the case of all species except *A. tessellatus*.

It has already been remarked that the ova when first laid are white. It was by no means unusual, especially with *A. aconitus*, to find a large percentage of a batch, still white, sunk at the bottom of the water, their floats unexpanded; and parti-coloured ova floating among black ones. For instance, of 65 ova laid by an *A. aconitus* 40 were black in colour and floated, and 25 white had sunk to the bottom of the bowl. None of either batch hatched. Such white ova as had sunk were found to have their floats unexpanded. But even brown and black ova, with floats unexpanded, were found to have sunk. This was especially the case with those of *A. tessellatus*. Thus 28 ova, normal in appearance except as to floats, and in another instance 88 similar ova, were found at the bottom of a basin of water. Sometimes floating ova never assumed their proper colour and failed to hatch. This was the case, for instance, with 30 white and 50 brown ova laid by an *A. fuliginosus* on 25th September, and with 46 brown ova laid by an *A. aconitus* on 19th October, and with 108 brown ova laid by an *A. hyrcanus* on 26th October; and such cases were numerous. Whole batches of ova, entirely normal in appearance and floating on water, sometimes failed to hatch. This was noted quite frequently with those of *A. umbrosus*, from females taken in Kuala Lumpur. Batches of these ova were sometimes kept for weeks on the assumption that incubation may be delayed. It was then thought that the medium on which they were placed might be unsuitable, but none hatched when they were transferred shortly after being laid to rainwater and to water from a natural breeding-place. It is not known whether the act of coitus in Anophelines is related, as concerns the female, to a meal of blood. If it takes place subsequent to such a meal the failure of the ova of *A. umbrosus* to hatch is explicable on the assumption that the virgin females had been compelled to leave their haunts, known to be remote from the town, in search of a meal, and had so escaped the attentions of males subsequent to it. Infertility of the ova as a cause of failure to hatch is suggested by a case in which 112 ova laid by an *A. vagus* on 5th August all duly afforded larvae, whereas of a second batch of 107 ova (39 black and 68 white) none hatched.

The ova were not always deposited on water. For instance, an *A. karwari*, enclosed in a dry tube plugged with cotton wool, was observed in the middle of the morning to be ejecting to a distance white ova, which blackened only on transfer to water, hatching in due course. An *A. barbirostris* deposited in a heap in a dry tube ova estimated at 56; these were floated off on water, becoming black two hours later, and all eventually hatched. Another *A. barbirostris*, kept in a glass tube for four days,



deposited on the fourth, in the water of condensation, a very considerable mass of ova in which incubation must have proceeded, for larvae hatched within 12 hours of their transfer to water. An *A. umbrosus* deposited about 40 ova in a dry tube, a few of which hatched in due course, having been placed on water.

It was frequently found, especially with the heavy, long-legged *Myzorhynchus* species, that unless supports, in the way of a few dead leaves, were provided, they were unable to rise from the water, and, becoming more and more involved in the surface film, eventually drowned. Ova were occasionally deposited on such leaves. Thus an *A. barbirostris* laid 58 ova on one floating leaf, and eight on another; an *A. hyrcanus* var. *paeditaeniatus* laid a batch of 36 ova on a leaf, rotting and mildewy; an *A. subpictus* var. *malayensis* laid a batch of about 88 ova on another leaf.

An interesting point arose as to whether the newly hatched larvae in such cases reached the water. All did so. In the case of the *A. paeditaeniatus* referred to, the ova on the leaf were about half an inch on all sides from the water; and the young larvae were found in the water on all sides of the leaf.

It was impossible at that time to take steps to ascertain if the ova are ever so deposited in nature, but on the assumption that this may be the case, the following experiment was devised with a view to enquiry whether newly hatched larvae have any special instinct for finding their way to water. Batches of ova, about to hatch, were transferred to a strip of blotting paper on a glass slide supported on the edge of two bowls of water so that the ends of the strip dipped into the water, which was in one bowl at a high level, in the other at a low. There being a slow current from the water at high level towards that at low level, it was thought possible that the larvae might find their way against stream, as they would do were the ova placed in nature on mud at the edge of pools. The larvae of various species were repeatedly watched wriggling by side to side movements in both directions, but more often than not there were fully as many in one bowl as in the other.

### Factors Controlling the Development of Ova.

The foregoing observation suggested an enquiry as to whether, as has been shown in the case of *Aedes argenteus* (*Stegomyia fasciata*), the development of Anopheline ova is retarded by their being placed under unfavourable conditions, an explanation offered by Ross to account for Anopheline periodicity in India, though this is less likely to occur in a climate so moist all the year round as that of the Malay States. A distinct advantage would accrue to some of the Anophelines, especially some of the small-pool breeders, were it the case that, in the event of such a breeding-place rapidly drying up, such ova as happened to be resting on the surface could resist the effect of desiccation, and could maintain their viability until such time as they again became fortunately circumstanced for hatching by the filling up of the pool. It was thought possible that such conditions, often accidentally produced in the laboratory by the gradual lowering by evaporation of the water level in basins containing ova, as a result of which the ova, which often collect through capillary attraction at the sides, become high and dry, might account for the marked inequality of size of larvae comprising one family; such ova hatching only when it became necessary to add a fresh supply of water. In a series of experiments the ova removed from the water at various stages of incubation were allowed to dry in the laboratory at ordinary temperature, and were restored to water at varying intervals, the results being noted. The following are typical of the many experiments so conducted:—

Of 36 ova laid by an *A. vagus* during the night of 18th to 19th May all were removed from the water at 8 a.m. on 19th, being then approximately under seven hours old. They were divided into two batches, (a) of 20, and (b) of 16, which were gradually dried on blotting paper in the laboratory. Twenty-four hours later batch (a) was restored to water, and at the end of a further period of 24 hours batch (b) also was restored. No hatching had taken place at the end of three days more,

and the bowl containing the ova was then placed in a sunny situation. This had no effect either; so that, unless the ova were unfertile, their removal for 24-48 hours at a very early stage of incubation, and their drying in this way, must have resulted in their death. Unfortunately no control ova were kept.

It was then thought possible that ova might retain their vitality if dried under more natural conditions—on mud. Accordingly, on 25th May, 63 ova, laid during the previous night by an *A. vagus*, were transferred at 10 a.m. to moist mud, which was then placed in a breeze to dry. By 4 p.m. all the ova were much contorted and the mud was apparently dry. After an interval of 48 hours water was gradually added so that the ova were floated off. They resumed their normal form in the course of the next 24 hours, but none hatched, though kept under observation until 10th June. A few kept on water as a control hatched in due course.

Ova at a very early stage of incubation being apparently unable to withstand even short desiccation, experiments were set on foot with a view to ascertaining whether ova at a more advanced stage were more resistant. The following are typical experiments of this nature:—

About 68 ova, laid two nights previously by an *A. vagus*, were removed on 1st June at 8 a.m., and were allowed to dry in the laboratory. Twenty-four hours later 25 of these were transferred to water. On examination a day later three larvae were found to have hatched, and towards the end of the morning 11 more were found. One larva hatched out on 9th June, fully 24 hours after its fellows. The rest of the 25 were kept under observation for many days; all failed to hatch. The balance of the ova removed, totalling 43, were allowed to remain in a dry and shrivelled condition for 24 hours still longer, till 9 a.m. on 3rd June, when they were restored to water. Microscopical examination at 2 p.m. showed that all had regained their shape, and after 24 hours 4 larvae hatched out. Emergence of the others proceeded so rapidly that at 10 a.m. there were 19 larvae and at 11.40 a.m. there were 23 in all. The rest of the ova failed to hatch.

Out of a batch of 138 eggs laid during the night of the 7th June by an *A. vagus*, having been on the water about 36 hours, 103 were removed on 9th at 11.30 a.m. in five batches and were kept in the laboratory to dry. Batch (1), consisting of 9 ova, was restored to water on 11th June at 2 p.m., being then, as the result of desiccation for more than 48 hours, rather flattened though fairly full in appearance; 18 hours later 2 larvae had hatched, and 1 was seen at 8 a.m. on 12th June in the act of emergence from the eggshell; four hours later 2 more had emerged. On 13th June, at 10 a.m. another larva hatched out; 3 more were found at 8 a.m. on 15th, evidently having just hatched out, and at 2 p.m. on that day still another came out. All 9 ova, therefore, which must have been in an advanced state of incubation, withstood successfully the effects of desiccation at ordinary laboratory temperature for rather over 48 hours, the larvae emerging between 12 and 84 hours after the restoration of the ova to water.

Batch (2) of 20 ova, which had been afforded an equal period of time for incubation, was allowed to dry for 24 hours longer, about 72 hours in all, and then restored to water. The hatching was by no means so good, for 9 only, rather less than 50 per cent., emerged within a period of 24 hours, between 8 a.m. 13th and 8 a.m. 14th June.

Batch (3) of 10 ova was allowed to dry for rather over 96 hours, batch (4) of 35 ova was allowed to dry for 120 hours, and batch (5) of 29 ova for 144 hours, being then each restored to water. No hatchings at all took place, though the eggs, which were mere flattened scales, duly filled out again. The control batch of 35 ova hatched out.\*

---

\* The awkward, varying and apparently arbitrary numbers employed in these experiments are accounted for by the extreme difficulty of separating batches of tiny eggs.

The previous experiments having afforded some guide as to the length of time the ova of *A. vagus* at an advanced state of incubation are able to withstand desiccation, the following experiment was conducted in an endeavour to reproduce some greater approximation to natural conditions. On 30th June 62 ova, about seven hours old, were transferred to a thin film of water covering mud brought in a few days previously from a breeding-place of the species. Twenty-four hours later the mud was found to be hard and fissured, in which state it was allowed to remain for 48 hours more. Water was then gradually added, so as to simulate the gradual filling up of a hollow by rain; within four hours 6 larvae were counted, and there was a gradual increase in number, until 5th July, when at 8 a.m. 36 were counted. The remainder failed to hatch.

This result, showing that some of the ova in which incubation must have been initiated are able to retain their vitality up to 72 hours when dried, was confirmed by experiments on other species than *A. vagus*. Thus, in the case of *A. barbirostris*, 88 eggs laid during the previous night were on 25th May divided into six batches, (a) of 12 ova, (b) of 8, (c) of 12, (d) of 13, (e) of 16, and (f) of 27. Batch (a) was left on the water to test the fertility of the ova. On 27th May 1 larva had hatched, and then unfortunately the bowl was upset so that the rest were lost. Batches (b), (c), (d), (e), and (f) were gradually dried; (b), (c), and (d) in the laboratory, (e) and (f) in the open air. When these ova were examined 24 hours later, all were found to be much wrinkled and distorted. At 8.30 a.m. on 26th May batch (b), having been off the water for the greater part of 24 hours, was restored to tap water, and the following hatchings of larvae took place:—

1 larva at about 8 a.m.	27th May.
2 " "	9 " 28th "
1 " "	9 " 30th "
1 " "	8 " 31st "

The remaining 3 eggs failed to hatch.

Batch (c) was restored to water on 27th May at 8 a.m., having been off for the greater part of 48 hours, a note being then made that they were exceedingly wrinkled and distorted. By 10.30 all had recovered their normal shape, and four days later 4 larvae were found to have hatched. The remaining 8 failed to hatch.

Batches (d) and (e) placed on the water on 28th May failed to hatch, death having presumably resulted from the length of time (three days) that they had been off the water, or from the exposure to which they had been subjected.

The ova of *A. barbirostris*, therefore, even in the preliminary stages of incubation, will withstand drying for 24 hours, and a percentage even for 48 hours. A further effect would appear to be the slowing of intra-oval development, whereby larvae may take from 48 to 144 hours before emergence, the normal hatching of *barbirostris* ova, when kept under usual conditions, having been found by repeated observations to take place within about 48 hours after the eggs have been laid.

The result was confirmed with other species. Thus, 38 ova laid by an *A. fuliginosus* were divided into two batches, (a) of 12, (b) of 16. Batch (a) left on the water afforded on the second day 4 larvae, and 24 hours later 6 more, the remaining 2 ova failing to hatch. Batch (b) was removed after about 31 hours on the water, and the ova were allowed to dry in the laboratory for 48 hours. They were then restored to water; 24 hours later 12 larvae had hatched out of the 16 ova, the rest having perished. In another experiment 18 ova were removed from the water, having been afforded the chance of incubating for about 31 hours, and 24 hours later exactly, at 11 a.m., they were restored in a wrinkled state to the water. By midday their shape was fully restored, and at 2 p.m. it was observed that 3 of the ova showed at one end a split, indicating the approaching emergence; 2 larvae emerged shortly afterwards, when 3 more ova showed the heads of larvae presenting, though only one was successful

in making its escape, the other 2 dying *in situ*. It is therefore to be presumed that, though the ova were at an advanced state of incubation when removed, the progress of development was checked, partly or entirely, only by their removal from the water.

The viability of the various ova was found to be very considerably extended beyond these limits when they were kept in a thoroughly moist atmosphere. This is shown by the following experiments :—

Of 20 ova laid during the night of 16th June by an *A. vagus*, 8 were left as a control, and in due course all afforded larvae between 18th and 22nd June. The remaining 12 ova were removed at 4 p.m. on 17th June to a Petri dish containing moist blotting paper. The dish was unopened for 82 hours, when, at 11.30 a.m. on 21st June, all the ova seemed to be unchanged except one which had yielded a larva that was lying close beside its egg-shell. One of the ova was then placed on water and kept under observation for several days; it failed to hatch, as did another placed on water 24 hours later. Five more of the ova still unchanged in appearance were transferred to water on 23rd June at 8 a.m. No hatching had taken place 24 hours later, but of the 4 ova still remaining in the Petri dish, almost seven days after they had been laid, 2 were seen to have their caps open. Both promptly hatched on transfer to water, and a third larva came out from one of the other two eggs later on in the same day. There then being every expectation that some of the 5 ova placed on the water on 23rd June were still alive, the bowl containing them was at 8 a.m. on 24th June put out in the open air in sunlight. At 4 p.m. 1 larva was found to have hatched, and another ovum, with its terminal cap slightly open, shortly afterwards produced a second. The bowl was placed in the sun for several days onwards, but none of the 3 remaining ova hatched.

One hundred eggs laid by an *A. vagus* during the night of 20th June were left on the water for 31 hours, and then removed to moist filter-paper enclosed in a Petri dish. The eggs, examined day by day, showed no change till the fourth day, when at 10.30 a.m. a single egg showed its cap open, and on transfer to water the larva forthwith wriggled out. Within five minutes every single egg had its cap off. On the transfer to water of 15, one by one, each produced a larva. Five of the ova transferred to water at 4 p.m. on the same day all hatched at once. With a view to testing what length of time larvae partly hatched can survive in a moist atmosphere, the remaining ova were transferred at intervals to water. On 8th June, at 8 a.m., 10 ova so transferred all hatched at once, but in the case of 18 ova transferred 24 hours later a considerable falling off in vitality was found. Three hatched almost at once; 5 after some minutes; the remaining 10 completely failed to hatch, but on 30th June were to be seen with the heads of the larvae presenting, as if these had died *in situ*, having insufficient energy to complete their exit. On 30th June the remainder of the ova were transferred to water. No larvae emerged forthwith, though all the ova had their caps off; 3 only were found hatched 24 hours later, and these were feeble and perished shortly afterwards.

Of 248 ova laid by an *A. vagus* 56 were left on water as a control, and the balance, after a possible incubation period of 24 hours, were placed in a moist Petri dish. From this 56, removed at the end of 90 hours, were again transferred to water; 6 hatched out almost at once, and by the end of 24 hours the larvae were so numerous that it was estimated that practically all must have hatched. A second batch of the same ova left in a Petri dish for 48 hours longer, *i.e.*, 138 hours in all, were then restored to water, being then 162 hours old; 2 hatched almost at once, and the majority within five minutes. The result was equally good with a third batch of 80 ova left in the moist atmosphere 162 hours and being then 186 hours old; 10 had their caps off; 9 of these hatched at once on restoration to water, and it was estimated at the end of eight hours that practically all had hatched.

Sixty-nine ova laid by a female *A. fuliginosus* during the night of 5th June were divided into two batches, (a) of 25 as a control, and (b) of 44 for experimental work.

Most of (a) hatched out in due course. Of batch (b) 9 were removed forthwith from the water and placed in a Petri dish in fairly moist filter-paper. On examination 48 hours later it was found that all these ova had their terminal caps pushed open by the larvae, which could be seen still *in situ*, 2 indeed having their heads presenting. On being placed in water all hatched out almost at once, 2 sinking to the bottom apparently in an exhausted state, and struggling to the surface, after a few seconds, only with difficulty. The others moved freely, though with their bodies bent at first out of the straight line, as if stiffened by having been for some little time in a strained attitude. Ten minutes after removal all were in a normal attitude in the water and all thrived subsequently.

Seven other eggs from batch (b) were allowed to remain on water until 7th June at 11 a.m., having been afforded a chance of incubating for 36 hours. They were then removed to very wet filter-paper on a Petri dish. On examination 24 hours later all these ova were observed to have their terminal caps widely open, the larvae having therefore probably emerged and wriggled away, for none could be found. Moisture alone, not necessarily a more ample supply of water, would therefore appear to suffice for the hatching of the eggs, and, indeed, so limited an amount of moisture that in the one case the larvae, though fully developed, were unable even to emerge completely. The balance of the eggs was used for other experimental purposes.

The evidence therefore suggests that the incubation of Anopheline eggs having once reached a certain stage may be retarded or even inhibited by adverse conditions; for in no instance did hatching take place for a considerable number of hours after the restoration of the ova to water. Such a provision of nature might, indeed, have been expected, for it must often happen that eggs laid on a small pool are left high and dry before fresh rain again affords the stimulus necessary for their final development. It is hardly to be wondered at that freshly laid eggs at once subjected to such disadvantageous conditions uniformly fail to develop, for so sudden a removal at an early stage from a moist to a dry spot cannot take place in nature, the mere fact of their having been laid by the parent at a selected spot, probably even the smallest pool, having ensured the environment requisite for them to attain, before the pool has had time thoroughly to dry up, a degree of development that will enable them to retain their vitality. But as has now also been shown, the resisting powers may be very much enhanced in the presence of a certain amount of moisture, and development, though delayed, is able to proceed as surely as if the eggs had been normally laid on water. It would appear also that, though the larvae are fully developed under such circumstances and are ready to hatch, their actual emergence may be deferred without prejudice for some little time until conditions have become more favourable to them.

The larvae of *A. vagus* may be found in small, drying pools in mud, in situations thoroughly well protected both from sun and breeze, and where, as observation has shown, when there has been no rainfall the mud will take many days to dry up completely. Eggs deposited in such a place may well retain their vitality even when there is insufficient water to stimulate their development.

Investigations conducted for the purpose of ascertaining whether irregularity in hatching occurs as a normal process with eggs floating on water have shown that it sometimes does, at all events in the laboratory. A batch of ova laid in the course of a night by a female Anopheline may all hatch out almost at the same hour, or may show, under exactly similar circumstances, differences in hatching of many hours or even of days. An *A. fuliginosus* laid during the night of 22nd to 23rd May 73 ova. At 8 a.m. on 25th May 48 larvae had emerged. These were removed; 24 hours later 14 more larvae had appeared; the remaining 11 ova failed to hatch. An *A. vagus* laid 152 eggs during the night 30th to 31st May; of these 68 were used for other purposes, and 84 were kept apart under observation. The majority of these, 48, hatched on 2nd June; the remainder were left just as they were until 4th June, when, on microscopic examination, 19 were found to have hatched; 17 were unhatched, though great care had been taken that at no time were any of these eggs left high

and dry by evaporation. These 17 ova were restored forthwith to water at 11 a.m. ; on examination at 11.40 on the same day 2 more larvae were found to have hatched. No further hatchings were noted until 7th June, when, at 8 a.m., another newly hatched larva was found, this being seven days after the eggs had been laid, five days after the first larvae had hatched, and three days after the two previous larvae had emerged. A day later, 8th June, at 8 a.m., still another newly hatched larva was found.

In the case of another *A. vagus*, 138 ova were laid during the night of 21st June ; 100 were removed for the purposes of another experiment and 38 were studied in regard to their hatching. By 8 a.m. on 23rd June, 15 larvae had hatched ; by 8 a.m. on the next day 3 more came out, and during the sixth night 2 more. There had been no change in conditions, and measures had been taken throughout to ensure that all the ova were floating.

Again, in the case of another *A. vagus*, 130 eggs were laid during the night of 7th June ; 35 were left as they were, the remainder being removed for the purposes of another experiment. No hatching took place until 10th June, when between 8 a.m. and 2 p.m. 7 larvae emerged ; 10 more larvae hatched out at intervals up till 17th June, no less than ten days after they had been laid.

In the case of *A. karwari*, 54 eggs laid by a captive female were found during the morning of 5th June. By the 8th of June larvae had emerged from the majority, but among 14 eggs then examined 5 were found with their shells still unbroken. These were then transferred to another bowl, and on 11th June a single larva hatched out at least 72 hours after the rest.

These phenomena of the hatching of Anophelines were found to be paralleled in *Stegomyia albopicta*. For example, a female laid on 12th May 32 ova, the bulk of which hatched out 24 hours later ; six days later, on 19th May, 6 more larvae hatched out. Again, out of 10 ova of this *Stegomyia* laid on 17th August, 2 hatched within 48 hours, and 3 more at intervals of five, ten, and eleven days respectively.

Yet, on the other hand, in some batches of ova hatching may be practically simultaneous, even though the conditions may not be uniform for all the eggs of the batch. Thus, on the 24th June, of 110 ova laid by an *A. vagus* in the course of the previous night, 22 were transferred to water from a running brook in which the larvae of *A. maculatus* were commonly obtainable ; 28 to an old infusion of hay ; 13 to water containing much organic matter, from a fish-pond ; 26 to water from a small muddy pool ; and 21 to tap water. At 10 p.m. on 25th June no hatching had taken place, but on examination at 8 a.m. next morning the whole 110 were found to have emerged.

Though the nature of the inhibiting influence is obscure, it is plain from the following observation that hatching may be delayed even though development within the egg is complete. On 9th August 20 ova of *A. vagus*, kept for about 54 hours after oviposition in a moist Petri dish, were transferred to water. Fifteen hatched at once ; the remaining 5, unhatched and floating on the water, were placed 24 hours later on a slide for microscopic examination ; in the course of this all 5 larvae were seen to wriggle out, possibly under the stimulus of a hot breeze.

A batch of 114 larvae of *A. vagus*, found in a breeding-bowl on 29th November, were on 30th transferred to another vessel. Hatching of the majority immediately occurred.

A rather interesting small observation was that ova kept in a moist atmosphere in a closed Petri dish often did not hatch, even for days, until the lid was removed, the larvae then all popping out within a minute or two, possibly by reason of the effect of change of air on their enclosed space, or of the effect on the egg-shell of a sudden change of temperature. The occurrence formed a convenient means of enabling one to watch at will the emergence of the larvae. These came out with their

thoracic plumes pressed back towards the tail end ; their caudal tufts directed towards the head. On emergence the former extend themselves at right angles, suggesting the function of the outriggers of a catamaran, which function they probably serve ; the latter extend backwards.

### Time of Oviposition.

This was noted in the case of all the open-country species as having been, as a rule, between 8 p.m. and 6 a.m. But there were a few exceptions. Thus an *A. vagus* that had been captured on 14th June deposited on 21st between 12 midday and 2 p.m. 106 ova. Another of the same species, taken on 14th June, laid, on 27th of the month, 69 ova between 8 and 10 a.m. ; a third, captured on 1st July, laid no less than 248 ova between 2 and 4 p.m. on 3rd July ; and, curiously enough, 4 others, out of 7 females of this species placed in captivity on 4th July with a view to obtaining eggs, oviposited on the same day, all between 8 a.m. and midday, one affording a batch of 67 ova, another 75 ova, the third 86 and the fourth 66. Another *A. vagus* laid a total of 248 ova between 2 and 4 p.m. one afternoon ; another laid a batch of 93 ova during the night of 3rd August, and was watched ovipositing in the afternoon of the following day, between 2 and 4 p.m., no fewer than 180 additional ova being the outcome. An *A. maculatus* was seen ovipositing at 10 a.m. on 12th October, 56 ova being obtained ; and another of the same species laid 73 ova at about 3 p.m. on 16th October.

### Length of Life-cycle.

This was found to vary very considerably, even in the case of larvae bred from ova laid on the same day and kept under the same conditions, the lack of uniformity of development being so constant as to make it difficult to arrive at any precise calculation of the normal length of the cycle.

The following are typical data obtained from bred families :—

Species.	Number of Ova.	Date of Oviposition.	First Offspring Emerged.	Length of its Life-Cycle.	Last Offspring Emerged.	Length of its Life-Cycle.	Total Offspring.
<i>A. vagus</i> ..	146	4.xii.20	14.xii.20	10 days	18.xii.20	14 days	75
<i>A. subpictus</i> var. <i>malayensis</i>	114	6.xi.20	17.xi.20	11 days	22.xi.20	16 days	60
<i>A. ludlowi</i> ..	120	25.x.20	5.xi.20	10 days	9.xi.20	14 days	36
<i>A. aconitus</i> ..	91	11.xii.20	29.xii.20	18 days	7.i.21	27 days	61
<i>A. hyrcanus</i> ..	117	13.xii.20	24.xii.20	11 days	30.xii.20	17 days	81
<i>A. hyrcanus</i> var. <i>paeditaeniatus</i>	147	7.ii.21	23.ii.21	16 days	2.iii.21	23 days	64
<i>A. barbirostris</i>	159	12.xii.20	31.xii.20	19 days	12.i.21	31 days	47
<i>A. maculatus</i> ..	123	17.xi.20	29.xi.20	12 days	4.xii.20	17 days	43
<i>A. karwari</i> ..	97	15.xii.20	29.xii.20	14 days	2.i.21	18 days	47
<i>A. fuliginosus</i>	46	22.i.21	6.ii.21	14 days	13.ii.21	21 days	40
<i>A. fochi</i> ..	110	8.x.20	19.x.20	11 days	24.x.20	16 days	84
<i>A. tessellatus</i> ..	?	27.xi.20	8.xii.20	10 days	11.xii.20	13 days	29

Apart from any irregularity of hatching, differences in the length of the life-cycle certainly depend, in part at all events, on inequality in the rate of growth of the larvae, even though kept under similar conditions. Thus, an *A. vagus* laid, on 14th May, 45 ova ; on 20th May the majority of the larvae were found to measure 5 mm. in length, and were considered as three-quarters grown ; but a small minority, looking as if they had only recently hatched, were barely 2 mm. in length. A second family of *A. vagus* larvae, all from ova deposited on 14th May, consisted, on the 20th of the

month, of a number of larvae varying in length from 1-3 mm. In the case of another family of this species, the ova were deposited on 11th May and the young larvae were first seen on 13th May. The family did not thrive. But on 21st May it consisted of 1 larva about 1 mm. in length, as small as if it had recently hatched; another 4 mm. in length, and thus well on the way to maturity; 1 pupa, a few hours in this stage of development; and of a male imago, just emerged.

A more striking instance of disparity in development occurred in a family bred from ova deposited during the night of 13th May by another *A. vagus*. Unfortunately no note as to the hatching of the larvae was made, but on 19th May when they were transferred to new water it was recorded that the larvae varied very considerably in size, some being barely 2 mm. in length, as if newly hatched, and others 5 mm. in length, and certainly more than three-quarters grown. By 2nd June, 7 of these had pupated and the imagos had duly emerged; the rest of the family then consisted, after 17 days, of 2 larvae, one 6 mm. in length, the other 2 mm. only. The diminutive size of the smaller larva was not due to any delay in hatching from the egg, for search was made for any unhatched ova on 21st May, when the only 4 found were removed.

The following were among the irregularities in development noted in the case of a number of *A. maculatus* families: The female parent, in one case, laid on 19th June approximately 143 ova, and two days later it was thought that all had hatched. At all events, all the larvae found on 23rd June were then transferred, one by one, to four bowls, in which, all being under similar conditions, 41 ultimately attained maturity. The first imago, a male, emerged during the night of 4th July, the life-cycle having therefore occupied 14 days. The other imagos came out at almost daily intervals right up to 29th July, the life-cycle of the last one having occupied no less than 39 days. There was, further, some evidence that the cycle might be extended over a considerably longer period; for at the end of the 39 days there were still 2 surviving larvae, one of which died during the night of 10th August, after a life lasting 50 days; the second survived a further five days. The interest of this particular observation lies, not only in the marked degree of irregularity of development, but also in its effect in having ensured the emergence of individual Anophelines of the same parentage throughout a long interval of time comprising both wet and dry seasons, the ova having been laid towards the end of a wet period.

In the case of a family bred from ova laid on 28th May by an *A. fuliginosus*, all hatched within 24 hours of each other and were kept in the same bowl. The first of the offspring, a male, emerged during the night of 8th June, the life-cycle from egg to imago having occupied 11 days. Thereafter imagos continued to emerge, one or two almost daily, up till the night of 18th June, 21 days, when the last imago, a female, came out. The life-cycle, therefore, of this female had occupied double the time of the first; and had it not been for the death of the single remaining pupa, the emergence of which was due in the night of 22nd June, the length of the cycle would, indeed, have been more than doubled.

The following case shows that the factors affecting the length of the life-cycle are both irregularity in hatching and irregularity in growth of the larvae. The ova deposited by an *A. kochi* on 10th May, and all kept in the same bowl with the water unchanged, afforded larvae which, ten days later, were found to vary from a bare 1 mm. to 5 mm. in length. On 18th May a note was made that there were 8 larvae, and on 21st 3 small additional ones were counted. The first pupa from this family was obtained on 20th May, and between then and 4th June there were 8 others, 4 of which died, and 4 yielded imagos (1 male and 3 females). On 31st May there were 2 larvae only, 1 barely 4 mm. long, the other about 6 mm. long. On 7th June there was discovered in addition a tiny larva looking as if newly hatched and unmistakably of this species by reason of the pearly patch on the thorax, which had characterised the other larvae in that bowl (a white one). One of these last 3 larvae died; the second pupated on the night of 10th June, no less than one month from the date on



which the ovum from which it sprang had been laid ; the third larva seemed then to be very little larger than when first seen.

The phenomena of the unequal hatching of Anopheline ova, and of the inequality in the rate of development of the larvae, must go far to ensure the continuance of a particular strain. Indeed, some such provision was to be looked for, since the Anophelines invariably deposit their ova in large numbers all at once. Were the offspring to emerge with equal precision the chances of the extirpation of the race either by natural enemies or as the outcome of unfavourable conditions would be enormously increased.

#### **Effect of Removal of Larvae and Pupae from Water.**

Larvae removed from water rapidly succumbed even in a moist atmosphere, but pupae removed on blotting paper and kept in a thoroughly moist atmosphere were able to survive and complete their metamorphosis within the usual limits of time. It is noteworthy, as affording evidence of the hardihood of *Stegomyia albopicta*, that the pupae will survive for hours, even when thoroughly dry. On one occasion a pupa thrown on a dry surface in the very early morning was seen at 3 p.m. to be struggling vigorously when attacked by small ants.

#### **Hours of Pupation.**

In the very great majority of instances pupation took place during the night, but some exceptions were met with. Thus a small family of 7 larvae of *A. vagus* all pupated, almost simultaneously, one afternoon, the pupae affording 3 males and 4 females. In a family of 42 larvae of *A. subpictus* var. *malayensis* 8 were recorded as pupating as 2 p.m., 7 at 3 p.m., and 6 between 2 and 4 p.m. In a family of 36 larvae of *A. ludlowi*, 21 pupated at about 4 p.m., 6 pupated in the morning (8 to 12), 1 at 10 a.m., 2 at noon ; and in three other families of this species there was also a high percentage of pupations in the day time. Of a family of 8 larvae of *A. karwari*, 5 pupated at 11 a.m. In a family of 20 larvae of *A. maculatus*, 1 was recorded as pupating at 9 a.m., 11 between 9 a.m. and noon, 1 at 11 a.m., 2 at 11.50 a.m., and 2 at 1 p.m. Similar data were obtained in the case of *A. hyrcanus*, *A. barbirostris*, *A. fuliginosus*, *A. kochi*, and *A. tessellatus*. In the case of *Stegomyia albopicta*, a day flier, it was the rule, rather than the exception, for pupation to take place by day.

It should be remarked that the exceptional pupations were not precipitated as the result of interference with larvae resting prior to pupation. In most species it was possible to infer some few hours beforehand the imminence of pupation by the change in appearance of the larvae ; in the *Myzomyia* group, in particular, there is a deepening of shade and an increase in the apparent density.

If at this stage the larvae were suddenly alarmed, as for instance by transfer to other water, pupation occurred instantly, the pupa scurrying away and gradually shedding the larval skin. This would seem to be the direct result of strong muscular action, the larval skin being already distended to its utmost limit.

It was rather astonishing to find that the pupal eye is at once functional. The pupae of a large number of *A. kochi*, known to be about five hours old, dived simultaneously, even when a shadow was slowly thrown on them by interposing an object between their bowl and an electric light at some little distance away. This was noted also with other species.

#### **Duration of Pupal Stage.**

Pupae formed during one night usually afforded imagos on the morning but one following, that is to say, within 36 to 48 hours. No instances of any prolongation of the pupal period were ever observed, though the data as to thousands were recorded.

### Times of Emergence.

This also commonly took place during the night, usually before 11 p.m., but occasionally just before dusk, as is usual with other night-flying insects, the Anophelines being on the wing within two hours after emergence. But exceptions to the rule were noted. Thus, out of a total of 1,093 pupae, comprising various families of *A. vagus*, only 9 imagos emerged during daylight; the whole of a small family represented by 3 males and 4 females, coming out during the morning at 11.50. This was not correlated with day pupation. A female *A. subpictus* var. *malayensis* was recorded as emerging at 4 p.m., the only instance in daylight out of 373. A single *A. ludlowi*, again, out of 113 was noted as emerging at 10 a.m. A single *A. karwari*, out of 93, came out at 11 a.m. Of *A. maculatus*, 16 out of 701 came out between 8 a.m. and 4 p.m. Out of 346 pupae of *A. hyrcanus*, 1 only was noted as coming out in the day time, at 11 a.m., and 1 imago emerged in the afternoon from 184 pupae kept under observation. Of *A. barbirostris*, 8 imagos only out of 377 came out in the day time; of 111 pupae of *A. fuliginosus*, all emergences took place at night, so also with 206 pupae of *A. kochi*. In the case of *A. aconitus* 235 pupae afforded 1 imago only by day; and out of 294 pupae of *A. tessellatus* 5 only came out by day.

The data are to be looked on as entirely accurate, for a very careful examination was invariably made of all pupae before the laboratory was shut at the close of the day's work.

### Proportions of Sexes in Bred Families.

Of *A. vagus* 49 families, some consisting of only 2 or 3 imagos, others of as many as 130, afforded 998 imagos, among which the sexes were represented in almost equal proportions—497 males and 501 females. In no single family was there marked inequality of the sexes. Twelve families of *A. subpictus* var. *malayensis* afforded 393 imagos (194 males and 199 females), the sexes being again almost equally represented in each family. Five families of *A. ludlowi* afforded 125 imagos (56 males and 69 females); 18 families of *A. aconitus*, a species difficult to breed, consisted of 105 males and 124 females; 23 families of *A. maculatus* afforded 697 imagos (354 males and 343 females); 6 families of *A. karwari* afforded 93 imagos (41 males and 52 females); 11 families of *A. hyrcanus* gave a total of 339 imagos (172 males and 167 females); 6 families of *A. hyrcanus* var. *paeditaeniatus* gave a total of 184 imagos (91 males and 93 females), all true to parent; 15 families of *A. barbirostris* afforded 394 imagos (199 males and 195 females); 6 families of *A. fuliginosus* afforded 111 imagos (62 males and 49 females); and 9 families of *A. kochi* afforded 205 imagos (93 males and 112 females). There was thus very nearly equality of the sexes in the families of all the species studied.

### House-frequenting Species.

Systematic captures of Anophelines were made at intervals, latterly twice weekly, throughout the year in different quarters of Kuala Lumpur—in native houses and cattle-sheds in the Malay kampong (village) near the town; in certain coolie lines at some distance from the town; and in servants' quarters attached to the house of a European in a residential suburb. The data are of interest as showing what are now the common house-frequenting species, and their proportions, a point of considerable importance, since some may be presumed to favour, as elsewhere, other hosts than man. They show also the proportion of the sexes, and afford further evidence as to the success of the campaign largely directed against the notorious *A. maculatus*, as a result of which its numbers are now so few as to be almost negligible. With a view to adding to the value of the data a record was kept of the actual numbers of such female Anophelines as had made a recent meal of blood, or had obtained one at no very recent date, as could be determined by inspection of the abdomen. Such a record is, of course, open to the objections that no positive evidence as to the source of the meal is presented (though when the replete Anophelines were taken in houses

presumably it was human), and that it is impossible to arrive by mere ocular inspection at any conclusions whether an Anopheline has had a meal a few days previously. The data are as follows :—

	Native Houses and Cattle Sheds.			Coolie Lines.			Servants' Quarters.		
	Males.	Females.	Replete Females.	Males.	Females.	Replete Females.	Males.	Females.	Replete Females.
<i>A. vagus</i> .. ..	26	57	26	966	2,419	2,045	538	830	426
<i>A. subpictus</i> var. <i>malayensis</i> .. ..	—	1	1	—	39	46	—	17	14
<i>A. maculatus</i> .. ..	—	2	2	7	7	7	6	6	9
<i>A. karwari</i> .. ..	—	—	—	1	1	1	5	16	19
<i>A. hyrcanus</i> .. ..	25	29	15	4	58	28	45	126	54
<i>A. barbirostris</i> .. ..	2	5	4	5	10	3	40	153	55
<i>A. aconitus</i> .. ..	2,149	1,294	775	165	292	233	227	1,626	1,175
<i>A. kochi</i> .. ..	29	7	2	29	7	14	29	7	2
<i>A. fuliginosus</i> .. ..	33	4	4	33	4	4	33	71	36
<i>A. umbrosus</i> .. ..	21	2	1	21	2	1	21	254	59
<i>A. tessellatus</i> .. ..	3	74	44	3	74	41	3	38	25

From the foregoing data it will be noted that there was a preponderance of *A. aconitus* in collections from the kampong (village), and from the servants' quarters near the house of a European, whereas in the collections from the coolie lines *A. vagus* was by far the most dominant species. This is explicable as to the kampong and coolie line collections by the proximity of breeding-places favourable to the two species. Near the former is a swamp from which were taken in the course of the year 13,091 Anopheline larvae, among which were no fewer than 6,381 larvae of *A. aconitus*; near the latter was low-lying ground under cultivation for sugar-cane and vegetables, and in the foul water between the furrows the larvae of *A. vagus* could almost always be obtained in abundance. The source of the Anophelines obtained at the house of the European was not determined as to *A. aconitus*; the other dominant species probably came mostly from a fish-pond at no great distance.

### The Habits of Larvae.

In a previous paper ("The Nature and Functions of the Caudal Tufts of Anopheline Larvae," Bull. Ent. Res., xii, p. 91) a description was given of the caudal hooklets wherewith the larvae of Anophelines are able to attach themselves to objects. In moving water it is probable that all species may employ these hooks to an equal degree to avoid being swept away. But there is a marked difference in habit between those larvae that are more usually found in moving water (though sometimes in still water) and those invariably found in stagnant water. The former, of which *A. maculatus* and *A. karwari* may be taken as types, invariably attach themselves to the nearest object; the latter, for example *A. kochi* and *A. vagus*, do not seek supports to anything like the same extent. When bowls containing examples of each were placed side by side, it was at once noticed that, whereas every single *A. maculatus* larva rested at right angles to its support, many of the larvae of *A. vagus* floated quietly, well out towards the centre, entirely unsupported and in this approximating to various Culicid larvae, for example, *Stegomyia albopicta*, which, owing perhaps in part to their having no means of attachment, are never found in moving water.

It is a little surprising that Anopheline larvae favouring stagnant water have any development of caudal hooks. The writer, speculating as to their presence in such larvae, had concluded that they might be evidence of an alteration of breeding habit,

such species having, perhaps, bred in running water before there were large numbers of small open pools available. But Dr. J. W. Scott Macfie has suggested a more probable explanation: that such larvae may use their hooks to prevent themselves being driven before the wind.

Such differences of habit may well have important practical bearings, as is suggested by the following observation in regard to *Stegomyia*. A considerable number of larvae of *S. albopicta* were found in water that had collected in an old iron pan under shelter. On the surface of this was poured a mixture of heavy oil (Mobil-oil A) and kerosene, and a complete film being formed, the larvae all perished. A few days later the film of oil was found to be no longer complete, for the volatile constituents had evaporated, and the residual globules had run together into masses which had collected largely at the sides. In the spaces free from oil young larvae of *Stegomyia*, which must have originated from eggs most carefully disposed by the female parent, were found. These thrived and increased in size, though the fact of their ultimate pupation was not ascertained. The presence of the larvae, indicating their instinct and ability to keep free from a broken film of oil, suggests the high importance of an investigation as to the relative fate, under such conditions, of species such as *A. maculatus*, with a strong tendency to attach itself to supports (round which globules of oil in incomplete film tend to accumulate), and of species, such as *A. vagus*, which tend to dispense with supports. Correlated with this there tends to be a difference of feeding habit between such as like to attach themselves to objects and those which more often than not swim freely. The former, anchoring at one spot, would seem to trust largely to chance to send them the necessities of life, either down current or wafted along the surface of the water by the wind. In this connection a definite tendency to collect all at one side was noticed in the case of larvae kept in bowls in the open, though its relationship to wind and to conditions of light was not investigated. The free-swimming larvae exhibit a more definite tendency to go in search of food. The larvae of *A. vagus*, for instance, were often watched repeatedly swimming slowly along the bottom moving their mouth-brushes and evidently feeding, in *Stegomyia* fashion. It is of interest that these small-pool breeders are among the more dominant varieties of Anophelines, for even so small an advantage of habit may be the factor accounting for their greater success compared with other species. The activity of the larvae of *Stegomyia* is certainly one of the factors which have resulted in the dominance of certain species. Those of *S. albopicta*, for instance, are far more active than those of any Anopheline. They are on the move constantly, either swimming at the surface and feeding on the surface material, often rotating as if the tail end was at a more or less fixed point; or groping at the bottom of water in receptacles, often of considerable depth, for food in the debris, into which they will often burrow so that their tails only can be seen. They can swim just as fish do in direct line, head first, altering their direction by a mere flick of the tail; they can rise or fall just as suits them without great effort; and, by reason of the greater flexibility of their bodies, they can wriggle violently in all directions so as to escape their enemies.

So it seems likely that the dominance among Anophelines of *A. vagus*, for instance, may be related to similar characteristics.

A surprising lack of wariness was exhibited by the young larvae of all the species of Anophelines. It was possible gradually to advance a spoon in the water and remove numbers at a time in their first and second ecdyses. But as the larvae became older, so they became more and more alert, until in the last ecdysis it was difficult to secure any, and with some species, *A. ludlowi* and *A. subpictus* var. *malayensis* in particular, almost an impossibility. The larvae in some families were more alert than in others of the same species, an indication possibly of a better state of health, and for this reason it was difficult to arrive at any definite conclusions as to the relative wariness of the different species. With *A. ludlowi* and *A. subpictus* var. *malayensis* a rapid advance towards the basins containing them was detected instantly, the larvae diving at once, so that on arrival not a single one was to be seen at the surface. All would be resting

at the bottom, usually ventral surface uppermost with the "tail" at an angle of 45 degrees to the body, an attitude often seen in dead larvae, whereby it would almost appear that they might be hoping to deceive enemies in search of them. It was noted that a family of larvae of *A. hyrcanus* var. *paeditaeniatas* were particularly wary, remaining under the turbid water so long (some for two minutes) that it would have been easy for any inexperienced passer-by to doubt their existence even in bowls. The larvae of *A. barbirostris* seemed to be less alert than these until a rather later age, but were yet quite wary in the last moult. A little experience with a family of these will probably be always remembered by the writer. There had been at first considerable difficulty in breeding the species, most of the larvae in families dying off and only a few attaining maturity. Trial with a new food material seemed to afford promise of far better results, for out of a very large family of half-grown larvae none had died for days, and all were active. It was with no small dismay, therefore, that on approaching the basins containing them, a day or two before the expected pupations, many were seen, lying at the bottom in various position of distortion and upside-down. At each previous inspection all had been quietly feeding at the surface, and it was felt that those at the bottom must be dead, for no movement at all was made. However, on an attempt to move them, the apparently dead larvae scuttled off elsewhere with great activity.

The alarm manifested by the larvae would seem to suggest that their instinct had apprised them of menace both from above and from the water itself; indeed, the polymorphism of species (*A. hyrcanus* and *A. barbirostris* in particular) breeding by preference in large open sheets of water, affording many varied conditions of environment (to which the variations both of colour and pattern are due, in part at all events—as some experiments by the writer, as yet unpublished, have shown) would seem to suggest constant attack from above. From lack of opportunity the nature of this attack was not, unfortunately, enquired into very fully; but Anthomyiid flies of the genus *Lispa*, described by Atkinson in Hong Kong and by the writer in Nyasaland as attacking Culicid larvae, were present often in very great abundance on the water at its edge, and may well take some toll of the Anopheline larvae. Furthermore, particular dragonflies were seen frequently stooping over the water, head down, as if at some prey on the surface. It is not beyond the powers of dragonflies to take creeping insects (the writer has repeatedly watched a particular species in Nyasaland catching ants), and so these may well be among the agents which cause the larvae of Anophelines to feel apprehension.

#### **Other Natural Enemies of Larvae.**

It is said that the larvae of Anophelines do not as a rule coexist in breeding-places with those of CHIRONOMIDAE. This is true of small muddy pools in the Malay States, in some of which the CHIRONOMIDAE larvae exist in enormous numbers. So far as could be ascertained their influence on the Anophelines is indirect only; for, in basins, the Chironomids collected up all the floating algae with such assiduity, for the purpose of forming their larva cases, that the Anophelines soon became reduced to the verge of starvation. No direct attack was ever seen.

In certain ponds in which no Anopheline larvae were ever found in the course of searches made between April and July, certain predacious insects existed in great numbers, particularly the larvae of Neuroptera, which were never bred out (but were not those of dragonflies), and some BELOSTOMATIDAE, the females of which were frequently seen with masses of ova on their elytra. The Neuroptera in particular were highly predacious, devouring each other when confined in basins, even though supplied with ample other food material in the shape of Culicid larvae.

The writer has to acknowledge his obligation to the most efficient staff, trained by Dr. H. P. Hacker, the officer in charge of the Malaria Bureau, and particularly to Mr. Daniel Rajamoney and Mr. M. C. Chuen, for their untiring enthusiasm and patience in carrying out the details of the tedious breeding experiments.

---