Studies on the breeding behaviour of Aedes (Segomyia) Aegypti L. In Southern Nigeria, including observations on the breeding site preferences and the variations in breeding intensity throughout the year

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(Abstract)

Title: Studies on the Breeding Behaviour of Aedes (Stegomyia) aegypti L. in Southern Nigeria, including observations on the Breeding Site Preferences and the Variations in Breeding Intensity throughout the year.

The area in which these studies were carried out was the village of Ilobi fifty miles North-West of Lagos. The village clearing, in late secondary forest was half a mile in diameter and had a population of fourteen hundred. To the North of the Village was farmland, to the East scrub running into late secondary forest, to the South early secondary forest and some farmland and to the West a cocoa plantation. The climate in the village was typically hotter and dryer than in the forest. A.aegypti breeding was restricted to the Village area and the pre-adults were most abundant in domestic clay water pots, whilst breeding intensity was greater inside than outside the houses. Throughout the year breeding was continuous in the Village but in the dryer and hotter months some breeding was observed beyond this area although never within the actual forest. The population fluctuations as indicated by pre-adult numbers were as follows. In the dry season (November to February) the numbers were at a minimum and at the commencement of the rains there was a sharp increase followed by a decline in mid-rainy season (May to July). There was a further increase in August and then a gradual decline toward the end of the year. Laboratory studies indicate some of the factors which control this pattern of breeding and activity. As the adult is almost exclusively anthropophilic the concentration of human population is considered to be a major factor controlling the distribution.

The adults show a marked preference for ovipositing in clear water as opposed to that which is fouled by organic debris. The breeding intensity inside houses is probably controlled by light intensity and water temperature as adults show a preference for darker and cooler ovipositioning sites.

STUDIES ON THE BREEDING BEHAVIOUR OF *AEDES (STEGOMYIA) AEGYPTI* L. IN SOUTHERN NIGERIA

Thesis submitted by Mr. G. Surtees for the Degree of Master of Science in the University of Durham. August 1958

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INTRODUCTION

Aedes (Stegomyia) aegypti Linnaeus has been found to be the main vector of the filterable virus which is the causative organism of urban yellow fever. Due to the plasticity of the behaviour of the mosquito there still remain extensive fields of study before the full importance of this species as a disease vector can be realised and before effective measures can be taken to control it. Furthermore, with the discovery of jungle yellow fever, the relation of this species to non-human virus reservoirs has still to be examined.

Throughout the ancient medical literature there is no mention of the disease which we know as yellow fever. If it had existed its typically violent outbreaks could hardly have escaped notice. It was first recognised as a disease entity in the Seventeenth Century and its origin was probably tropical Africa or Central America, communication having existed between these two areas since the Fourteenth Century. Garrison, (1929) states that the term "yellow fever" was first used in a history of the Barbados written by one Griffin Hughes in 1750. Nott, (1848) was perhaps the first to suggest that a mosquito was the agent of transmission for this disease and for malaria, while Findlay, (1881) proposed the theory of propagation of yellow fever by a mosquito in a paper which he gave to the Royal Academy of Havana.

The nature of the causative organism was not finally established for sometime, although Sanarelli, (1897)

claimed to have discovered it in what he called Bacillus icteroides. Reed and Carroll, (1899) studying yellow fever cases, considered that this bacillus was only a secondary invader belonging to the colon group and was not, in fact, the causative organism of the disease. Later, Reed and his co-workers tried to isolate B. icteroides Sanarelli from the blood of yellow fever patients in Cuba and were forced to conclude that this organism stood in no causative relation to the fever. This team of workers then pursued the mosquito-vector line of inquiry and were able to report three yellow fever cases transmitted by A. aegypti adults which had previously been fed on patients clinically ill with the fever (Reed, Carroll, et al., 1900). Two further achievements of this team were, demonstrating that the causative organism could be passed through filters which would stop the smallest known bacteria, (Reed and Carroll, 1902) and sanitary measures directed against the mosquito which eradicated the disease from Havana within a year.

Up to the formation of the International Health Commission of the Rockefeller Foundation in 1913 the epidemiologic concept of yellow fever was a simple one. It was thought that the disease, caused by a filterable organism, could only be acquired by a bite of A. aegypti which had previously fed upon a yellow fever patient. In the mean time the findings of Reed and his team in Havana had been confirmed by workers elsewhere and Rockefeller Commissions commenced studies throughout South and Central America. Similar studies were

initiated in West Africa in 1916, although these were not fully established until the arrival in Lagos of the Rockefeller Foundation Yellow Fever Commission in 1920. During 1916 Bacot studied various aspects of the biology of A. aegypti, the results of which were published as an entomological report for the Commission. This commission produced a progress report, but in 1925 the newly formed West African Yellow Fever Commission under Dr. Harry Beeuwkes set about the problem in a more intensive manner. Their aim was to study the epidemiology of the disease in West Africa, to isolate the causative organism, to discover the mode of transmission and to identify the endemic areas. In 1927 Stokes and others took blood from two yellow fever patients in Accra, the serum of which was injected into a rhesus monkey; this developed the fever, thus opening up a new field of possible laboratory studies of the virus. This strain of the virus was propagated. They also demonstrated that this organism was a filterable virus, that it could be transmitted from monkey to monkey by A. aegypti and that the adult mosquito, after having once bitten an infected host, remained infective throughout its life (Stokes, Bauer and Hudson, 1928). Bauer, (1928) reported transmission of the virus by using Aedes (Stegomyia) luteocephalus Newstead, A. (Aedimorphus) apicoannulatus Edwards and Eretmapodites chrysogaster Graham, whilst Philip, (1929) also reported transmission by Aedes (Stegomyia) vittatus Bigot, A. (Stegomyia) africanus Theobald and A. (Stegomyia) simpsoni Theobald.

After the discovery of jungle yellow fever, (Shannon, Whitman and Franca, 1938) the most rapid advances were made in South and Central America and in East Africa. Studies upon A. aegypti in West Africa during this time were largely confined to a few workers, including Dalziel (1920), Dunn (1927, 1928), Philip (1931, 1933), Kumm (1931), Kerr (1933) and later Mattingly (1945, 1947). The results of these studies may be briefly summarized as follows. The concensus of opinion was that A. aegypti was typically a breeder in domestic containers and that its breeding habits kept it in intimate association with man. This element of domesticity was considered by Mattingly to play some part in the distribution and in the dispersal of the species. At the same time it was apparent that such domestic behaviour would prevent it from disseminating the disease into the forest, unless of course areas were located where the species had a more plastic behaviour. Dalziel and Kumm found the species breeding to a limited extent in tree-holes, whilst Dunn found that of the tree-holes he examined 87 per cent. were positive for A. aegypti. This latter result is in some conflict with the other findings, whilst Mattingly recorded that in his experience the species was absent from these habitats in this area. Dunn found that the species would breed up to 500 yards beyond human habitations, although both he and Philip found that the intensity dropped off considerably beyond 100 yards. Dunn also recorded that there was a sharp increase in numbers at the beginning of the rains. The findings of these previous studies may perhaps be best

summed up in the words of Mattingly.

> "The only species regularly found in small containers made of metal or pottery, or any inorganic substance, or in containers in or immediately about houses is Aedes aegypti."

In the second report of the Expert Committee on Yellow Fever of the World Health Organisation (1949) the following statement was made.

> "In view of the lack of sufficient knowledge concerning the ecology of Aedes aegypti in Africa and the problems involved in its eradication the Committee recommend that the World Health Organisation consider co-operation with the Governments concerned in an experiment aimed at the eradication of Aedes aegypti in a selected area in Africa where Aedes aegypti is known to be responsible for man-to-man transmission of the yellow fever virus."

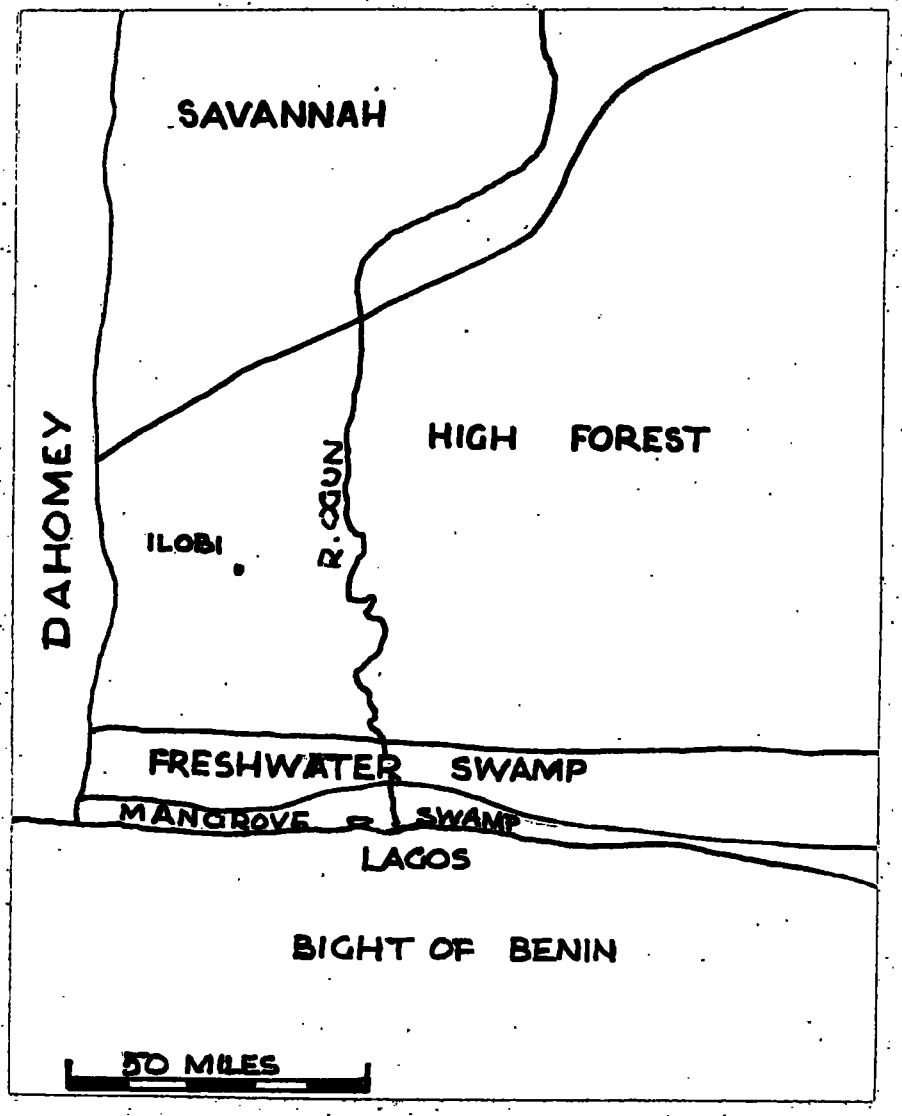
In 1954 the West African Council for Medical Research was set up and the Virus Research Unit, centred in Lagos, concentrated its studies on yellow fever. In September 1955 the author joined this Unit as Research Entomologist. With the recommendation of the Expert Committee in mind a systematic, localised study of the ecology of A. aegypti was commenced, with particular emphasis on the breeding behaviour, the results

of which are given here. The area where these studies were carried out, which will be described in detail below, was a small village some 45 miles north-west of Lagos by road, situated in the rain forest. This study fell naturally into two parts, namely, field and laboratory investigations.

The field investigations were made up of a survey of the natural larval habitats, a study of the distribution of the species in and around the village, with observations on any seasonal movements of the breeding population and, thirdly, a study of the numerical fluctuations of the species throughout the year. These numerical studies were carried out on the immature stages of the mosquito and will be described in the relevant sections. The laboratory studies consisted of a description of the biology of the species relating to its breeding and development and an investigation into the limiting factors which may control the observed pattern of breeding in the field.

Fig. 1.

Showing the main vegetational zones of South-western Nigeria.



PART I

THE TOPOGRAPHY AND CLIMATE OF THE AREA

a) South-western Nigeria.

South-western Nigeria can be divided into three major vegetational zones, mangrove swamp which runs parallel to the coast, freshwater swamp which extends up to ten miles inland and high forest which is found up to two hundred miles inland (Fig.1) High forest is a term employed to indicate a tropical forest having a leaf canopy of approximately one hundred feet or more (Rosevear, 1953).

The geology of this area was determined by two main subsidences, one at the beginning and another at the end of the Cretaceous period. In the early Cretaceous sinking of land allowed the sea to cover Southern Nigeria and in the late Cretaceous the water retreated leaving large areas of swamp. At the end of the Cretaceous further subsidence resulted in the deposit of sandstone and shales. The subsequent Eocene beds are rich in marine fossils and above these are seams of clay and sand. In the late Tertiary peneplains were formed and these became covered with thick layers of laterite.

The main drainage system of South-western Nigeria is formed by the Ogun River. The major tributaries enter some seventy miles inland and a large number of

smaller streams enter between here and the outlet on the coast.

The mangrove swamp is typical of much of the Nigerian coastal region, occupying most river deltas and inlets which may become flooded with brackish water at some time during the year. The major tree species of this swamp is the red mangrove (Rhizophora sp.). Mammals are restricted to the tree tops and only monkeys (Cercopithecus mona mona Schreber) and bats (Epomophorus gambianus Ogilbly, Rousettus smithii Thomas, Taphozus peli Temminck, Eptesicus platyops Thomas, E. tenuipinnis Peters, E. moloneyi Thomas and Scotophilus gigas Dobson) are abundant, but within these swampy areas patches of forest are to be found on hard, dry ground where rodents are more common.

Immediately inland from the mangrove swamp is the freshwater swamp which although low lying does not have its rivers inundated by tidal waters, although in the wet season much of the area is submerged. Apart from the swampy nature of the ground the freshwater swamp resembles the more typical forest zone further inland. Arboreal animals are typical of this area and include such species as flying squirrels (Anomalurus fraseri Waterhouse and A. beecrofti Fraser) and mona monkeys (C. mona mona).

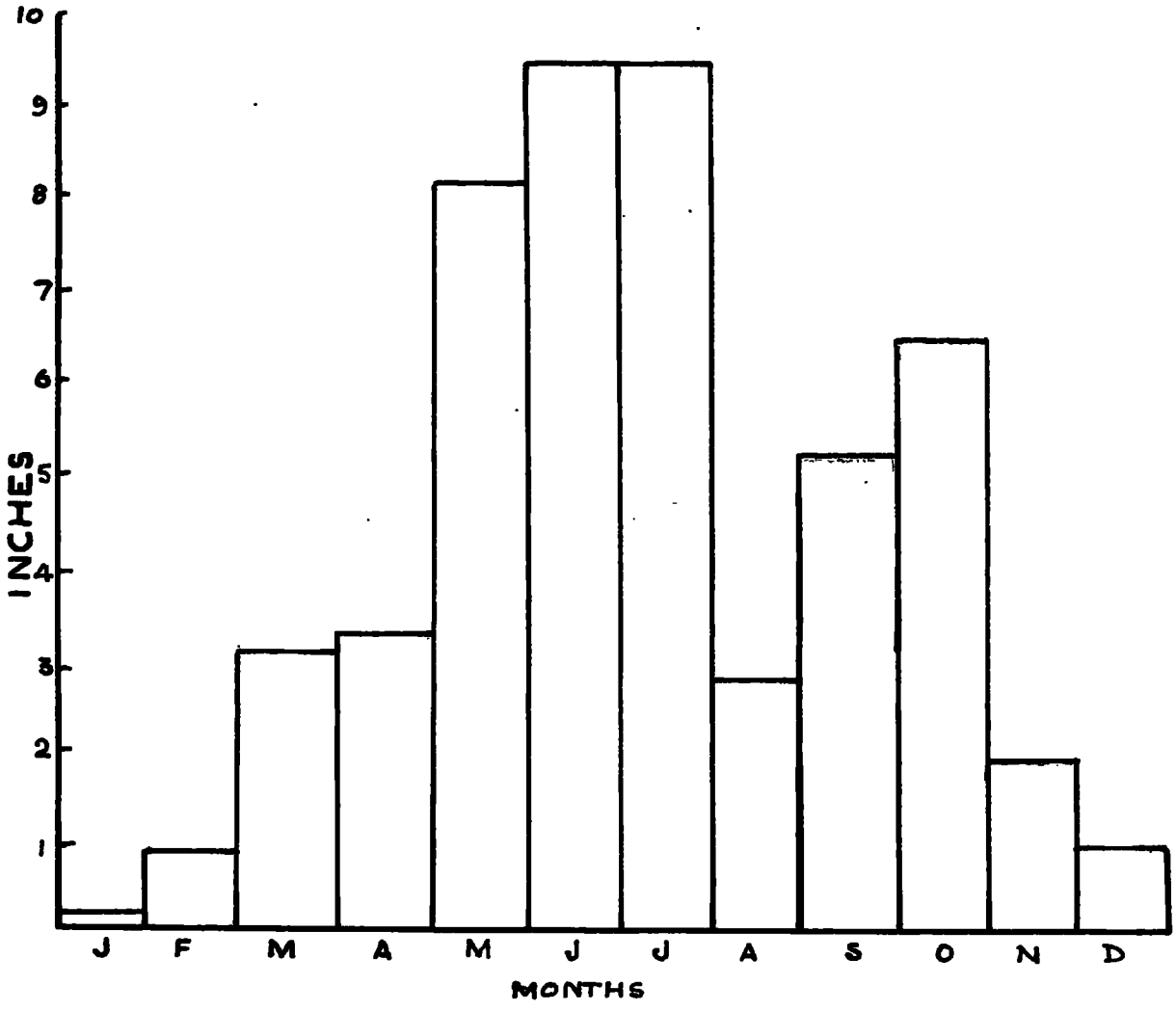
The rain forest in South-western Nigeria is much reduced toward its western edge due to the action of the south-west rain carrying winds which blow onshore

in Ghana and much of Nigeria, but parallel to the Dahomey coast. In this latter region the absence of rain forest constitutes the "Dahomey Gap" and although the rain forest commences once more in Nigeria it is at first reduced to an area near the coast. This gap is reflected by the animal distribution inasmuch that there is an unbroken distribution of savannah species extending from Nigeria to Ghana, whilst the forest species of these two territories are separated and distinct. Much of the high forest in Southern Nigeria is in a late stage of development and is characterised by the presence of emergents which typically have buttressed roots and penetrate the main canopy formed by the more shade enduring trees. There is a wide variety of mammalian species in this zone including mongooses (Crossarchus obscurus Cuvier), bush babies (Galago demidovii Fischer), pottos (Perodicticus potto Miller), hyraxes (Dendrohyrax dorsalis sylvestris Temminck), tree-squirrels (Funisciurus anerythrus Thomas), dormice (Graphiurus hueti Rochebrune) and Cercopithecus mona mona.

This three-fold division of South-western Nigeria has superimposed upon it changes due to clearing and farming. The forest is broken by scattered clearings, each containing a village surrounded by its area of farmland. The original forest is considered to be largely cleared and the present forest is termed "secondary". This is found in various stages of development, from cleared farmland with incipient tree growth, through mixed farmland and early secondary forest to typical

Fig. 2.

Showing the average mean monthly rainfall (inches) over the past five years for Lagos, Nigeria.



late secondary forest. From the point of view of animal ecology this presents a wide variety of plant communities, each of which may be expected to have a characteristic fauna.

Nigeria is situated between the latitudes of 6° and 11° north of the equator and there are typically two seasons, a hot dry season and a cooler wet season, the latter lasting from April to October. The prevailing wind system is from the south-west and on the coast the average wind speed is about seven miles per hour. During the year there are some thirty to thirty-five line squalls, usually at the beginning or the end of the rainy season, which are characterised by winds of high force and heavy downpours of rain. In December the hot, dry "Harmattan" blows southwards across the country from the Sahara Desert, causing reduced humidities, and in the south this dust laden wind causes low night temperatures and early morning mists. At this time of the year the minimum relative humidity in the southern region may fall as low as sixty per cent. The average yearly rainfall in the coastal region is between fifty and sixty inches but this value decreases further inland. Between November and March there is typically little rain but showers become frequent in April before the main rains in May to July. In August there is slightly less rain before the last rains in September and October. The mean monthly rainfall over the past five years for Lagos is shown in Fig. 2 (Table 1, Appendix I). The maximum temperature throughout the year ranges between 26° and

Fig. 3.

Showing the average monthly means of maximum and minimum temperatures (°C.) over the past five years for Lagos, Nigeria.

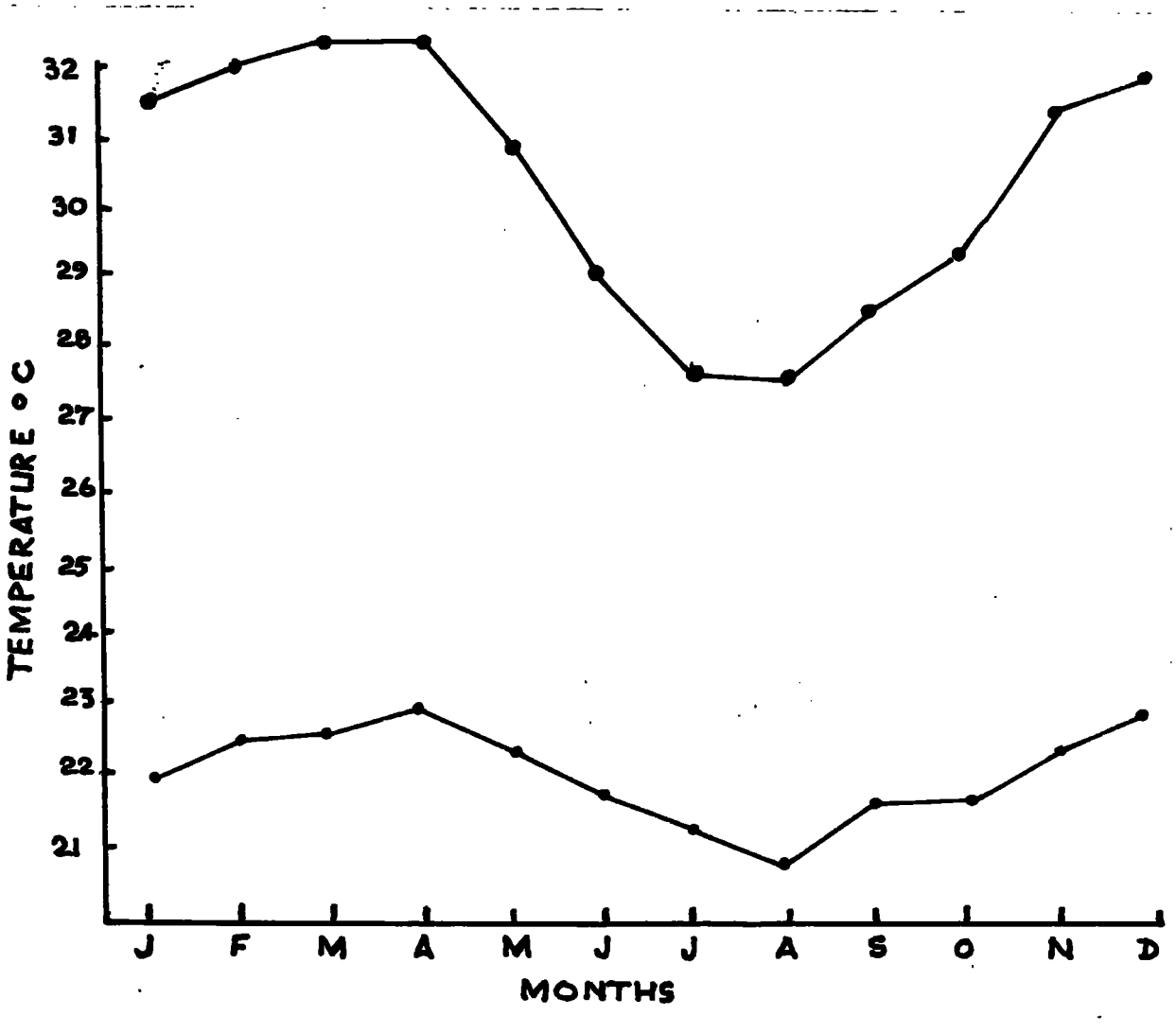
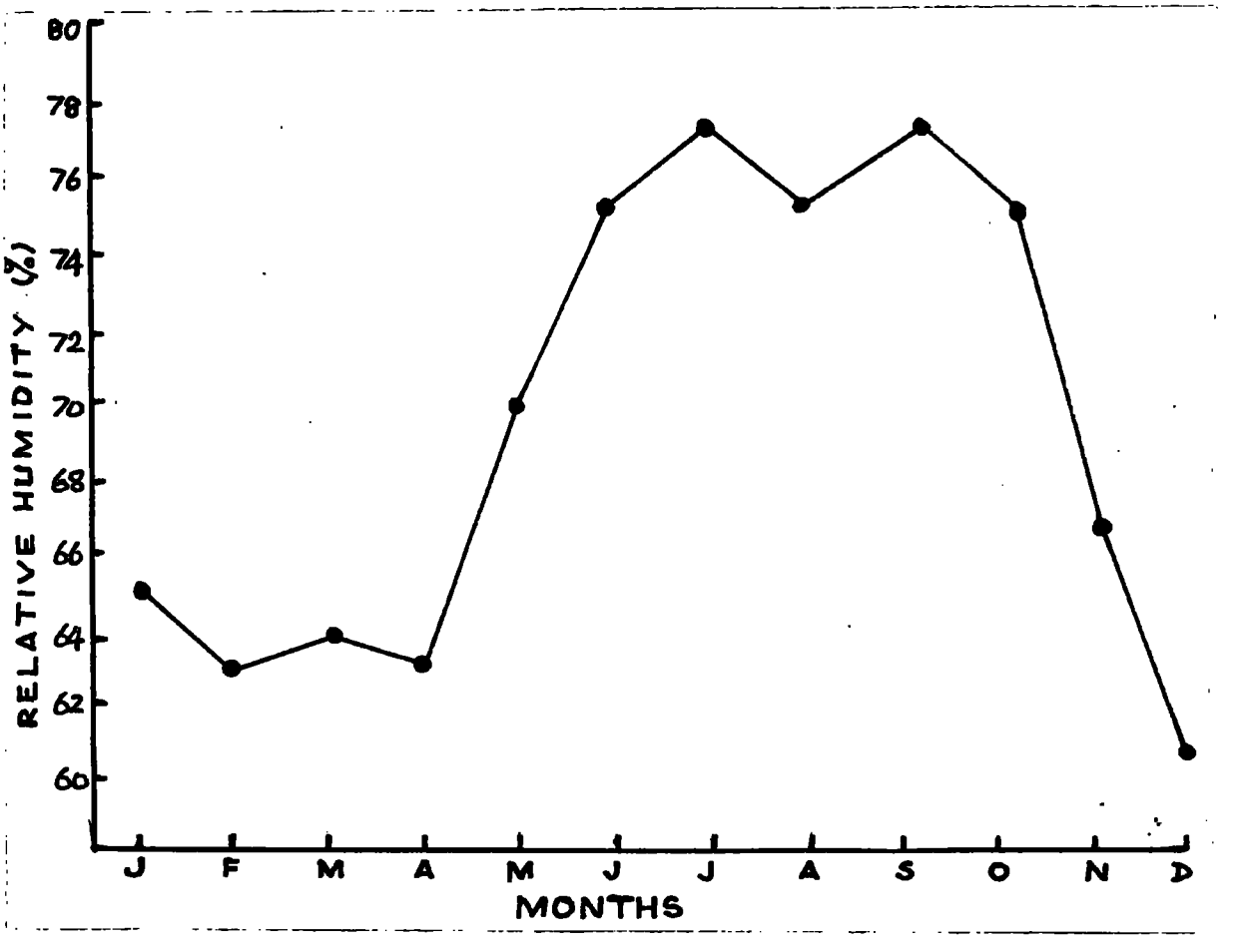


Fig. 4.

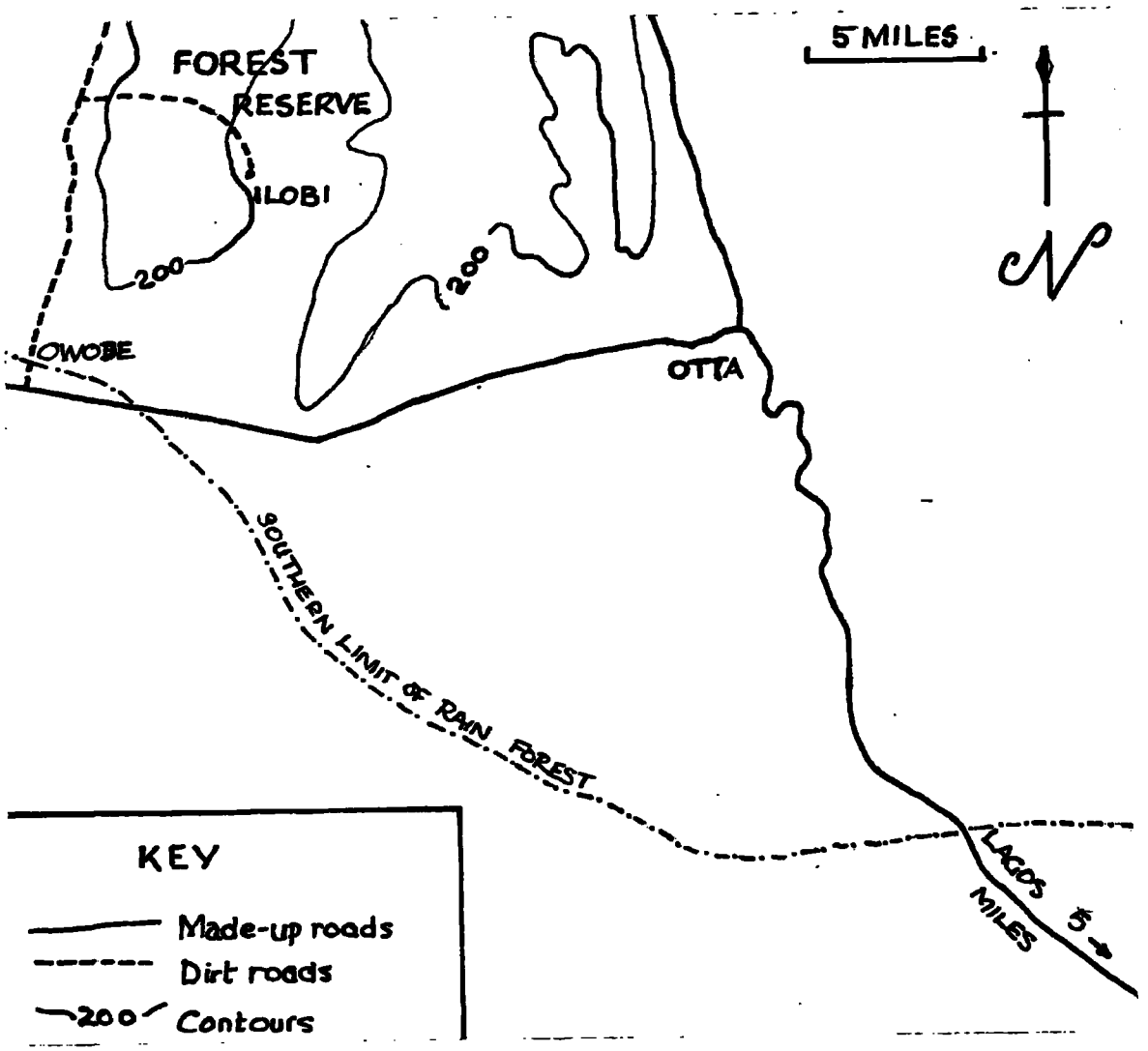
Showing the average mean monthly minimum relative humidity (%) over the past five years for Lagos, Nigeria.



34°C., the lower temperatures occurring in the wet season. The minimum temperature remains in the region of 22° to 24°C. at all times of the year, Fig. 3 (Table 1, Appendix I). The relative humidity has a constant maximum value of about ninety-nine per cent. whilst the minimum value varies throughout the year between sixty and eighty per cent., the lowest value being in the dry season, Fig. 4 (Table 1, Appendix I).

Fig. 5.

Showing the position of Ilobi village.



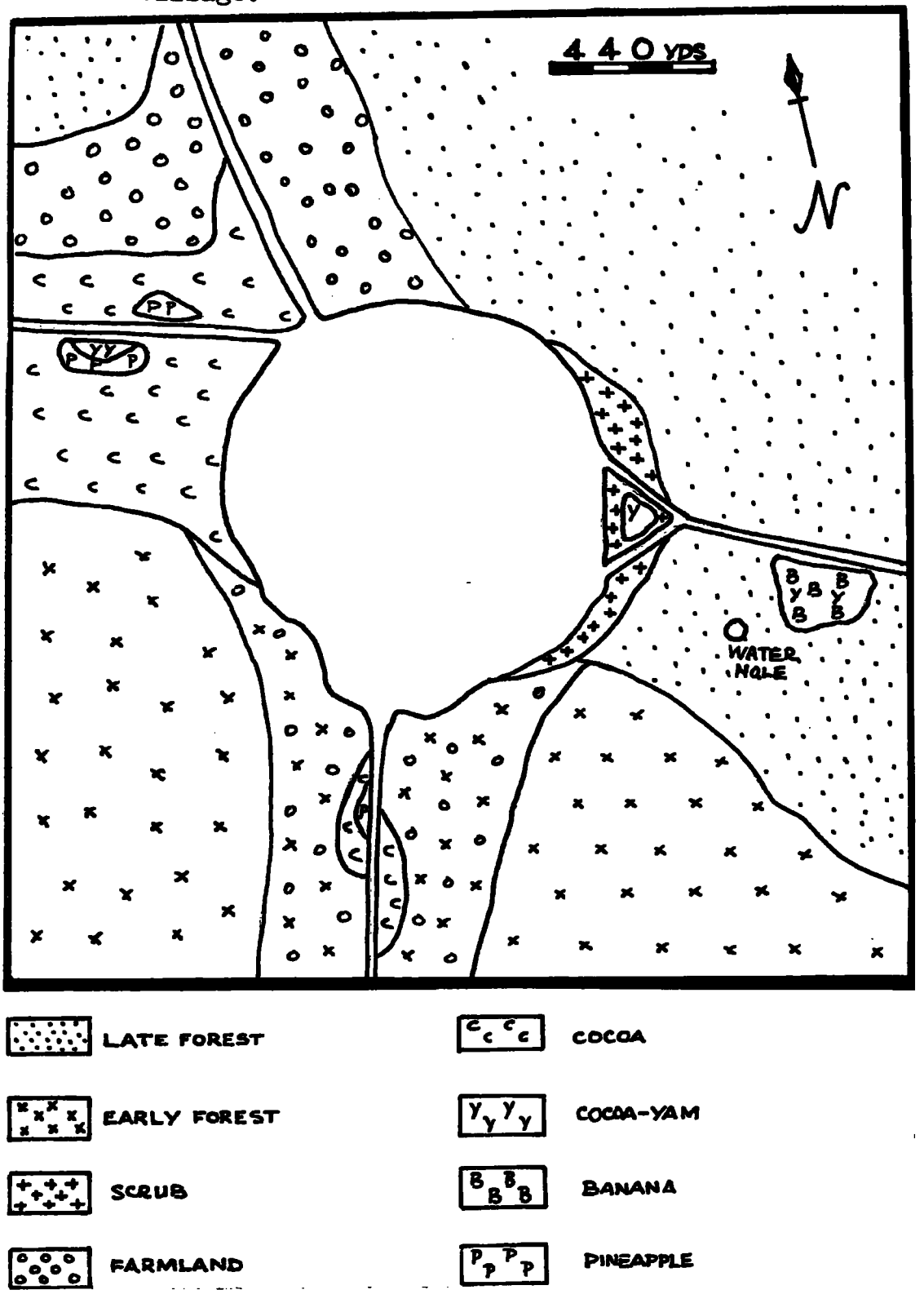
b) Ilobi Village.

Ilobi Village (6° 45' N. 3° 5' E.), in which these studies were carried out, is forty-five miles north-west of Lagos by road, it is two hundred feet above sea level and is surrounded by rain forest, Fig.5. The village clearing is roughly circular, half a mile across, the houses are built of compacted laterite mud and within the village there is very little plant growth. The population, numbering about 1,200, belongs to the Yoruba tribe and the people are mostly farmers. The village is divided into a number of family units, or compounds, each being surrounded by a mud or bamboo wall which will thus enclose the main house and subsidiary buildings. In the centre of the village is the "court house" in which most of the administrative and judicial functions of the village are conducted. There is also a church and a school. Around the village there are a number of well defined vegetational zones, Fig. 6.

To the north of the village there is an area of mixed farmland, the main crops being cassava (Manihot sp.), yam (Dioscorea sp.), West African cocoa-yam (Xanthosoma sagittifolium and some cocoa; each family plot being marked off by a bamboo fence. The road into the village runs through this area of farmland. On the eastern side there is a belt of scrub which extends for some two hundred yards beyond the village boundary and here there is some cocoa-yam growth and a few gourd trees apart from the general scrub and incipient tree growth.

Fig. 6.

Showing the main vegetational zones around Ilobi village.



This area represents an early stage in forest recolonisation of cleared farmland. Beyond this is late secondary forest forming part of a forest reserve, having dense canopy and a fairly thick herb and shrub layer. These latter characteristics distinguish late secondary forest from true primary forest, where the ground layers are typically absent. The major tree species in the forest are African Maple (Triplochiton schleroxylon), Iroko (Chlorophora excelsa), Bosquiea angolensis and Sterculia rhinopetala. Triplochiton and Bosquiea have extensive buttresses and above these the girth of the tree may be as much as fifteen feet. Chlorophora on the other hand has no buttresses and the main trunk may be free of any branches up to about seventy feet. Sterculia is a straight boled tree about one hundred feet tall and has short, narrow buttresses. Just inside the forest there is a large plantation of bananas (Musa sapientium) in which there is some cocoa-yams. To the south of the village there is an area of mixed farmland and early secondary forest where the canopy is less dense and the shrub layer thicker than in the late forest. About two hundred yards beyond the village there is a thick patch of pineapple (Ananas comosus) and some more, with scattered cocoa-yams, at about four hundred yards. The western side of the area is taken up by an extensive cocoa plantation in which there is some scattered pineapple growth. The plants which are of importance from the point of view of mosquito breeding are pineapples, bananas and cocoa-yams, all of which have water retaining leaf axils, and cocoa trees which provide husks capable of holding

Fig. 7.

Showing the mean monthly maximum and minimum temperatures (°C.) for Ilobi village and Lagos. Jan - Dec 1956.

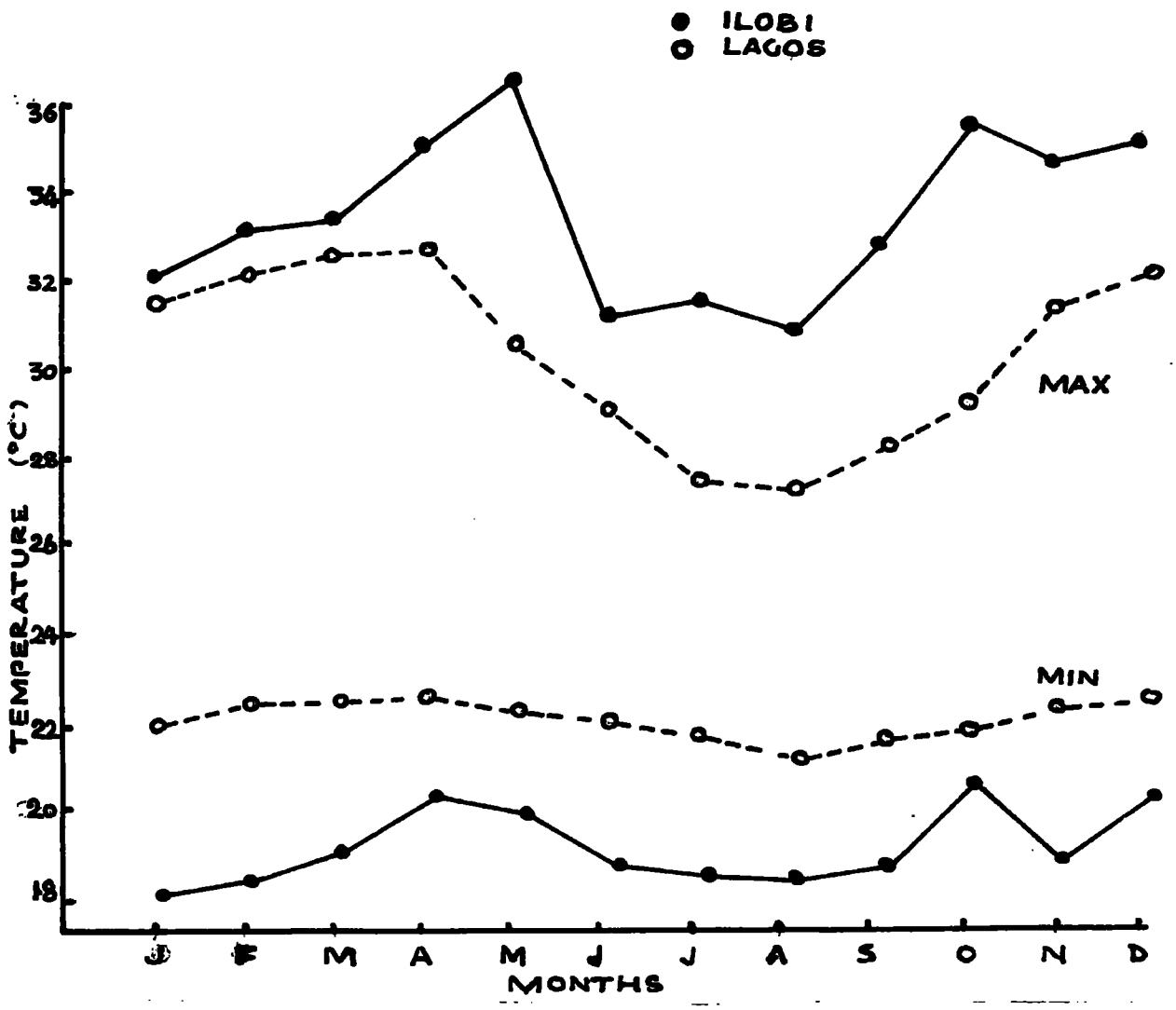


Fig. 8.

Showing the mean monthly maximum and minimum temperatures (^{o}C.) for Ilobi village and forest. Jan - Dec 1956.

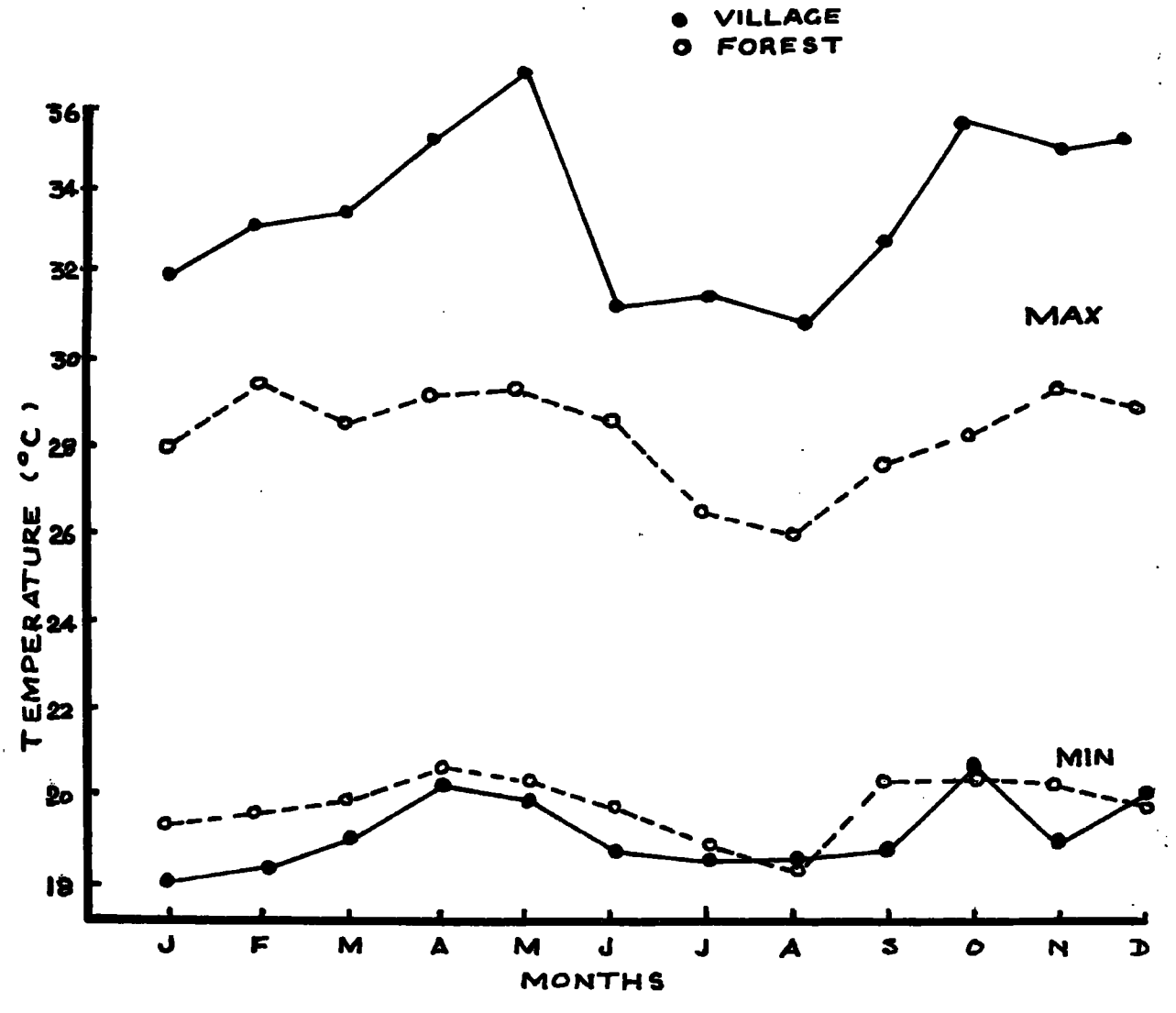


Fig. 9.

Mean hourly values of temperature (°C) and relative humidity (%) for Ilobi village and forest, taken over one week in November 1956.

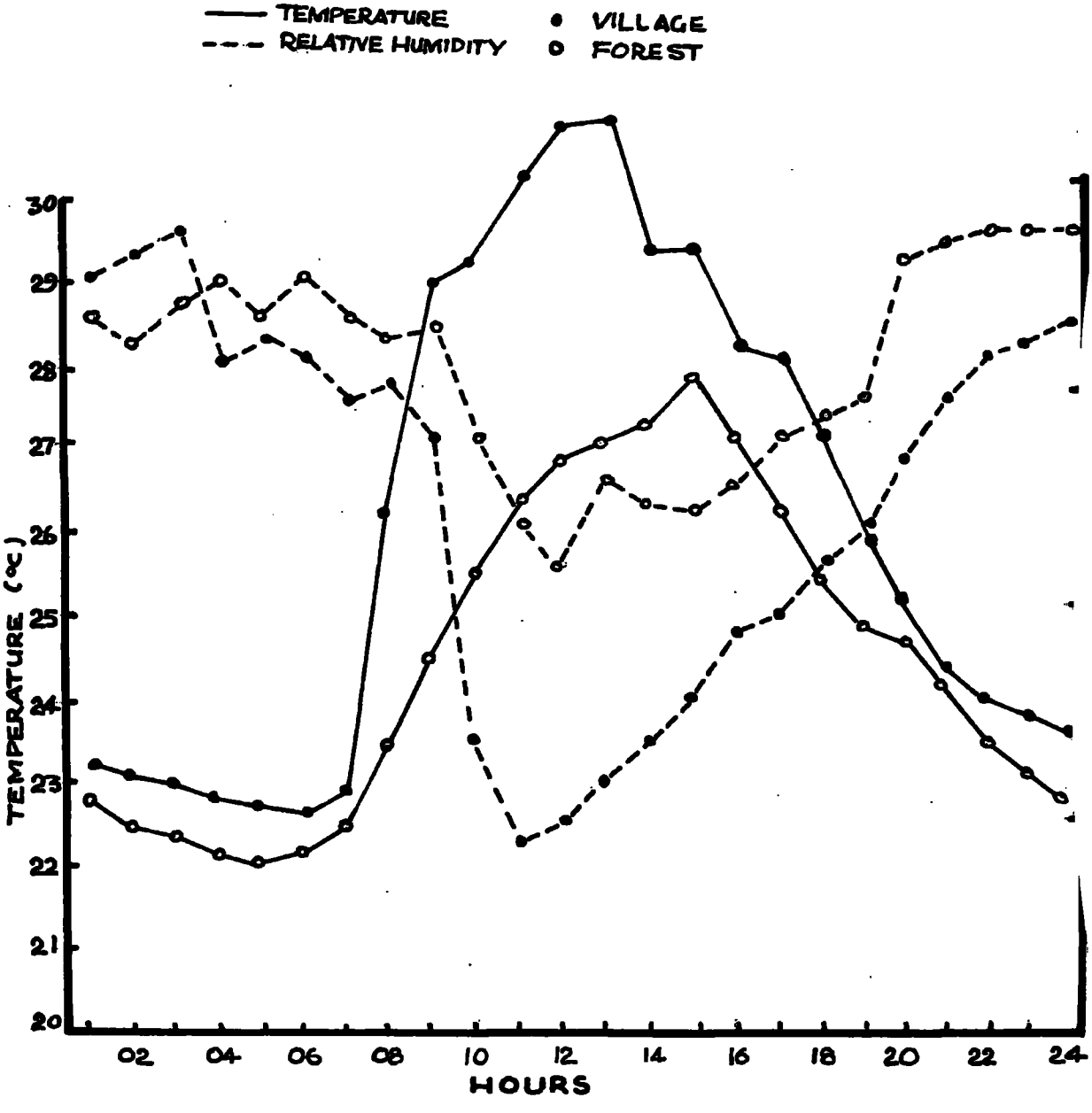
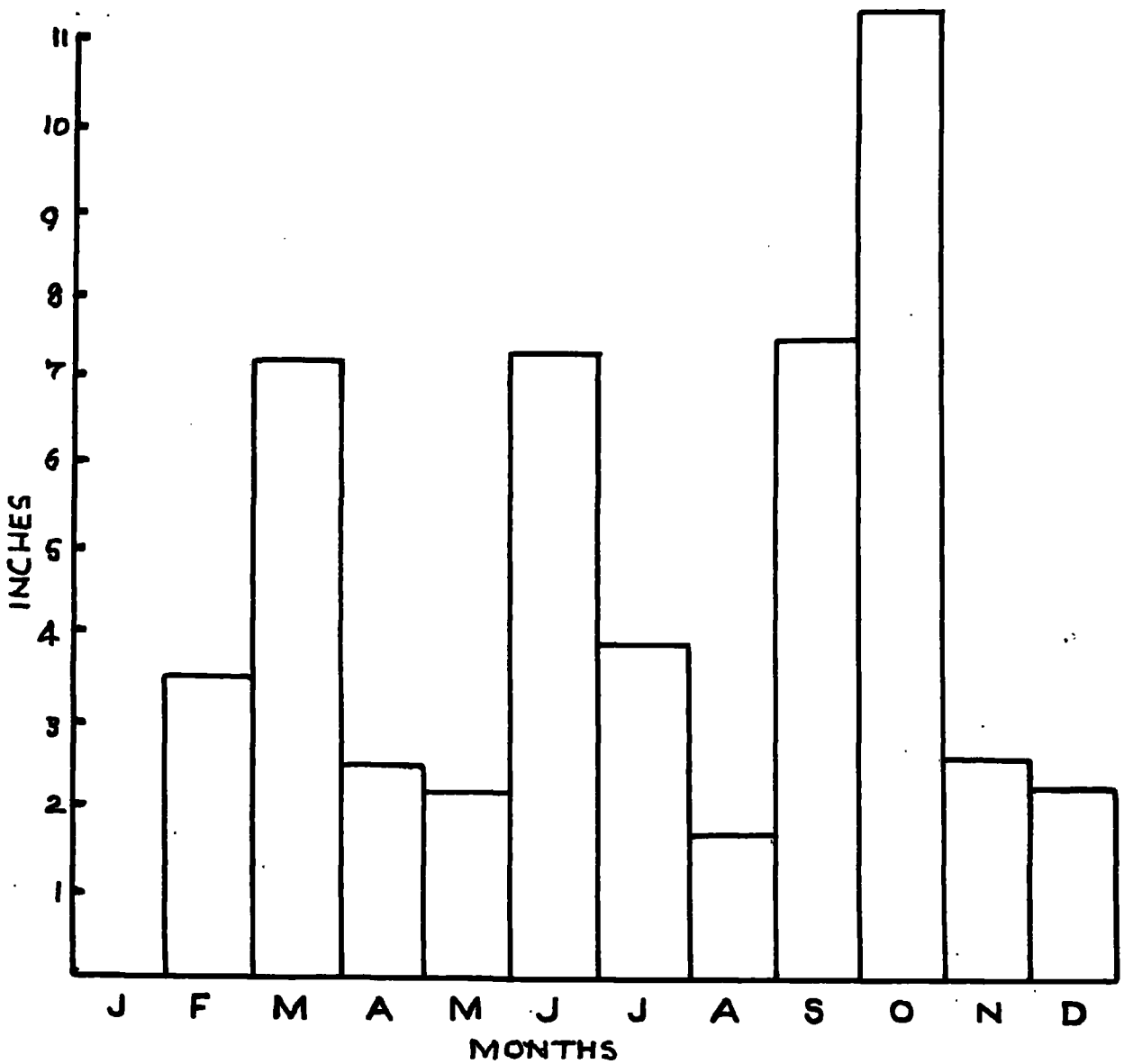


Fig. 10

Rainfall (inches) at Ilobi, 1956.



varying quantities of water.

During the year January to December 1956, in which the field studies were carried out, temperature records were kept for the village and the late secondary forest. The records for the village have been compared with those for the coastal region over the same period in Fig.7 (Table 2, Appendix I) and with the forest in Fig.8 (Table 3, Appendix I). These comparisons indicate that the village is subject to greater climatic variations than either the coastal region south of the rain forest boundary, or the forest immediately beyond the village. Further studies to investigate the difference in the climate between village and forest were done by taking continuous readings of temperature and relative humidity in both areas. These readings were taken at hourly intervals over a period of one week in November and the mean hourly values are plotted in Fig. 9 (Table 4, Appendix I). The results indicate that the forest is cooler and more humid than the village, with a reduced range of variation of both temperature and relative humidity. It is also seen that the midday peak of temperature is delayed in the forest by some two hours, further more the forest temperatures both increase and decrease less abruptly than those in the village. The rainfall at Ilobi throughout 1956 was recorded and the monthly values are shown in Fig. 10 (Table 5, Appendix I). It is seen that 1956 did not follow the regular pattern, in that April, May and July were dryer than usual whilst March and October were very wet.

PART II

GENERAL BIOLOGY OF THE SPECIES

a) Nomenclature.

Culex aegypti Linnaeus 1762
Culex argenteus Poiret 1787
Culex fasciatus Fabricius 1805
Culex calopus Meigen 1818
Culex formosus Walker 1848
Culex inexorabilis Walker 1848
Culex insatiabilis Bigot 1859
Stegomyia nigeria Theobald 1901
Stegomyia fasciata Theobald 1901
Aedes argenteus Theobald 1901
Aedes (Stegomyia) aegypti Barraud 1934

Mattingly (1958) has recently examined the taxonomic situation with regard to this species and has come to the conclusion that there is a type form, a sub-species and a variety, each of which have distinct morphology and biology. The findings of this inquiry may be summarized as follows.

A. aegypti sens. str. The type form, variable in colour but always paler and browner than the blackish African sub-species. May also have pale scaling on the first abdominal tergite.
Common in the Indomalayan and Pacific areas, coastal in Africa with some penetration. Mainly domestic but has some capacity for

breeding in natural habitats.

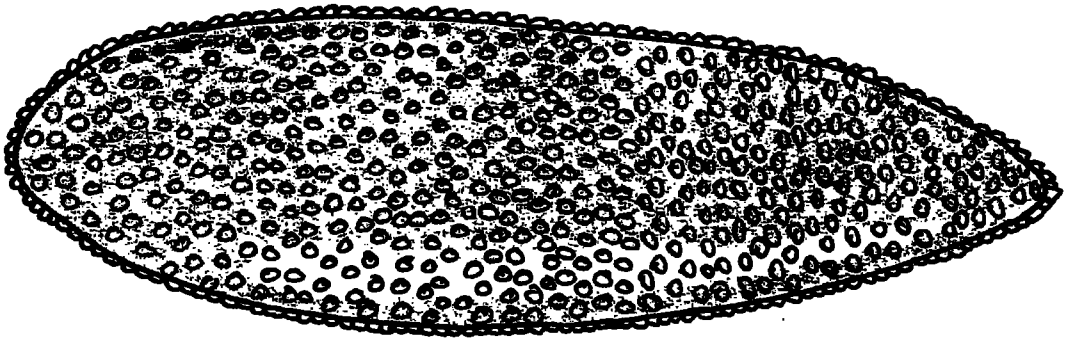
A. aegypti spp. formosus Walker 1848. Has a markedly blackish appearance of the darker areas of the thorax and abdomen and entirely lacking in any bleaching or extension of pale scaling on any part of the body.
Confined to Africa south of the Sahara.
Can exist in the fully wild form but will become domesticated under rural conditions.

A. aegypti var queenslandensis. This was described by Theobald in 1901 from Northern Australia and includes any of the following forms. Those with bleaching of the scales of the metanotum. Encroachment of pale basal bands on the abdominal tergites on to the apices of the preceding segment. Extension of these bands to form basal triangles in the mid-line or continuous longitudinal pale line. Those with scattered pale scales on the dark areas posterior to the pale tergal bands or on the normally dark areas of the legs.
These forms are common in Mediterranean areas, probably common in parts of India and Australia and coastal in Africa.
They are entirely domestic.

The mosquitoes discussed here are the type form of A. aegypti.

Fig. 11.

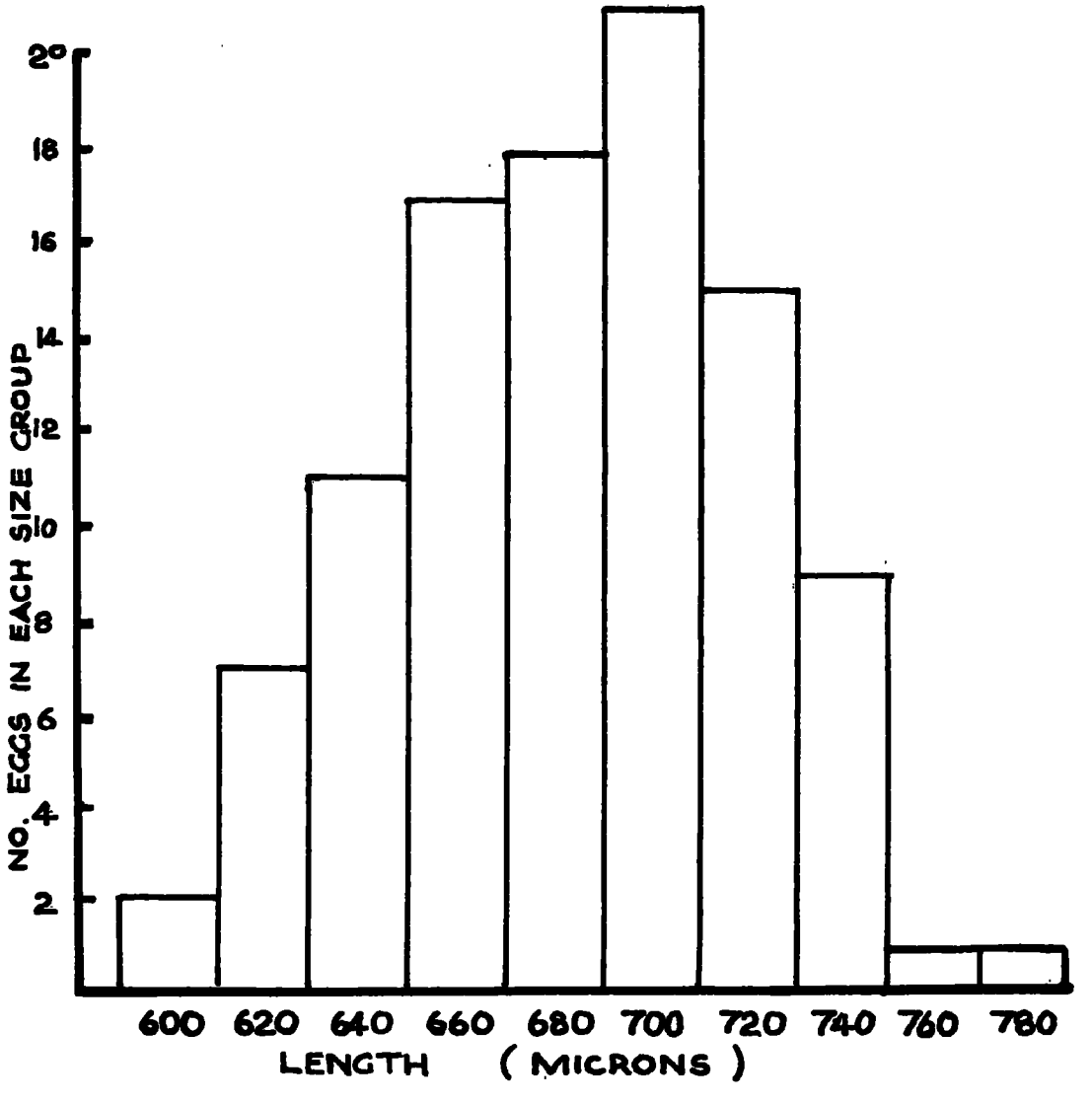
A diagramatic representation of the egg of A. aegypti showing the sculptured exochorion and peripheral floatation chambers.



X100

Fig. 12.

Showing the size distribution in microns of egg lengths of A. aegypti.



b) The Egg.

The eggs are laid in batches of about one hundred per female and they are scattered individually on the moist surface immediately above the water level or, less commonly, on the actual water surface. They are black, ovoid, slightly wider at the anterior end and do not have lateral floats common to Anopheles eggs, although the periphery is marked by a series of small air chambers which aid floating. The exochorion is heavily sculptured giving it the appearance of being deeply pitted, Fig.11. The size of the eggs varies particularly with regard to the length, Fig.12, (Table 6, Appendix I). Measurements of 103 eggs of this strain of mosquito showed that the length varied between 600 and 780 microns (mean= 683 microns) whilst the greatest width varied between 160 and 200 microns (mean= 184 microns). The operculum forms an asymmetrical cap at the anterior end, usually occupying about $1/6$ of the total length of the egg.

The maturation of the egg varies with the conditions in which it is kept. It has been observed that a period of drying is necessary before hatching will take place. Eggs laid and maintained on a water surface in the laboratory at 26°C. will remain in the unhatched state for at least a month. On the other hand eggs which have been kept in a dry state at 28°C. and 60 per cent. relative humidity for 24 hours will hatch in 15 minutes. The eggs have been shown to withstand long periods of desiccation, this facilitating the survival of the species over the dry season (Surtees, 1958a). During the field

studies to be described later it was found that at the beginning of the rains there was a greater increase in larval numbers than would be expected if only a few adults had survived the dry season. The following experiments indicate that this may have been due to the residual population from dried eggs which had survived the dry season and commenced to hatch when the rains started. The eggs used were laid between 9:10:55 and 12:12:55 in the department's insectory. The adult females had been fed on laboratory white mice and the eggs were laid on damp filter papers placed around the inside of beakers in the cage. The eggs, still on the filter papers, were placed in test tubes lightly stoppered with cotton wool and were thus exposed to the same changes of humidity and temperature throughout the period of storage as they would have had in the field. Commencing on 24:1:56 and then at 4-weekly intervals, samples of each egg batch were placed in water to hatch. The number of adults resulting from each egg batch was expressed as a per-centage of the number of eggs used (Tables 7 & 8, Appendix I). The eggs were counted under a dissecting microscope and 600 were used in each test. Within the period of these experiments no rain fell between 12:11:55 and 9:2:56. The percentage hatch of each of the egg batches falls off with increase in time from laying. It is also indicated that the later batches produced a smaller percentage of adults at the 20 and 24 week stages than the ones laid earlier in the year. It would seem that the eggs laid at the end of the previous rainy season had a greater resistance to desiccation

Fig. 13.

Showing the grouped results of the tests on hatching of A. aegypti eggs. Age of eggs plotted against percentage hatch

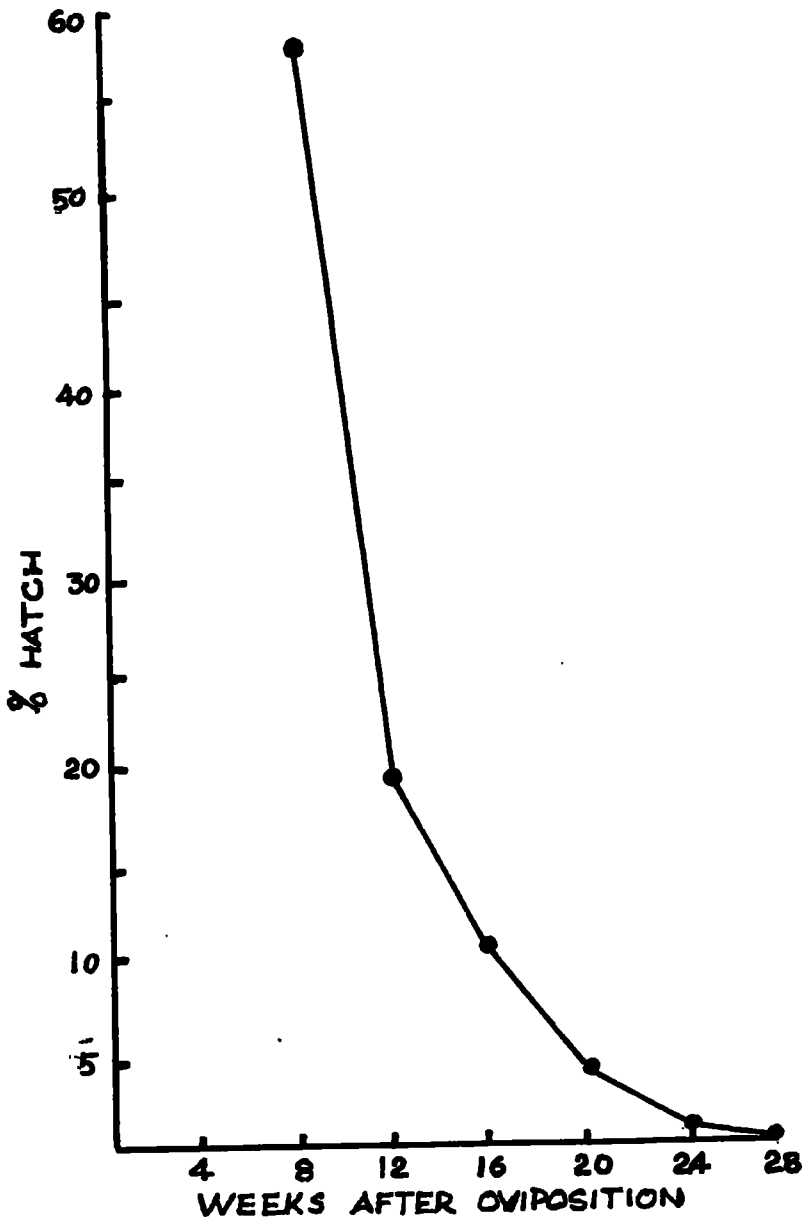


Fig. 14.

Showing the rate of hatching of A. aegypti eggs in water at various temperatures. The time of hatching of the first 50 eggs in each batch of 2000 is plotted.

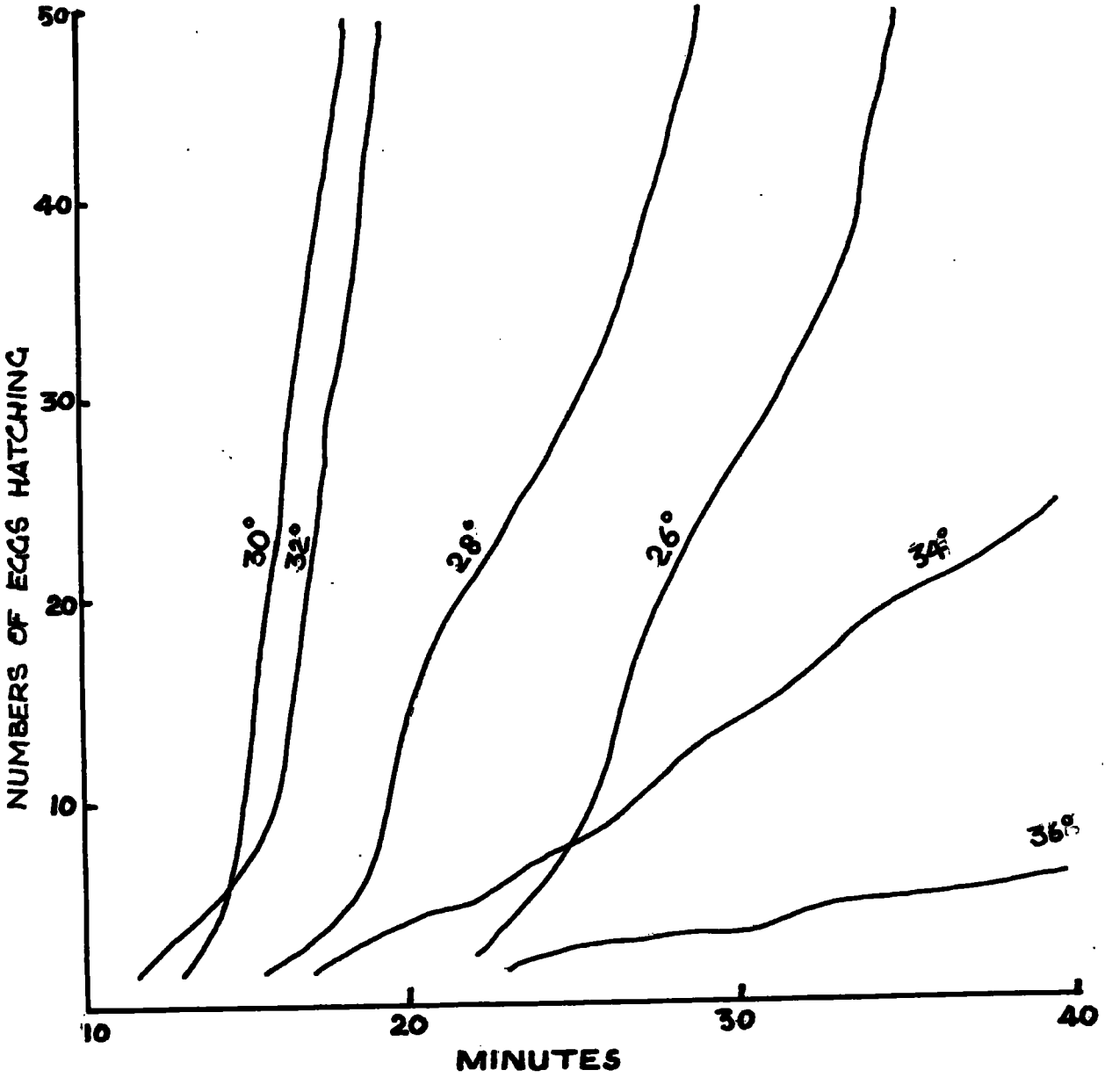
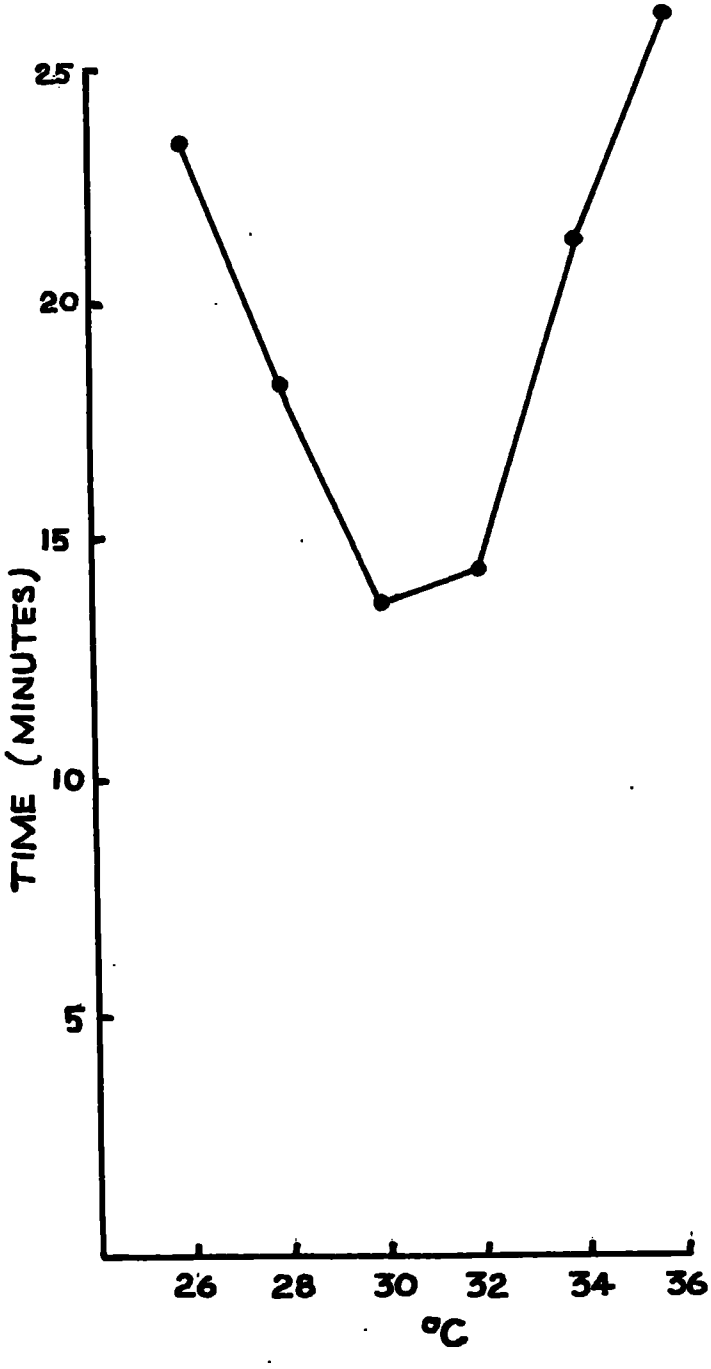


Fig. 15.

Mean time taken to hatch of mature A.aegypti eggs in water at various temperatures. 50 eggs used at each temperature.



than those laid during the actual dry season, nevertheless all the egg batches survived in some degree until the next rainy season. (q.v. Fig.13.)

It has also been found in the laboratory that the rate of hatching and the mean time taken to hatch for matured eggs varies with temperature. This was demonstrated as follows. The eggs used in these tests were obtained from a freely breeding colony maintained at 26° to 28°C., the eggs being laid on damp filter papers. They were stored for 2 days after laying at a relative humidity of 60 per cent., the experiments being carried out on the third day. Batches of about 2,000 eggs were used in each test, these being placed in a litre of water maintained at the required temperature. The water temperatures ranged from 26°to 36°C. and the time at which each of the first 50 eggs hatched was plotted. It was found that hatching was most rapid at 30° to 32°C. but below and above this point the rate was retarded, Fig. 14. At temperatures above 40°C. no hatching response was obtained. It was also observed that after the first few eggs had hatched the rate increased, this being less obvious at higher and lower temperatures.

Similar tests carried out under conditions ranging from 26° to 36°C. revealed that the mean time for eggs to hatch was shortest at about 32°C., the time increasing at higher and lower temperatures, Fig.15 (Table 9, Appendix I).

Fig. 16.

Showing the process of eclosion of A. aegypti larva.

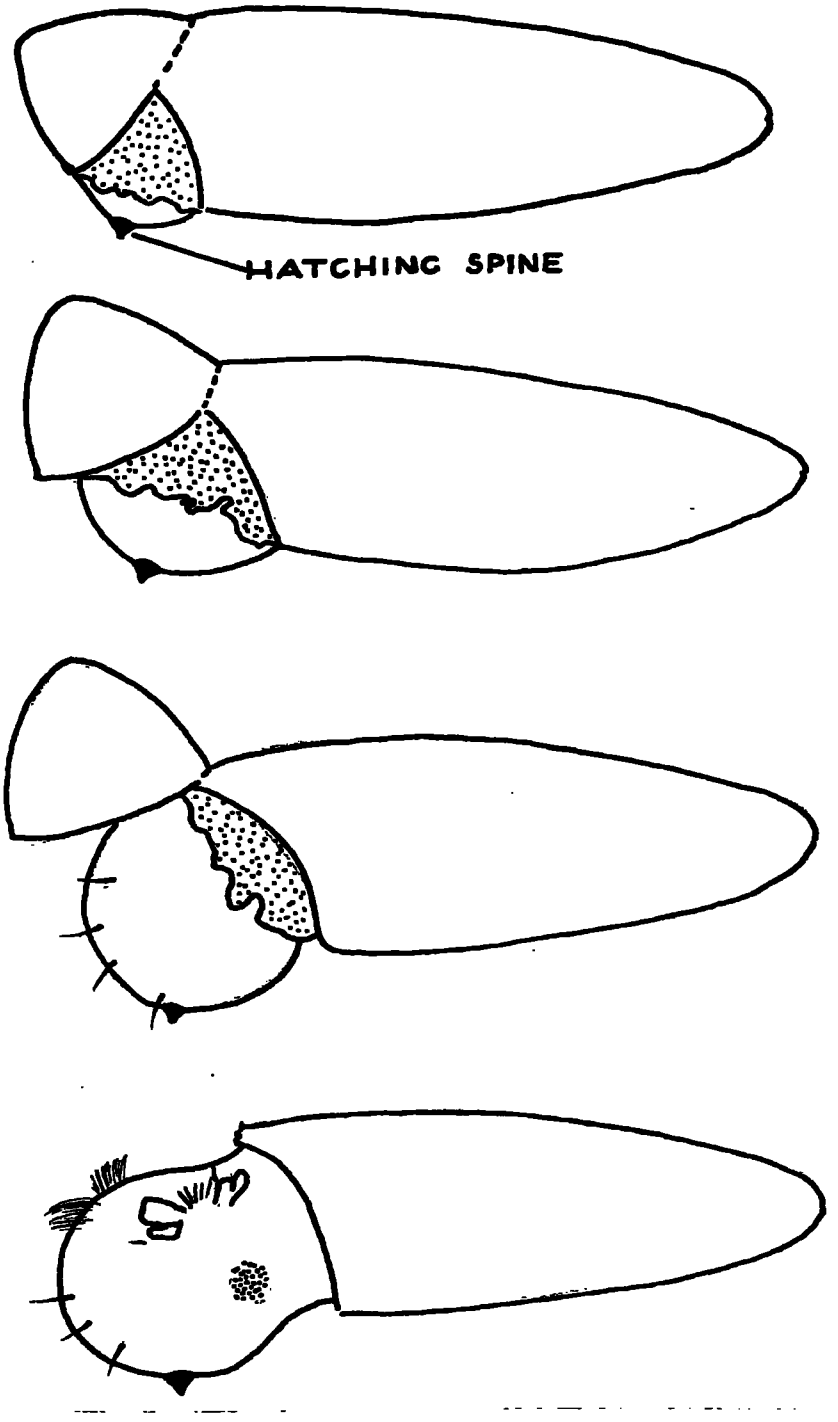
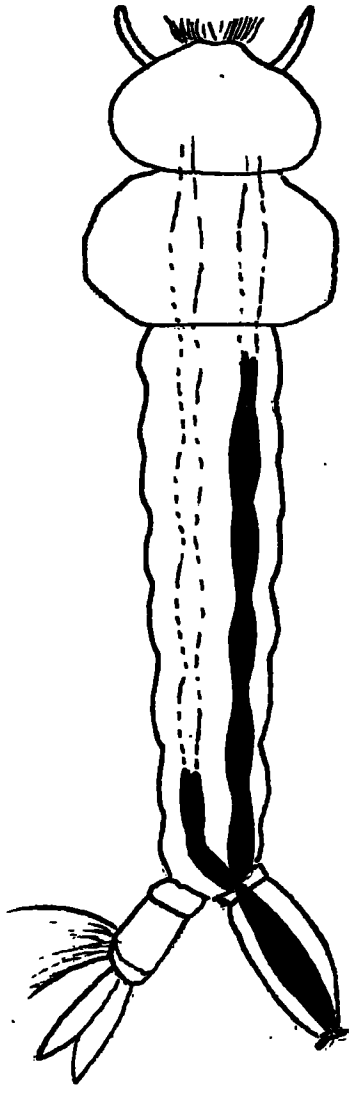


Fig. 17.

Showing the two major air trunks in the first instar larva some 30 minutes after eclosion.



c) The Larva.

Eclosion from the egg takes place when the mature egg is either on the surface of, or submerged in, water. The first sign of hatching is the appearance of a split at the anterior end. This gradually widens and the embryonic membranes are seen to bulge outwards, Fig. 16. Soon after this the dorsal surface of the head of the larva becomes visible, the hatching spine being the most prominant feature, and as the head is forced into the split the latter widens. The head continues to be extruded, swelling as it does so and rupturing the remaining membranes. At this stage the opercular region may break away altogether. As the head becomes more free the mouthparts, eyes and antennae become visible and with a final contortion the larva frees itself. The hatching spine of the first instar is well chitinised but is cast off with the exuvium at the first ecdysis. Once the larva becomes free it has been observed that the main tracheal trunks extending from the posterior siphon into the head begin to fill with air. This does not happen simultaneously in both trunks, one usually being at least half expanded within the first half hour while both are expanded within the first hour of free life, Fig. 17.

The four larval instars vary little in morphological features and such variations as have been observed will be discussed later. The general features of the larva are best demonstrated by the fourth instar which is

Fig. 18.

Showing the prominent metapleural spine of the fourth instar larva of A. aegypti.

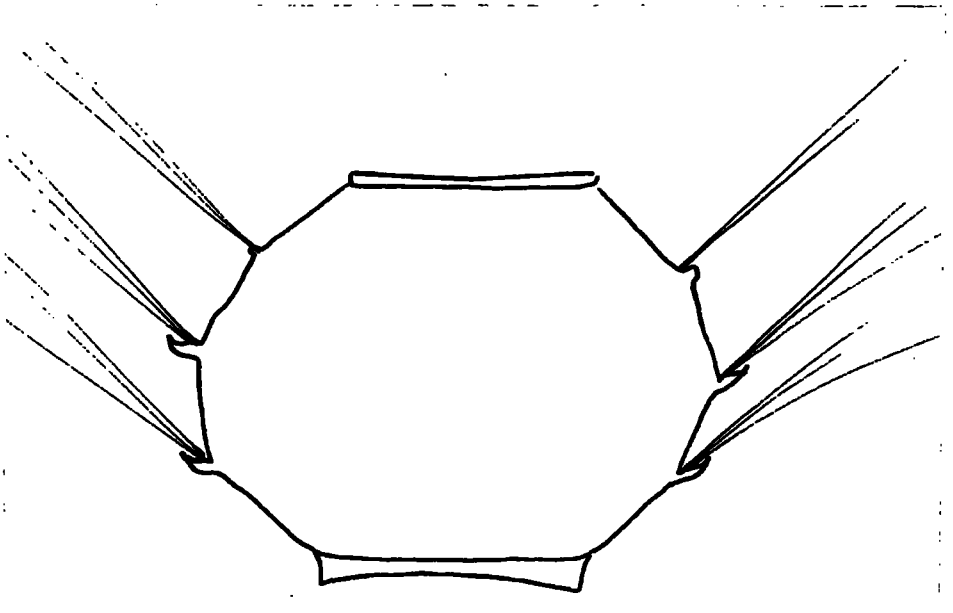


Fig. 19.

Details of the terminal segments of fourth instar larva of A. aegypti.

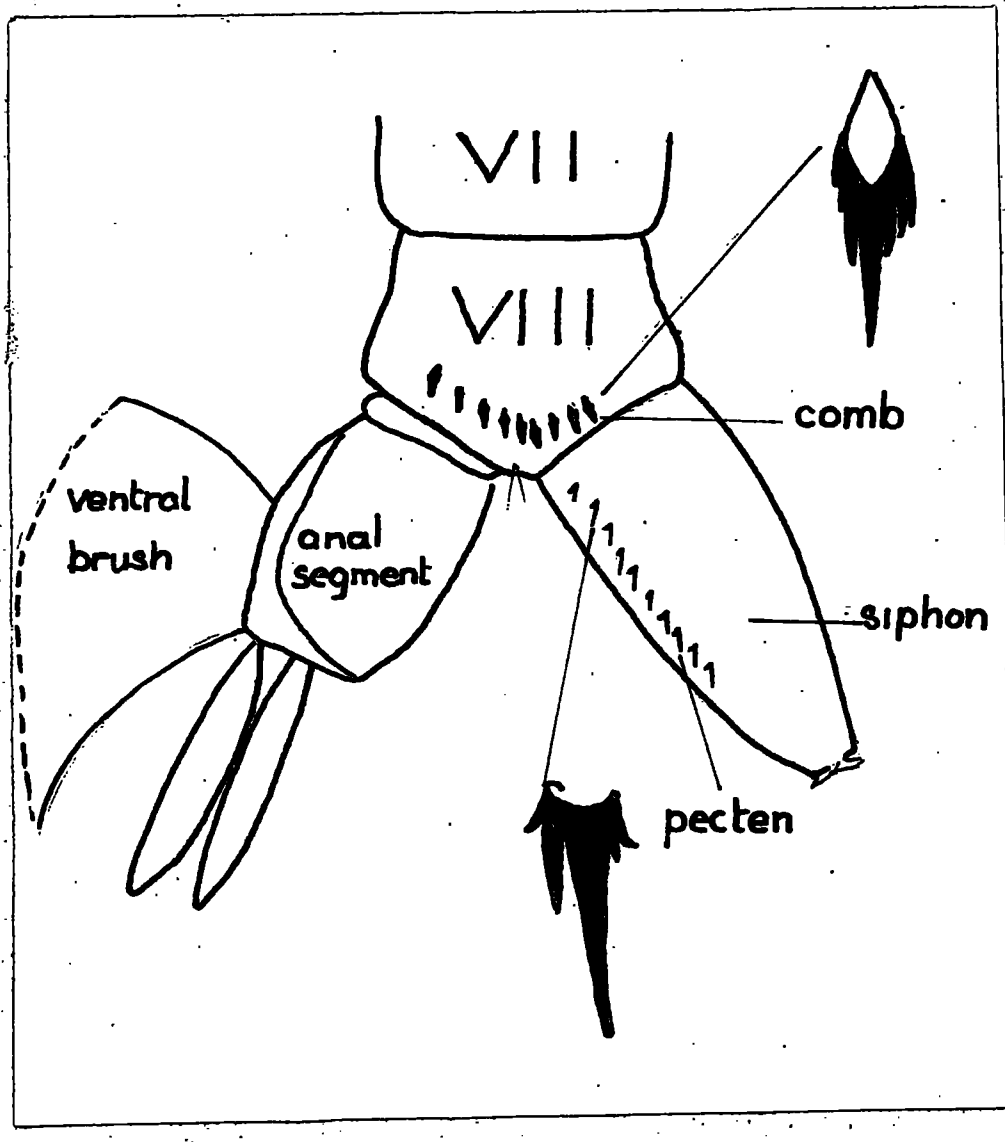


Fig. 20.

Showing the variation in the number of comb spines in the fourth instar A. aegypti.

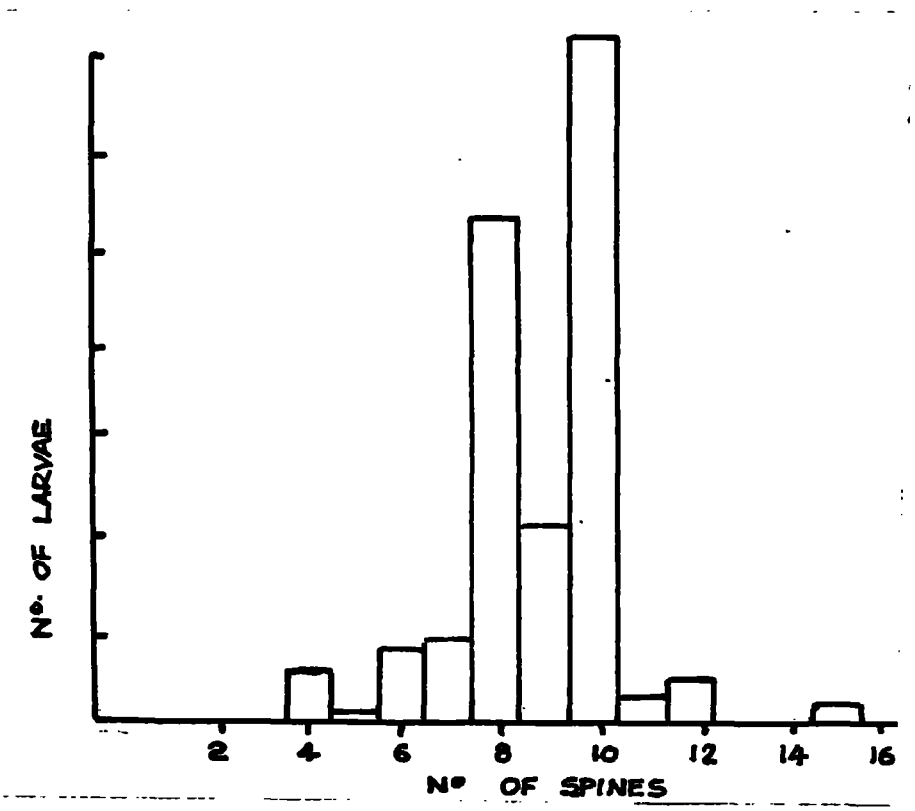
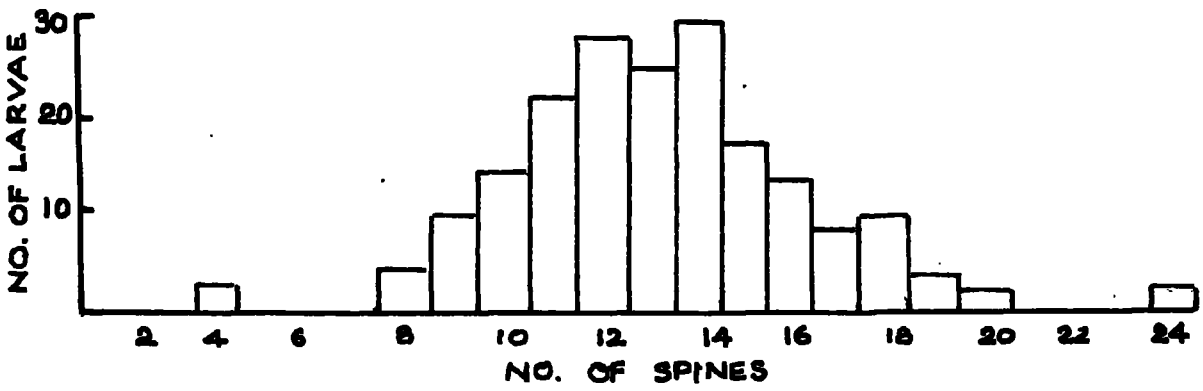


Fig. 21.

Showing the variation in the number of pecten spines in the fourth instar of A. aegypti.



PART V

DISCUSSION AND GENERAL CONCLUSIONS

Prima facie consideration of the data resulting from research upon Aedes aegypti in West Africa indicates that this species is typically a domestic one. This latter term is used here to delineate a species which has an ecology closely associated with man. Empiricle evidence of the underlying causes of this domestic behaviour have hitherto been inconclusive. It is the aim of this discussion to integrate the data presented in this thesis so that for this particular area, these underlying causes may be demonstrated. It is emphasized that these conclusions must primarily be applied to the type form of the species in this area, for as will be shown, the ecology varies throughout the African continent. Before proceeding to the main discussion however, attention must be paid to some general features of the insect's biology.

Buxton and Hopkins (1927) working in the south-west Pacific region, found that the eggs of this species varied in length between 624 and 704 microns and in width between 144 and 192 microns (means, 664 and 170 microns). Measurement of Ilobi eggs showed variations between 600 and 780 microns and 160 and 200 microns (means, 683 and 184 microns). It is likely that the species investigated in the Pacific was A.aegypti sensu stricto in which case egg dimensions may be of taxonomic value. Buxton and Hopkins similarly measured the eggs of A.variegatus but

By day 14 cultures I and II had completed development, half of the larvae in culture III had reached the fourth instar whilst the others had died and in the remaining cultures the mortalities were high. After day 14 there was no further development in cultures IV to VII.

f) Influence of population density on the survival and development of larvae.

As the monthly fluctuations in numbers found in the field did not appear to be directly related to any feature of the climate, it was thought that the mid-year decline in numbers may have been due to population density rather than any extrinsic environmental factor. Some attempt was therefore made to study the influence of population density upon the survival and the development of larvae. A series of cultures was maintained each containing different numbers of larvae but in otherwise standard conditions of food supply and water volume. To ensure constant food supply a solution of the standard larval food, (Appendix III), was made up as follows. 10 grammes of solid were placed in 200 cubic centimeters of water and heated to 100°C., the resulting filtrate being used as the food solution. 40 cubic centimeters of this were placed in 1000 cubic centimeters of water and 7 of these cultures were made up and in them were placed 10, 20, 30, 40, 50, 60, and 70 newly hatched larvae. respectively. A daily record was kept of mortality and development and on day 10 the situation was as follows.

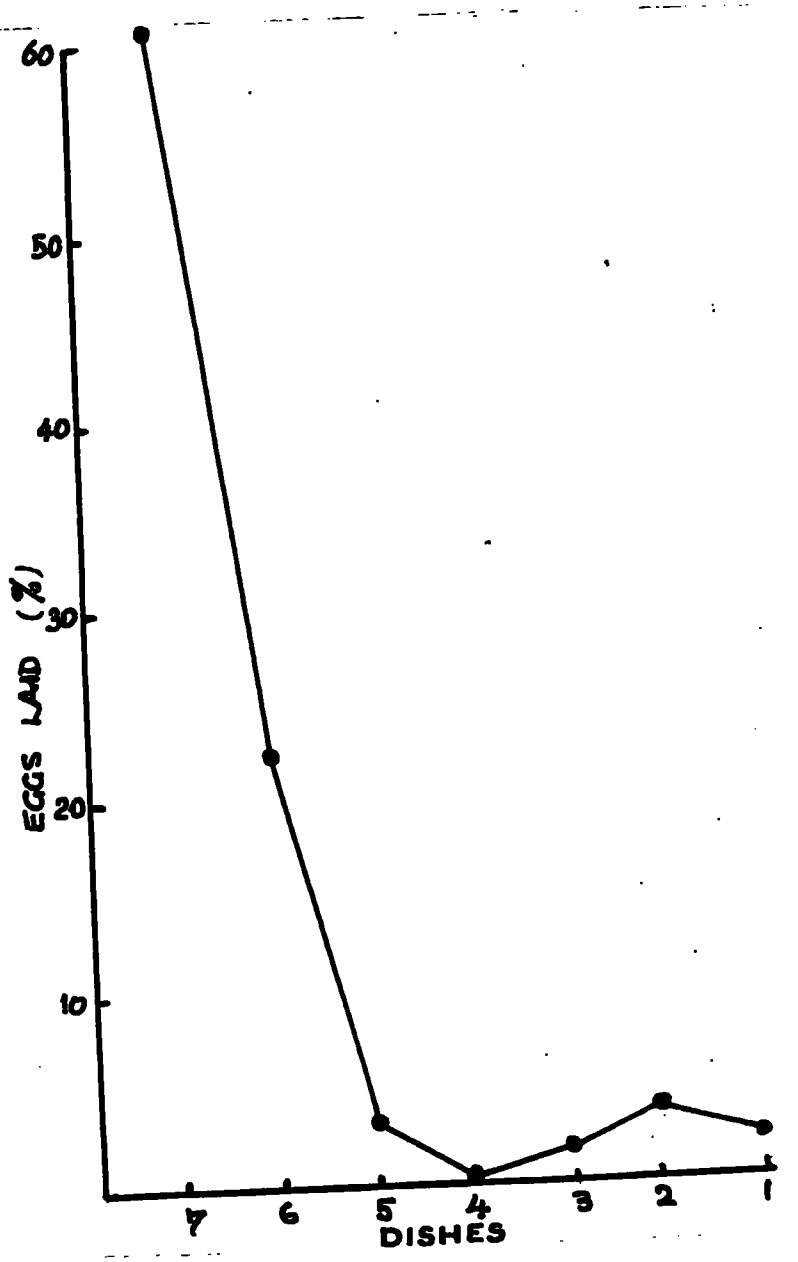
Culture I	(10 larvae)	8 at fourth instar, 2 at third.
Culture II	(20 larvae)	All at third instar.
Culture III	(30 larvae)	All at third instar.
Culture IV	(40 larvae)	Mostly at second instar.
Culture V	(50 larvae)	Mostly at second instar.
Culture VI	(60 larvae)	Second instar only, mortality 20 to 30 per cent.
Culture VII	(70 larvae)	

e) Influence of light intensity on egg-laying.

To study the influence of light intensity on egg-laying a colony was maintained in a cage measuring 2½ feet x 1½ feet x 1½ feet which was placed inside a larger compartment with a black interior but provided with a single light source. The adults were allowed to feed on blood and glucose as already described. Variation in light intensity was maintained by covering the top of the smaller cage with black cork at one end of which there was a 3 inch diameter aperture. Above this was placed a 100 watt electric light bulb and heat insulation was obtained by placing a petri-dish of water between this and the aperture. The floor of the cage was white. Due to the light source at one end, a light gradient was maintained along which was placed a series of petri-dishes for the purpose of egg-laying. The eggs laid in each of these dishes were counted daily. The results (Fig. 35; Table 29, Appendix I) show that there was a marked preference for egg-laying in the lowest light intensities whilst the brightly illuminated dishes were largely avoided. Dishes in conditions of uniform light intensity showed an even distribution of eggs.

Fig. 35.

The number of eggs laid in containers in varying light intensities. The dishes are numbered in order of decreasing light intensity.



similar to that found inside and outside houses under field conditions.

The apparatus used was a wire gauze tube, 8 inches long and 2 inches in diameter. A blacked out 40 watt electric light bulb was placed in a tin compartment beneath one end of the tube. It was found that by this method a column of heated air could be made to pass upwards through the tube so that there was a sharp division between the heated and cool ends. A thermometer was placed in each end of the tube so that the temperature levels could be maintained. The mosquitoes were intoduced by means of a small hatch in the middle of the tube. Olfactory attraction was reduced to a minimum by a glass plate between the apparatus and the observer. Uniform light intensity was maintained throughout by placing the apparatus on a bench beneath a daylight-type fluorescent tube.

In each test 15 females were placed in the tube and allowed to settle down at random. The air temperature at one end was then raised to 32°C. and maintained for 2 minutes. The number of mosquitoes at each end of the tube and at any intervening points was then counted. Tests of this kind were conducted upon 210 females and in all cases there was a marked preference for the cooler end of the tube, (Table 28, Appendix I). It was also observed that if the females were introduced into the tube after the temperature difference had already been attained, they at once all flew to the cooler end.

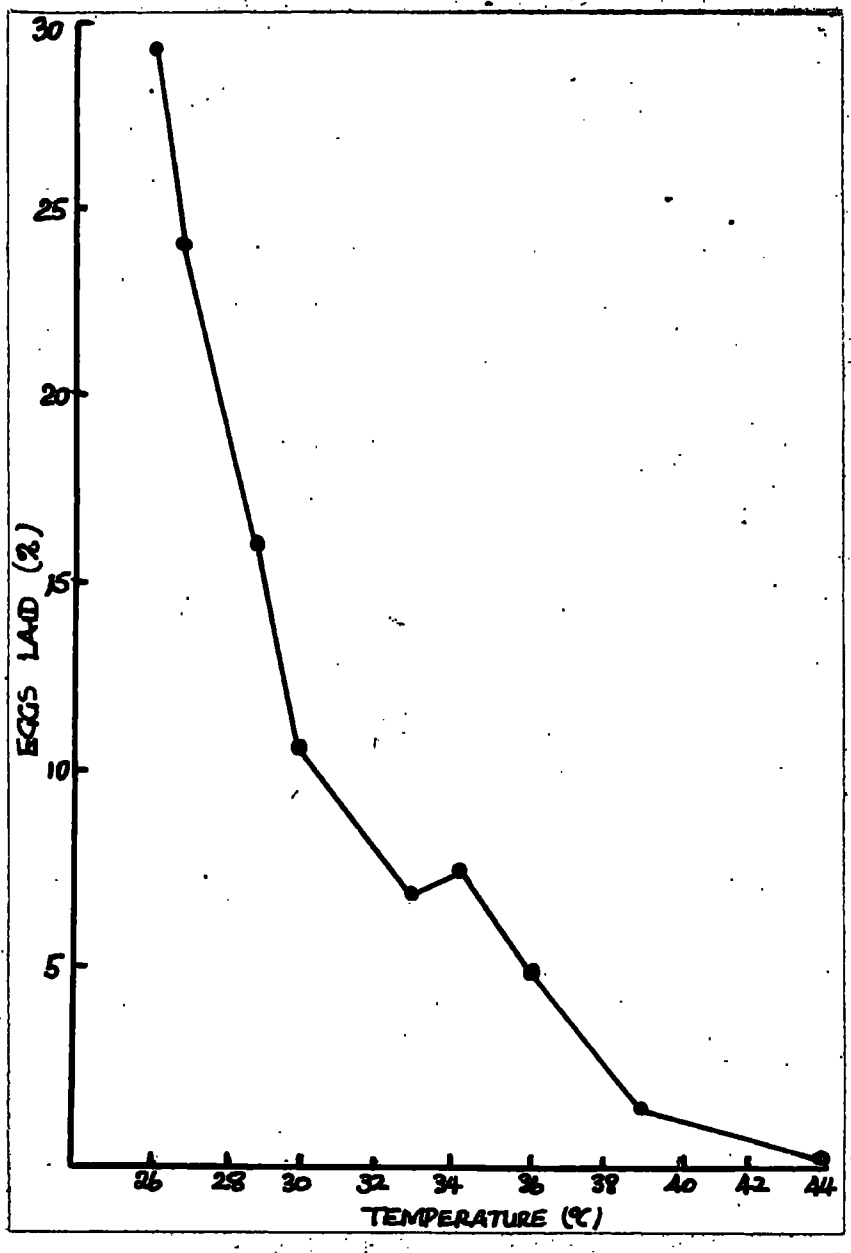
d) Influence of water temperature on egg-laying.

The influence of water temperature on the egg-laying behaviour of the female was investigated in the following way. A series of 100 cubic centimeter beakers was placed in a line along the floor of a cage in which a colony of mosquitoes was maintained, one end of which was heated by a blacked out 100 watt electric light bulb. This produced a temperature gradient so that the water in the beakers was maintained at different temperature levels, the range extending from 26°C. to 44°C. A strip of filter paper was placed around the inside of each beaker for the ovipositing female. The experimental cage was placed in a larger one in which a constant light source was maintained throughout the experiment. The females were allowed to feed daily on white mice and the males on 10 per cent glucose. The eggs laid in each beaker were counted daily. The results (Fig. 34; Table 27, Appendix I) show that whilst no lower limit for egg laying was established the upper limit was in the region of 44°C. and that there was a marked preference for egg-laying in the cooler water. This result, which indicated a reaction on the part of the female to water temperature, was further investigated in the following way.

In a colony of the Ilobi strain of mosquitoes the female were fed on blood when two days old and the tests described here were carried out 48 hours later when egg-laying would normally have been taking place. The aim of the experiment was to find if the gravid female exhibited a temperature choice when presented with a variation

Fig. 34.

The number of eggs laid in water at various temperatures.

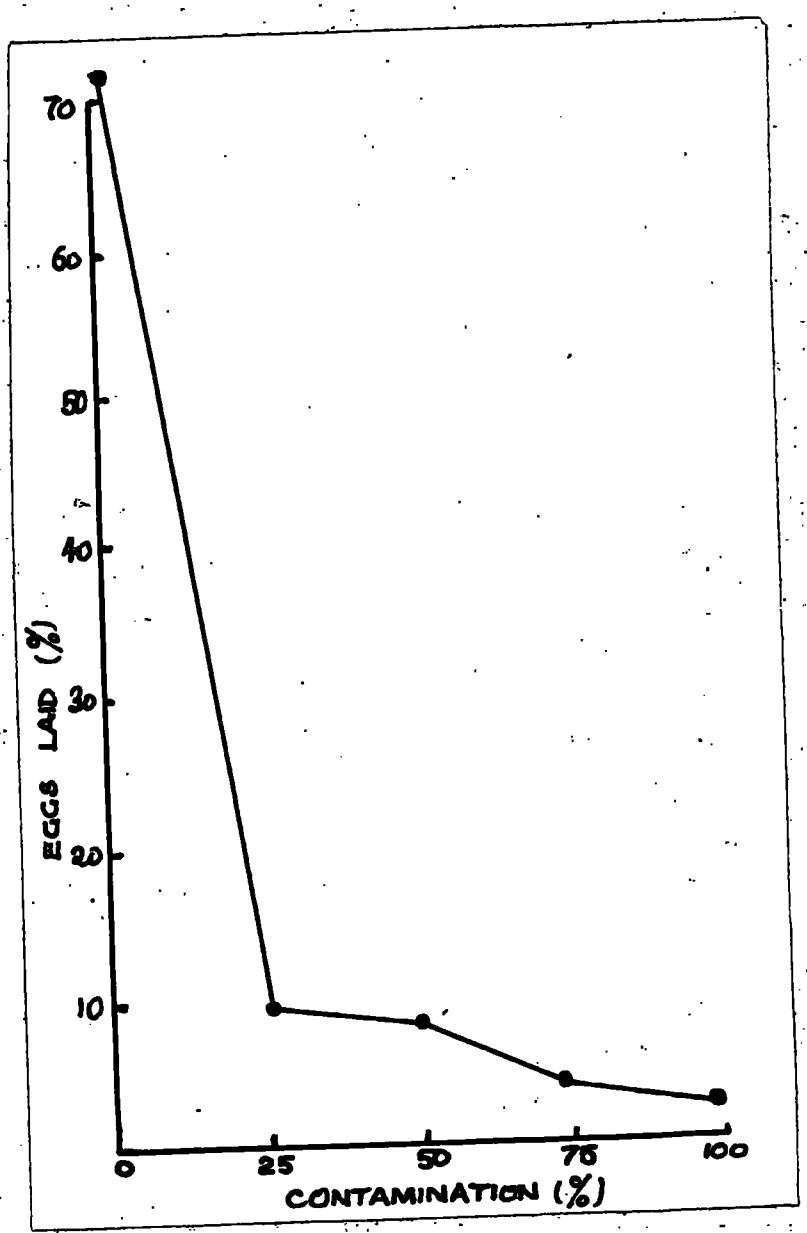


c) Influence of vegetable contamination on egg-laying.

It had been found in the field that if any site was contaminated with decaying vegetable matter, A.aegypti pre-adults would be absent from it. The influence of this factor on egg laying was therefore investigated. Leaves of Hybiscus sp., a common shrub of the area, were used to make up the contaminating matter. To every 200 cubic centimeters of water were added 10 grammes of crushed leaf and the mixture was maintained at 45°C. for 48 hours. The resulting solution contained small particles of leaves, was deep green in colour, malodorous and in every way resembled the contaminated water contained in the natural sites. The pH was 7.0. Five petri-dishes were placed in a mosquito colony and these contained, concentrated leaf mixture, 75, 50 and 25 per cent. of the leaf mixture diluted with distilled water and a dish of tap water. All of these dishes were of the same dimensions and contained the same volume of liquid, the solutions being renewed each morning when the eggs were counted. The results (Fig. 33; Table 26, Appendix I) show that the greatest number of eggs were laid in the uncontaminated water whilst there was a marked avoidence of the other sites. A further point of interest is that eggs placed in similar solutions, hatched, and the larvae developed normally.

Fig. 33.

The number of eggs laid in water containing varying degrees of organic contamination.

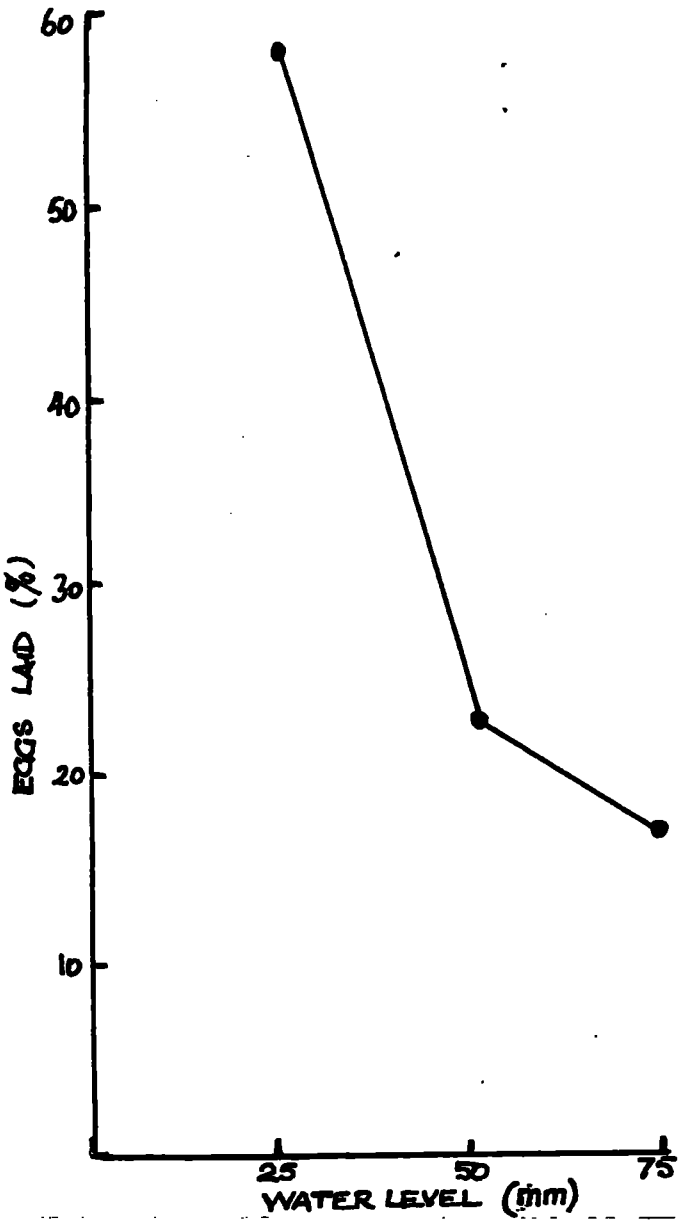


b) Influence of water level on egg-laying.

In nature it was found that A.aegypti pre-adults were typically absent from water which was some way below the level of the container, the series of experiments described here demonstrating that the egg-laying female has a preference for sites with high water levels. Three beakers in which the water was at different levels were placed in a colony similar to that described in the previous section. The levels of the water surfaces below the rims of the beakers was 25, 50 and 75 millimeters and filter paper was placed around the inside of the beakers upon which the female could rest whilst ovipositing. The eggs laid in each beaker were counted daily, care being taken to note the number of eggs laid at any points above the water surface. Throughout the experiments 1187 eggs were laid. It was found that the greatest number were laid in the beaker with the highest water level, less being laid in the deeper ones (Fig. 32) and it was also found that the greatest number of eggs laid in each beaker were between 0 and 5 millimeters above the water surface, (Table 25, Appendix I). Furthermore in the 25 and 50 mm beakers, 77 and 78 per cent. of the eggs were laid in the 0 to 5 mm. band but in the deepest one there was a greater degree of spread toward the upper margin. Vessels in which the water level was the same did not show this distribution of eggs.

Fig. 32.

The number of eggs laid in containers with the water level at 25, 50 and 75 mm.

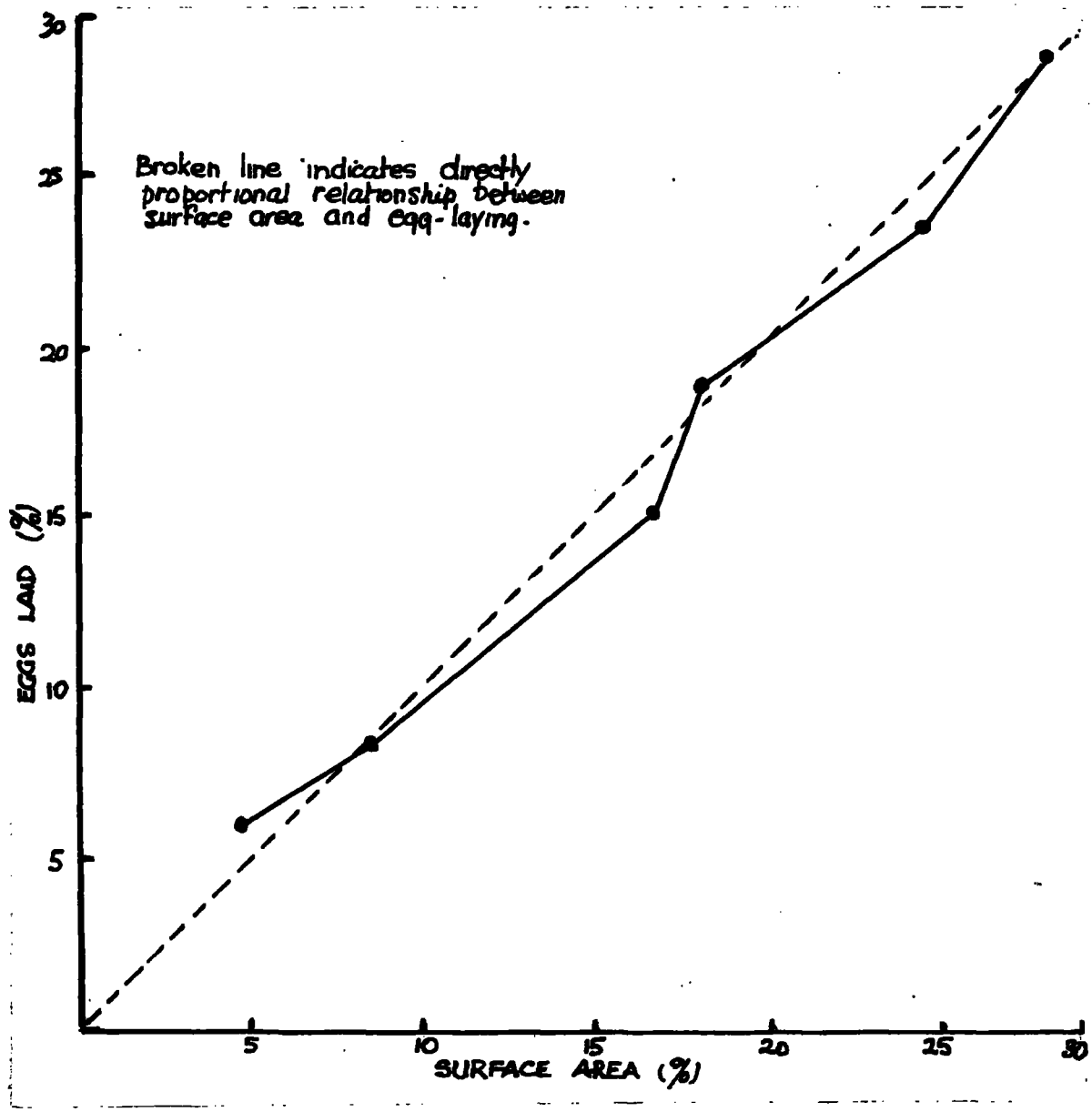


a) Influence of surface area on egg-laying.

To study the egg-laying behaviour of the female with respect to the surface area of the egg-laying site, a colony of mosquitoes was maintained in a cage measuring 3½ feet x 3½ feet x 3½ feet made of wood and mosquito-gauze. Throughout the experiment the females were allowed to feed daily on white mice and the males were supplied with a 10 per cent. glucose solution on cotton wool pads. Egg-laying commenced two days after the females had fed. Petri-dishes, providing surface areas of various dimensions were placed in the cage and their positions changed daily. The number of eggs laid in each dish was counted each morning and it was observed that most of the eggs were laid around the water margin. Egg-laying was allowed to continue for about a month by which time some 18,000 eggs had been laid, the results being given in Table 24 (Appendix I). These indicate that there is some relationship between the surface area of an egg-laying site and the number of eggs laid therein. This is more clearly shown if the surface area of each dish is taken as a percentage of the total surface area exposed and the number of eggs laid in each as a percentage of the total laid, Fig. 31.

Fig. 31.

Showing the surface area of each dish as a percentage of the total area exposed, plotted against the number of eggs laid in each dish as a percentage of the total laid.



> "Finally there are those mosquitoes which breed in artificial containers.....of these A.aegypti is undoubtedly the most important. As receptacles of this kind are closely associated with man, any mosquito breeding in them is bound to be peridomestic and peridomesticity means intimate contact with man. If such a mosquito can transmit yellow fever, it is almost certain to be of importance in the epidemiology of the disease. Obviously therefore because of its breeding habits and susceptibility to the virus of yellow fever A.aegypti is an outstanding vector of the disease as it occurs in urban areas."

It remains therefore to demonstrate the causes of the domestic pattern of breeding as observed for this species in the Ilobi experiment. It is suggested that there are two main factors which limit the breeding of this species to the immediate vicinity of the village, namely water contamination and the dimensions of the egg-laying site. It has been pointed out that with the change from village to forest conditions , there were recognisible several gradients with regard to the nature of the egg-laying sites, one of these being the increase in the degree of decaying vegetable matter found in the water. In the laboratory it was found that when a freely breeding colony was given a choice of egg-laying dishes, the greatest number of eggs were laid in the one containing the least contaminated water. In the field, both natural breeding places and experimental pots beyond the village were found to be contaminated to some degree with falling leaves or other vegetable debris. Thus it may be suggested that this factor limits the breeding behaviour of the egg-laying female in which case it would be expected that pre-adults would

be absent from sites in which the water was contaminated. The neuro-physiological basis of this behaviour pattern was not investigated by the writer but Wallis (1954) demonstrated that a chemoreceptive reaction was involved in the avoidence of contaminated water by the egg-laying female A.aegypti. Females with abdominal movements restricted so that contact between the ovipositor and the test solution was impossible, could still detect salinity in egg-laying sites. Furthermore, removal of palpi, proboscis or antennae did not result in loss of sensitivity. Investigation of the tarsi showed that sensitivity was present in all tarsomeres and distribution of curved, thin-walled spines similar to chemoreceptive structures in other insects, was correlated with the extent of the sensitive areas.

A further alteration in the nature of the egg-laying sites associated with the change from village to forest conditions is that of size. The natural sites beyond the village typically had small dimensions compared with the domestic containers found in the village. The laboratory studies on the influence of surface area and water level on egg-laying, indicated that these factors were significant in determining the distribution of eggs by the female. This result was most pronounced in the study of surface area and it may well be that in the field similar factors are operative. During the field studies the greatest number of pre-adults at any one station were to be found in the clay as opposed to the bamboo pot (with some exceptions on the south transect). In the laboratory studies, the number of eggs laid in each dish was directly

proportional to the surface area and it is known that evaporation rate is also directly proportional to surface area. Whilst it was not empirically demonstrated, it may be that this observed distribution of eggs was the result of a hygroreceptive response on the part of the gravid female. On the other hand those dishes having the largest surface areas stood the greatest chance of being located.
The influence of water volume on the growth and development of larvae has been shown and it is of interest that if the water volume per larva is calculated for the surface area experiment, the results are very constant. The depth of water in each dish was 1 centimeter and commencing with the largest container the volumes of water per larva were as follows, 0.23, 0.19, 0. 17, 0.20, 0.18, and 0.19 cubic centimeters respectively. Whilst such a situation would be of obvious survival value to the species it is one which would be rarely found in the field where egg-laying sites of various volumes occur irrespective of their surface areas. It may be concluded therefore at this point, that water contamination and size of egg-laying site play a part in determining the breeding behaviour of the species as indicated by the distribution of pre-adults.

It is considered that temperature and light intensity limit the breeding behaviour of this species so that egg-laying is most intense inside houses. When gravid females were given a choice of temperatures approximating to the range found in the field, a choice was made for the coolest end of the tube. Martini and Teubner (1933) found that when A.aegypti was placed in a temperature gradient extending from 15 to 32°C., 50 per cent of the adults went

to the coolest end. Thomson (1938) experimenting with Culex fatigans Weidmann obtained similar results and he observed that the most sriking feature of the behaviour at all stages was the strong avoidence of high temperatures.

Buxton and Hopkins (1927) suggested that temperature was an important factor in determining the egg-laying behaviour of the female but did not demonstrate this experimentally. In the studies described in this thesis there was evidenced a marked distribution of eggs by a freely breeding colony with respect to water temperature, most of them being laid in the coolest waters. This fact links up with the female preference as previously mentioned. Such a well marked pattern of behaviour has rarely been obtained with this species although both Hecht (1930) and Thomson (1940a) were able to demonstrate a choice with Anopheles claviger Meigen and A.minimus Theobald. In both of these cases the females laid their eggs in the water at the coolest end of the temperature range.

A further point of interest is that small volumes of water in the village, e.g. snail shells, would be subject to greater ranges of temperature than would larger volumes. These would be expected to become much hotter than water in clay pots where evaporation tends to reduce the maximum temperature. This fact may well prevent eggs from being laid in small containers in the village.

The investigation of the influence of water temperature upon egg hatching showed that optimum conditions existed

above and below which hatching was retarded and in the region of 40°C. ceased altogether. Thus it may be said that the egg-laying behaviour of the female enhances the chances of successful hatching, whereas random distribution of eggs, some of which would be laid in exposed water, would result in an increased egg and larval mortality. Unfortunately, literature relating to the influence of temperature on egg maturation and hatching is sparse, but this subject could profitably be pursued.

Bates (1949) pointed out that in insects the relationship between the rate of development and the temperature is direct so that if the length of time required for development is plotted agaist temperature, the resulting curve is a hyperbola. In the present series of experiments the time of the onset of pupation increased with temperature and the writer has also found (unpublished data) that temperatures in the region of 15 to 18°C. cause an extended developmental period. Buxton and Hopkins (1927) observed that low temperatures caused prolonged larval life but pointed out that this may have been due to starvation because of the death of the microbiota. Both Wigglesworth (1953) and Uvarov (1931) have reviwed the problem of the relationship between temperature and insect development. Bar-Zeev (1958) found that with A.aegypti larvae, development was most rapid at 32°C. and that at 14°C. it was irregular whilst the mortality was high. At 36°C. development was slow and at 38°C. all the larvae died before reaching the third instar. His studies support in general

the observations made in this thesis.

It was observed that the thermal death-point of larvae was in the region of 34°C., the term being employed to denote the lowest temperature at which complete development failed to take place. Thomson (1940a) defined this term as the lowest temperature at which all larvae were killed by an exposure of five minutes, the temperature being raised over a period of 1 to 1½ hours. His results for Anopheles species were as follows. A.insulaeflorum Swellengrebel and Swellengrebel 41.0°C., A.minimus 41.0°C., A.hyrcanus Pallas 43.0 to 43.5°C., A.barbirostris van der Wulp 43.5°C. and A.culicifacies Giles 44.0°C. Bates (1940) concluded that species with a high thermal death-point are apt to be found in relatively warm waters. This could apply in a converse way for A.aegypti in the present study where a low thermal death point is associated with breeding in relatively cool water. It is of interest to note that at Ilobi Anopheles gambiae Giles was found breeding in fully exposed pots which would be expected to reach high midday temperatures, whilst deMeillon (1934) had previously recorded this species from pools at 39°C.

We may therefore conclude from a consideration of the field observations and the laboratory experiments, that the female behaviour is such that eggs are laid in conditions most favourable to pre-adult survival and development. Also that temperature may be considered as one of the factors limiting the behaviour of the gravid female with the result that the majority of egg-laying takes

place inside houses where relatively cool atmospheric and water temperatures are to be found.

Subsequent to establishing that most of the egg-laying took place within houses in the village, it was found in the laboratory that egg-laying was largely restricted to containers in dark conditions. Buxton and Hopkins (1927) by using two glass containers, one of which was blackened on the outside, found that most of the eggs of this species were laid in the latter rather than the fully illuminated container. They further noted that different degrees of illumination did not in any way influence the survival or the development of the larvae. Fielding (1919) had previously found that the larvae developed equally well in dark or light conditions. The presence of pre-adults in poorly illuminated sites would therefore appear to be the result of selective behaviour on the part of the female.

It may be concluded that both the factors of light and temperature play a part in determining the breeding behaviour of this species as indicated by the greater density of pre-adults inside as opposed to outside houses. But this conclusion is only valid if egg-laying takes place during the day. If this were not so, there would be no difference in conditions of light and temperature inside or outside houses. Haddow and Gillett (1957) clearly demonstrated that in this strain of A.aegypti egg-laying takes place during the hours of daylight. Under constant conditions of temperature and humidity but with normally fluctuating daylight they found that there was a regular

cycle of oviposition with a peak of major activity in the afternoon. They also found that under controlled constant light conditions, this pattern broke down and egg-laying became aperiodic.

Turning now to the distribution of the species as indicated by the weekly sampling of experimental pots along the eastern transect, four possible causes must be considered which may have accounted for the observations obtained. The distribution may be due either to egg-laying site factors as discussed above, climatic factors, flight range or the action of predators. It is seen that the records of breeding beyond the village were obtained in the warmer months of the year. It may be that although this species typically breeds in the village, extreme conditions, particularly of temperature may cause some breeding beyond the normal range. In the scrub-zone and inside the forest for example the temperature is typically reduced so that breeding records for these areas may indicate atypical breeding behaviour in extreme climatic conditions.

The flight range of the species may also play an important part in determining the distribution. Although no direct studies have been carried out by the writer, it would seem from previous work that the species has a flight range capable of carrying it well beyond the village into the forest, providing no other factors were operative. The findings of other workers giving the observed maximum of flight range of the species may be summarized as follows.

Shannon et.al. (1930).. ..	120 meters
Shannon and Davis (1930) ..	300 meters
" " " (1930) ..	1000 meters
Chwatt (1949)	1000 meters

The third factor which may play a part in determining the pattern of distribution of the species is the predatory activity of other larvae. In this area two major larval predators encountered were Eretmapodites chrysogaster Graham and Megarhinus brevipalpis Theobald. Both of these bred beyond the village. Breeding throughout the year was not continuous but even when these species were absent from the pots in the scrub-zone and the forest, A.aegypti larvae were not found.

The relative importance of these four factors may be compared and the following conclusions drawn. If the climate were a major controlling factor, the species would be found breeding beyond the village at all times of the year, for as has been shown, egg-laying is most intense in relatively cool and dark situations, both of these conditions being found in the forest. Nevertheless water contamination and breeding site dimensions restrict breeding to the actual village. Thus although extreme conditions of temperature may cause some breeding beyond the village, climate cannot be considered as a major factor in determining the observed distribution of this species. The flight range may be considered as permitting breeding 300 to 1000 meters beyond the village so that the restriction to the immediate vicinity of the village cannot be considered as primarily due to a short flight range.

Predation may to some extent prohibit the survival of the pre-adults in sites beyond the village although the spasmodic records of the predatory larvae make this somewhat doubtful. Furthermore, in the survey of natural larval habitats E.chrysogaster was found breeding mainly in cocoa husks whilst M.brevipalpis was taken from leaf axils and only on 3 occasions from domestic vessles. It may therefore be concluded that water contamination is the major factor controlling the distribution of the species as indicated by pre-adult records from natural and experimental breeding sites. Breeding site dimensions are also considered to be important. These factors limit the breeding to the immediate vicinity of the village, whilst temperature and light intensity determine the relative intensity of breeding within houses.

The underlying causes of the fluctuations in numbers of this species throughout the year present the most difficult problem in this present study. Three possible answers may be considered, namely, climatic factors, changes in water temperature and environmental limits. Taking the village as giving the typical picture of the monthly fluctuations (57 per cent . of all pre-adults were taken here), the pattern over the year may be taken as follows. The figures of pre-adults were taken to indicate the changes in density of the egg-laying population and for the sake of this discussion the value N (number of pre-adults per pot per month) will be used. It is felt that this will give a truer index of the fluctuations in the actual village population.

$$N = \frac{\text{Number of p-a taken from all pots in a month}}{\text{No. experimental pots in village X No. of weeks sampled}}$$

During the first four months of the year, the value N increased from 24.3 to 135.0 this being followed by a sharp decline in May (52.6) a slight increase in June (71.2) and then a continued decline until October (21.3). There was then a final build-up reaching a peak in December (71.2).

Considering the climatic factors first, the mean monthly tempeature in the village continued to rise throughout the first 5 months of the year reaching a maximum in May. The temperature was lower in June to September with a gradual increase in the last 3 months of the year. If temperature was influencing the population density in a dynamic way, it would be expected that a change in temperature would be reflected in a population change in either the same or the succeeding month. But this is not the case.

A similar argument may be applied to a consideration of the changes in water temperature which would be expected throughout the year. Relatively low temperatures may retard the hatching and maturation of the eggs so that during the rainy season the production of adults would be spread over a longer period. This, combined with the mortality of the existing adults would result in a drop in numbers of the population. It has been found in the laboratory that if newly laid eggs are maintained on a water surface at a reduced temperature, they will not hatch for some weeks. But for the present study, a comparison of the fluctuations in numbers with the temperature and rainfall records does not support this argument.

The third postulated cause was that of environmental limits. In the laboratory experiments on the influence of population density upon the survival and the development of larvae, each of the cultures described may be considered as a successive stage in the growth of a population. Thus the smallest culture may be taken to represent the commencement of the breeding season with the onset of the rains and each successive culture, say, two-weekly stages in the population growth. It was found that in otherwise standard conditions, the greater the number of larvae the longer the developmental period and higher the mortality. Thus it may be suggested that in the field the population increased and the limits of the environment were reached and increased larval mortality resulted in a reduced egg-laying population. In other words, when a certain population maximum was reached as indicated by the number of larvae per pot, the food supply of the pots would be so reduced that the larvae would either die or take relatively longer to reach maturity. This decline was then followed by a recovery. From this postulation of environmental limits it may be suggested that this factor is a major contributing one to the monthly fluctuations of the species. These fluctuations are therefore density dependent.

At Ilobi the natural larval habitats indicated that the breeding behaviour of this species was constant for the area, a fact which is not true for the species throughout the whole of the Ethiopian region. In West Africa it has been recorded mainly from domestic containers but also from tree-holes (Kumm 1931) crab-holes (Dalziel 1920) and rock

pools (Surtees 1958b). At Ilobi whilst the larvae were also taken from snail shells and a leaf axil, these habitats were found more typically to containe other genera, (Surtees 1958c). Dalziel in his studies found that of the domestic containers he examined, 68 per cent. contained aegypti larvae whilst only 28 per cent of the tree-holes and 7.3 per cent. of the crab-holes were positive for the species. In East Africa A.aegypti has an even more plastic breeding behaviour which in some cases is associated with changes in adult behaviour. Gillett (1951) working in Uganda found that at Entebbe the species bred in domestic containers and the adults were anthropophilic but that at Bwamba they bred in tree-holes and the adults were non-anthropophilic. Similarly Garnham et.al.(1946) found that in Kenya the species bred in rock pools and tree-holes in the forest but was rare in the villages. On the other hand Harris (1942) and Haddow et.al. (1951) both found the species breeding in domestic containers. Harris considered that the original breeding place was tree-holes but pointed out that it was now more generally found in water which was not contaminated with decaying vegetable matter. The writer is also of the opinion that originally A.aegypti was a tree-hole dweller as indicated by the morphology of the larval mouthparts which conform to the browsing facies typical of tree-hole dwelling larvae (Surtees 1958d). Haddow (1948) records that out of 35,000 leaf axils examined, A.aegypti larvae were only taken on 10 occasions.

That this species is able to breed in a wide variety of habitats has probably contributed to its wide distribution, although its domestic behaviour will also have played a part. It would seem that ubiquity is closely associated with

domesticity as in the case of the species in question and also others such as Culex fatigans Weidmann (=quinquefasciatus Say) and C.pipiens Linnaeus. Edwards (1932) pointed out that Aedes aegypti occured in tropical and sub-tropical regions of the world except Japan, New Zealand and some parts of the Pacific. Its northerly and southerly limits are probably determined by climate as the eggs are killed by freezing (Davis 1932). Also the colonisation of domestic habitats has greatly facilitated its role as a disease vector. Allee et.al. (1949) records that when this species was introduced to South America it spread through domestic environments, breeding in domestic containers but did not invade those natural habitats from which it had been recorded in Africa. It would seem that alth ough widespread, there is no physiological diversity between populations in various parts of the world (Mathis 1934).

Finally some mention must be made of the application of a study such as this to the control of yellow fever. A clear distinction must be maintained between disease control and vector control, these results being applicable only to the latter problem. Furthermore it must be clear what is meant by the term eradication. Control could mean measures directed against the vector so as to maintain it at a low level thus interfering with the man-to-man transmission of the virus, whilst eradication must be taken to mean the total elimination of the species from a definite area. In the WHO recommendation quoted in the introduction to this thesis, it was suggested that the experiment should be

aimed at the eradication of A.aegypti from a selected area in West Africa. It is to be doubted whether such an object is attainable over a large area due to the plasticity of the species with respect to the breeding behaviour and to the purely mechanical barrier involved in attacking all the possible breeding sites. Control on the other hand is more likely. Carter (1931) pointed out that as A.aegypti density is lowered by anti-larval measures, the number of imagoes present eventually becomes too small for the virus to be maintained and the yellow fever gradually dies out in the controlled area.

Close contact with human habitations renders this species more susceptable to control measures than if it were non-domestic.but care must be taken to employ a method which takes the greatest advantage of the observed breeding behaviour of the species. In this particular area, however, although dealing with a domestic mosquito, the fact that most of the breeding takes place inside houses complicates any control measures, in a social way, which may be employed.

Toward the end of 1957 two experiments were carried out to test the efficiency of insecticidal measures against A.aegypti in Ilobi village. During these experiments the writer made certain entomological observations which are recorded here. In the first experiment the village was sprayed from the height of 50 feet by means of an aircraft. DDT was used in oil at the rate of ½ lb. per gallon, this was applied at 1 gallon per acre and the mean droplet

size was 40 microns. In the second experiment a method of ground fogging was used. Dieldrin was the insecticide and this was applied in a cloud of large droplets. During both sprayings, fourth instar larvae were placed out in clay pots in various parts of the village, including inside houses. The larval mortality was acessed 24 hours after spraying had taken place. As a result of the aerial spraying the mortality inside houses was only 1 to 2 per cent. and it was felt that in the light of the previous breeding studies, this method was inadequate as a control measure. The ground fogging achieved a greater degree of success in that mortalities of between 80 and 100 per cent were obtained even in the most sheltered positions.

In view of the widespread reports of insecticide resistance in mosquitoes and the rapidity with which A.aegypti developes resistance (Surtees 1958e), care should be exercised in embarking upon a campaign which seeks control of an insect by chemical means. It is salutary to recall the results of Carroll and Reed mentioned in the introduction, where sanitary and drainage measures alone eradicated yellow fever and malaria from Havanawithin a year. As we are dealing with a domestic mosquito it should be possible to attempt a similar project in an area such as Ilobi. The study of the monthly fluctuations in numbers combined with the knowledge that most of the pre-adults found during the first rains are from residual eggs, suggests that the early months of the year would be the most ideal for an attempted sanitary control scheme. This would use supervised labour which would involve less expense than a chemical method, and the means employed would consist of pot

examination and emptying where necessary. It is suggested that such a method would meet with a degree of success which would fully justify its employment for an experimental period.

SUMMARY

PART I. Topography and climate.

South-western Nigeria is divided into three geographical regions, the coastal mangrove swamp, the freshwater swamp extending up to 10 miles inland and the high forest. This 3-fold division has superimposed upon it changes due to clearing and farming. There are two seasons, the hot, dry season extending from November to March and the cool, wet season extending from April to October. An average of 60 to 80 inches of rain fall per year in this region, the maximum temperature reaches 34°C. and the minimum relative humidity falls to 60 per cent. in the dry season.

Ilobi village, in which these studies were carried out was situated in the rain forest some 45 miles north-west of Lagos. There was farmland to the north, late forest to the east, early forest and some farmland to the south and a cocoa plantation to the west of the village clearing. The village was subject to greater climatic variations than the coastal region although the forest was relatively cool and humid.

PART II. General biology of the species.

The species discussed is Aedes (Stegomyia) aegypti Linnaeus 1762, sensu stricto (Mattingly 1958). It is typically domestic. The eggs can withstand periods of desiccation up to several months and hatching is most rapid at 32°C.

The eggs will not hatch in water above 40°C. Larval development is most rapid at 32°C. and the development from egg to adult is not completed at temperatures above 36°C. Overcrowding of larvae was found to alter their growth form. The typical method of larval feeding is by browsing. The adult female is anthropophilic although feeding has been observed to take place on chickens, dormice, galagoes and rhesus monkeys. Mating lasts approximately five seconds and once mated the female will lay fertile eggs for the rest of her life. The mean number of eggs laid per female has been found to be 73.7

PART III. Field studies.

In the field the pre-adults (larvae and pupae) were found most commonly in domestic containers in the immediate vicinity of the village. Egg-laying as indicated by the presence of pre-adults was most intense inside houses. Some records of breeding were obtained from experimental pots beyond the village for the warmer months of the year and it is suggested that this was atypical behaviour due to extreme climatic conditions. Throughout the year the numbers of pre-adults were found to fluctuate and these fluctuations were taken as an index of the changes in density of the breeding population. There was a build-up in numbers in the early part of the year followed by a decline in the wet season. there was another minor build-up in the latter months of the year.

PART IV. Laboratory studies.

In the laboratory it was found that when freely breeding colonies of the Ilobi strain of A.aegypti were given choices of egg-laying sites, the greatest numbers of eggs were laid in those with the largest surface areas, highest water levels and least degree of water contamination. Egg-laying was most intense in relatively cool water and dark situations. It was found that in otherwise controlled conditions, the greater the density of larvae the longer the developmental period and higher the mortality.

PART V. Discussion and general conclusions.

It is concluded that water contamination (degree of plant debris) is the major factor limiting the breeding of this species to the immediate vicinity of the village, egg-laying site dimensions also playing a part. Anthropophilism will also restriet the range of the species to some extent. Temperature and degree of illumination are considered to limit the breeding of the species so that more than half (57 per cent.) of the eggs are laid inside houses. The female behaviour is such that the eggs are laid in situations most favourable to hatching and pre-adult survival and development. The monthly fluctuations in numbers are considered to be density dependent, such that at a critical maximum of pre-adult population the limits of the environment are reached and extended developmental periods and increased mortalities cause a drop in the population. Sanitary measures as a means of control are discussed.

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APPENDIX I

TABLES

Table 1

Average monthly means of rainfall (inches), maximum and minimum relative humidity (per cent.) and maximum and minimum temperature (°C.) over the five years 1951 to 1955 for Lagos, Nigeria

Month	Rainfall	Rel. humidity		Temperature	
		Max	Min	Max	Min
Jan	0.3	99.0	65.0	31.5	22.0
Feb	0.9	98.0	63.0	32.0	22.5
Mar	3.1	98.0	64.0	32.4	22.6
Apr	3.3	98.0	63.0	32.4	22.8
May	8.3	99.0	70.0	30.9	22.5
Jun	9.6	99.0	75.0	29.1	21.8
Jul	9.6	98.0	77.0	27.6	21.4
Aug	2.9	99.0	75.0	27.4	20.9
Sep	5.1	99.0	77.0	28.4	21.7
Oct	6.3	99.0	75.0	29.3	21.7
Nov	1.9	98.0	67.0	29.3	22.3
Dec	0.8	99.0	61.0	32.0	22.6

Table 2

Monthly mean maximum and minimum temperatures (°C) for Ilobi village and Lagos. Jan. to Dec. 1956.

Month	Ilobi		Lagos	
	Max	Min	Max	Min
Jan	32.0	18.0	31.4	22.0
Feb	33.0	18.5	32.0	22.5
Mar	33.4	19.0	32.5	22.5
Apr	35.0	20.2	32.5	22.7
May	36.4	19.8	30.5	22.3
Jun	31.0	18.4	29.0	22.0
Jul	31.3	18.4	27.5	21.6
Aug	30.8	18.3	27.3	21.0
Sep	32.8	18.4	28.0	21.5
Oct	35.1	20.6	29.0	21.6
Nov	34.5	18.5	31.0	22.2
Dec	35.0	20.0	32.0	22.2

Table 3

Monthly mean maximum and minimum temperatures (^{o}C) for Ilobi forest. Jan. to Dec. 1956

Month	Maximum	Minimum
Jan	28.0	19.2
Feb	29.5	19.4
Mar	28.5	19.8
Apr	29.1	20.4
May	29.2	20.2
Jun	28.5	19.8
Jul	26.5	18.6
Aug	26.0	18.1
Sep	27.5	20.0
Oct	28.0	20.5
Nov	29.0	20.0
Dec	28.5	19.5

Table 4

Mean hourly readings of temperature (°C.) and relative Humidity (per cent.) for Ilobi village and forest.

Time (hours)	Village		Forest	
	°C	RH%	°C	RH%
0100	23.3	97.0	22.8	97.0
0200	23.1	97.5	22.5	96.5
0300	23.0	99.0	22.4	97.5
0400	22.7	96.0	22.2	98.0
0500	22.5	96.5	22.0	97.0
0600	22.2	96.0	22.2	98.0
0700	23.0	95.0	22.5	97.0
0800	26.3	95.5	23.5	96.5
0900	29.0	94.0	24.5	97.0
1000	29.4	87.0	25.5	94.0
1100	30.0	84.5	26.2	92.0
1200	30.9	85.0	26.8	91.0
1300	30.9	86.0	27.0	93.0
1400	29.3	87.0	27.3	92.5
1500	29.3	88.0	27.8	92.5
1600	28.2	89.5	27.0	93.0
1700	28.0	90.0	26.2	94.0
1800	27.2	91.0	25.4	94.5
1900	25.9	92.0	24.8	95.0
2000	25.1	93.5	24.6	98.0
2100	24.4	95.0	24.2	98.5
2200	24.0	96.0	23.5	99.0
2300	23.8	96.5	23.0	99.0
2400	23.6	97.0	22.8	99.0

Table 5

The rainfall (inches), number of rainy days and rain index for Ilobi Village, 1956

Month	Inches	No. rainy days	Rain Index
Jan	0.0	0	0.0
Feb	3.5	6	0.7
Mar	7.2	11	2.6
Apr	2.4	8	0.6
May	2.1	6	0.5
Jun	7.2	13	3.2
Jul	3.8	11	1.3
Aug	1.5	8	0.4
Sep	7.3	15	3.6
Oct	11.2	11	4.0
Nov	2.4	8	0.6
Dec	2.0	2	0.1

$$\text{Rain Index} = \frac{\text{Inches of rain} \times \text{No. rainy days}}{\text{No. days in month}}$$

Table 6

Size distribution of mature eggs of A.aegypti.
Measurements in microns. 103 eggs measured.

Length		Width	
Microns	Number	Microns	Number
600	2	160	1
620	7	180	78
640	11	200	24
660	17		
680	18		
700	21		
720	15		
740	9		
760	1		
780	1		
mean = 683 m.		mean = 184 m.	

Table 7

The percentage adult hatch obtained from eggs of A.aegypti at various intervals after laying.

Date eggs laid	Time of test (Weeks after laying) 8	12	16	20	24	28
9.10.55	NT	NT	NT	14.8	2.0	0.0
24.10.55	NT	NT	9.4	3.9	2.0	0.0
7.11.55	NT	3.9	8.8	3.3	2.0	0.0
19.11.55	NT	24.5	13.6	2.0	0.0	0.0
28.11.55	NT	32.0	18.0	1.7	1.6	0.0
12.12.55	58.0	16.0	8.0	2.0	0.0	0.0

(NT = no test)

Table 8

Grouped results of tests on hatching. Mean percentage hatch and mean age in weeks.

Age of eggs:	8	12	16	20	24	28
% hatch :	58.0	19.1	11.6	4.6	1.9	0.0

Table 9

Mean time taken to hatch (minutes) of mature eggs of A.aegypti at various water temperatures (^{o}C)

Water temperature	Mean time to hatch
26	23.2
28	18.2
30	13.6
32	14.1
34	21.1
36	26.0

Table 10

Variation in the number of comb spines in the fourth instar larva of *A.aegypti*.

Number of spines	Number of larvae.
4	5
5	2
6	7
7	8
8	53
9	20
10	71
11	2
12	3
13	0
14	0
15	1

Table 11

Variation in the number of pecten spine in the fourth instar larva of A.aegypti

Number of spines	Number of larvae
4	1
5	0
6	0
7	0
8	3
9	8
10	13
11	22
12	28
13	24
14	29
15	16
16	13
17	8
18	9
19	0
20	0
21	0
22	0
23	0
24	0
25	1

Table 12

Increase in the mean number of comb and pecten spines in successive instars of *A.aegypti*

Instar	Mean number of spines	
	comb	pecten
I	4.4	4.6
II	8.5	7.9
III	11.2	8.9
IV	11.8	9.2

Table 13

The larval mortalities of A.aegypti for successive instars when bred in water at various temperatures

Water temperature (°C)	% mortality of each stage					Total % Mortality
	I.	II.	III.	IV.	Pupa.	
44	100	0	0	0	0	100
40	100	0	0	0	0	100
36	0	50	30	10	0	90
32	0	0	10	0	0	10
28	0	0	0	4	0	4

Table 14

The onset of pupation (days after hatching) of A.aegypti larvae bred in water at various temperatures (°C)

Water temperature	pupation
44	0
40	0
36	10
32	6
28	6

Table 15

The mean length and width of the head capsule of fourth instar larvae bred in various water volumes.

Water Vol. (cc)	No. larvae	Mean length (mm)	Mean width (mm)
35	100	0.758	0.898
200	100	0.768	0.916
900	100	0.757	0.926
1500	100	0.754	0.954

Table 16

The number of eggs laid by female A.aegypti after blood meal on white mice.

Female	No. eggs
1	7o
2	60
3	46
4	55
5	30
6	63
7	45
8	70
9	30
10	69
11	57
12	68
13	66
14	42
15	56
16	109
17	76
18	80
19	117
20	87
21	70
22	113
23	82
24	62
25	96
26	115
27	120
28	110

mean = 73.7

Table 17

a) Results of initial test on Ilobi strain of A.aegypti

% DDT	% mortality
0.01	100
0.001	100
0.0001	100
0.00005	100
0.00001	85
0.000005	80
0.000001	64

b) Results of tests upon successive generations of fourth instar larvae

Generation	% DDT	% mortality
I	0.000001	81.87
II	0.000001	86.19
III	0.000001	74.37
IV	0.000001	32.68
V	0.000001	2.45

Table 18

Mean water temperature in clay water pots, 1200 hrs. (^{o}C.)

Atmospheric Temperature		Position of pots		
Sun	Shade	Sun	Semi-shade	full shade
32.0	29.5	28.9	28.3	26.9

Table 19

Distribution of A.aegypti pre-adults in natural habitats.

Habitat	Total sampled	No. times A.aegypti present
Domestic containers	237	49
Tree-holes	25	..
Snail shells	252	2
Cocoa-husks	730	..
Gourd shells	31	..
Cocoa-yam axils	279	..
Banana axils	88	1
Pineapple axils	280	..
Dried leaves	50	..

Table 20

Breeding indices for A.aegypti in experimental pots in Ilobi Village

Position	No. pots exposed	% containing larvae after 1 week
Inside houses	90	54.3
Outside houses (sheltered)	90	31.3
Fully exposed	120	14.4

Table 21

The weekly distribution of A.aegypti pre-adults in clay and bamboo experimental pots along the eastern transect, January to December 1956

Week	Clay	Bamboo	Week	Clay	Bamboo
1	1.2.3.	1.2.3.7.	27	2.3.	1.3.
2	1.2.3.6.	1.2.3.	28	1.2.3.	1.3.
3	1.2.3.	3.	29	1.3.	1.3.
4	1.2.3.4.9.	1.2.3.	30	2.	3.
5	1.3.4.5.	1.2.3.5.8.	31	1.2.	3.
6	1.2.3.	1.2.3.	32	1.2.6.	1.3.
7	2.3.4.5.8.	1.	33	1.	1.3.
8	1.2.3.	1.2.3.	34	1.	1.3.
9	1.2.3.	1.3.	35	1.2.	3.
10	2.3.	2.	36	1.2.3.	3.5.
11	1.2.3.	2.	37	2.3.	1.3.4.
12	1.2.3.5.	1.9.	38	1.2.3.4.	1.3.
13	2.3.4.	3.	39	1.9.	3.
14	1.2.3.	1.2.3.	40	3.4.	1.3.
15	1.2.3.4.	2.3.	41	1.3.4.	2.
16	1.2.3.4.	1.2.3.	42	1.2.3.4.5.6.	1.
17	1.2.3.	1.2.3.	43	1.2.	2.3.
18	1.2.3.	1.2.3.	44	1.2.3.4.	1.2.3.
19	1.2.3.	2.3.	45	1.2.3.4.	1.3.
20	1.2.3.	1.2.3.	46	1.2.3.	1.2.
21	1.2.3.	2.3	47	1.2.	1.2.
22	1.2.3.	2.3.	48	1.2.	1.
23	1.2.3.	2.3.	49	1.2.3.	1.2.4.
24	1.2.3.	1.2.3.	50	1.2.	1.2.4.
25	1.2.4.	1.2.3.	51	1.2.10.	1.2.3.
26	1.3.4.	1.2.3.4.	52	1.2.3.	1.2.3.

Table 22

Total number of times A.aegypti pre-adults recorded from each station along eastern transect.

Station No.	Pre-adult records.
1	64
2	58
3	66
4	17
5	6
6	4
7	2
8	3
9	1
10	1
11	0

Table 24

The number of eggs laid by A.aegypti in dishes of various surface areas

Surface area (cm^2)	Number of eggs laid
19.6	842
28.3	1501
50.2	2943
63.6	3187
78.5	4387
95.0	5011

Table 25

The number of eggs laid by A.aegypti in containers with the water at various levels below the rim

5-mm bands above water surface	Water level (mm) 25	50	75
0-5	533	218	139
5-10	94	44	47
10-15	35	14	18
15-20	24	8	13
Total	686	284	217

Table 26

The number of eggs laid by A.aegypti in leaf solutions of various concentrations

% leaf solution	No. eggs laid
100	58
75	65
50	140
25	160
Tap water	1130
Total	1553

Table 27

The number of eggs laid by A.aegypti in water at various temperatures, (^{o}C.)

Water temperature	No. eggs laid
26	622
27	500
29	330
30	225
33	155
34	165
36	112
39	55
44	0

Table 28

Distribution of gravid females of A.aegypti in temperature choice tube

Test No.	Distribution of females		
	32°C	28°C	Intermediate points
1	2	13	0
2	1	14	0
3	4	11	0
4	2	13	0
5	5	9	1
6	2	13	0
7	2	12	1
8	2	13	0
9	3	12	0
10	1	14	0
11	0	15	0
12	4	11	0
13	2	13	0
14	3	7	5

Table 29

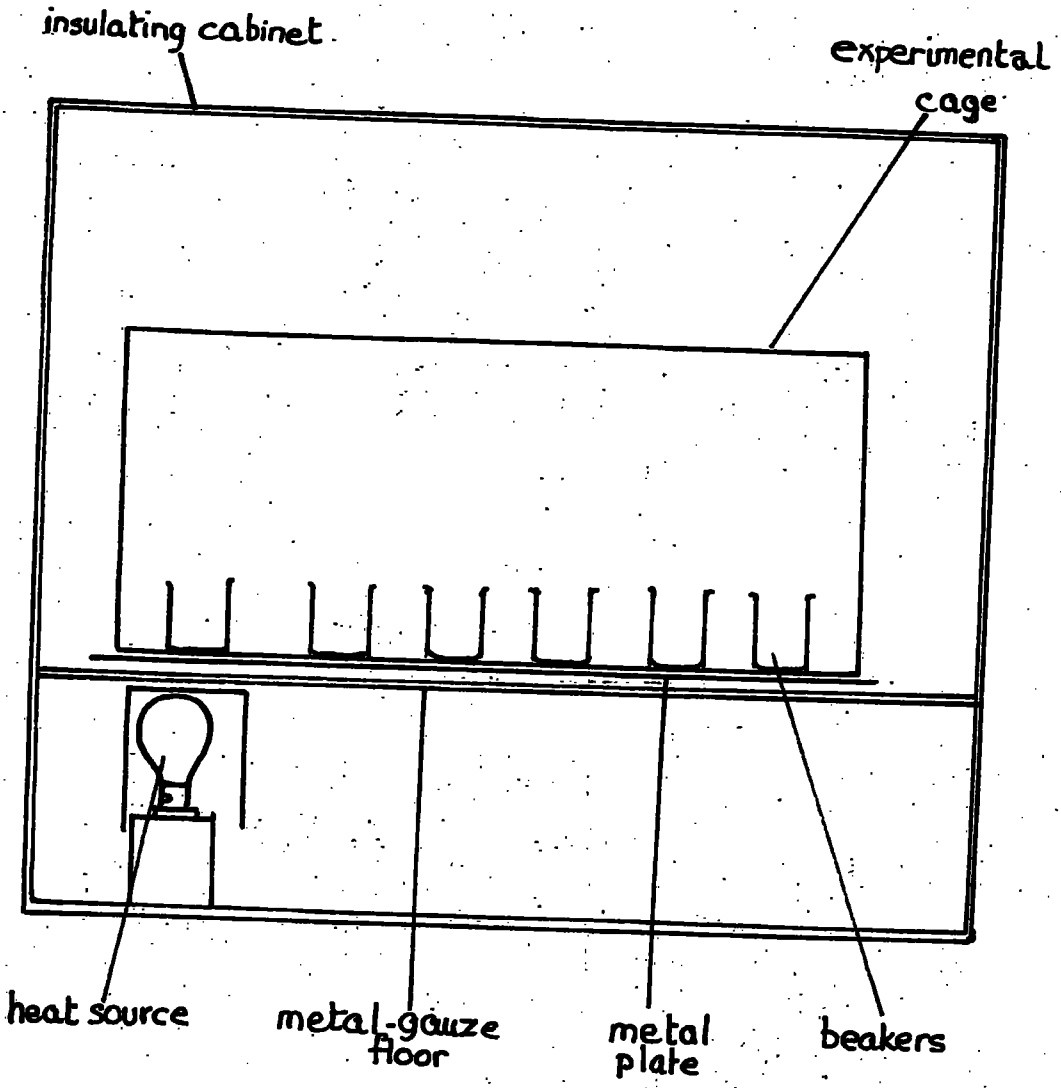
Number of eggs laid by A.aegypti in containers in various light intensities
(Containers numbered in order of decreasing light intensity)

Dish No.	No. eggs laid
1	55
2	105
3	45
4	12
5	50
6	420
7	1115

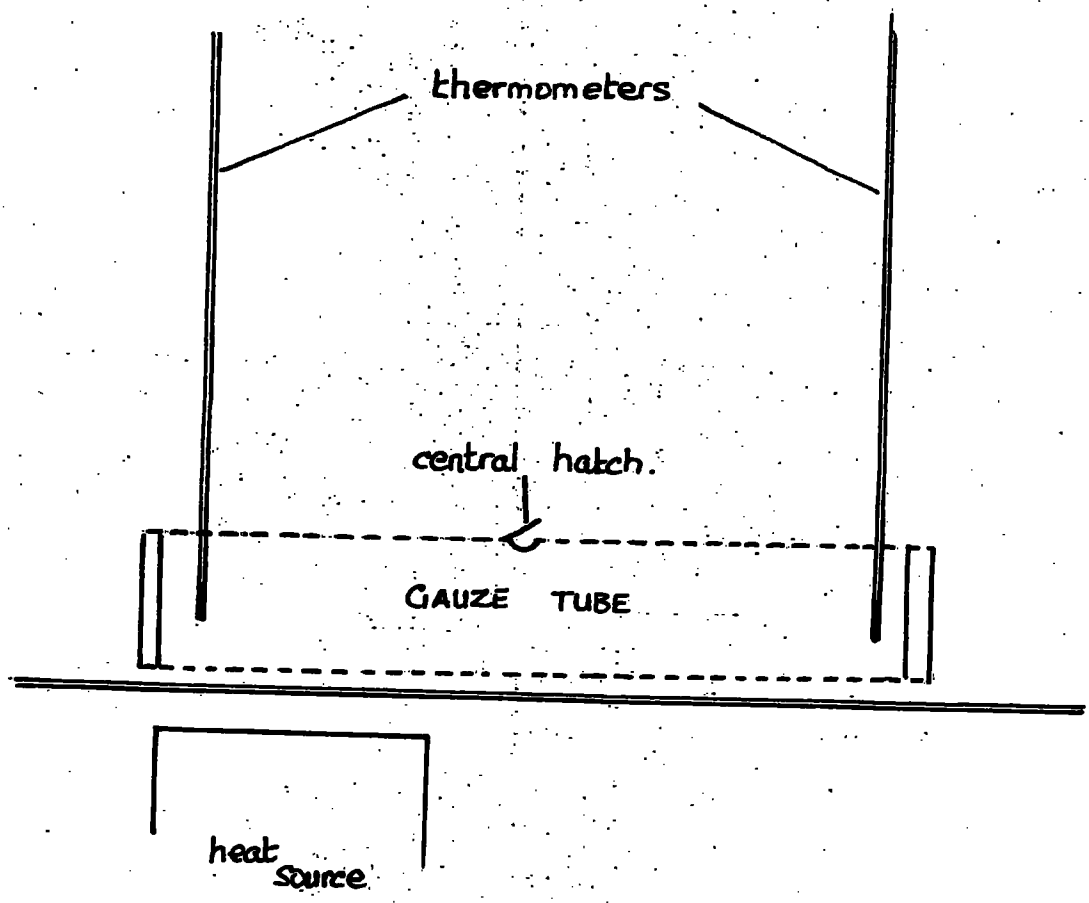
APPENDIX II

Apparatus used in the laboratory experiments.

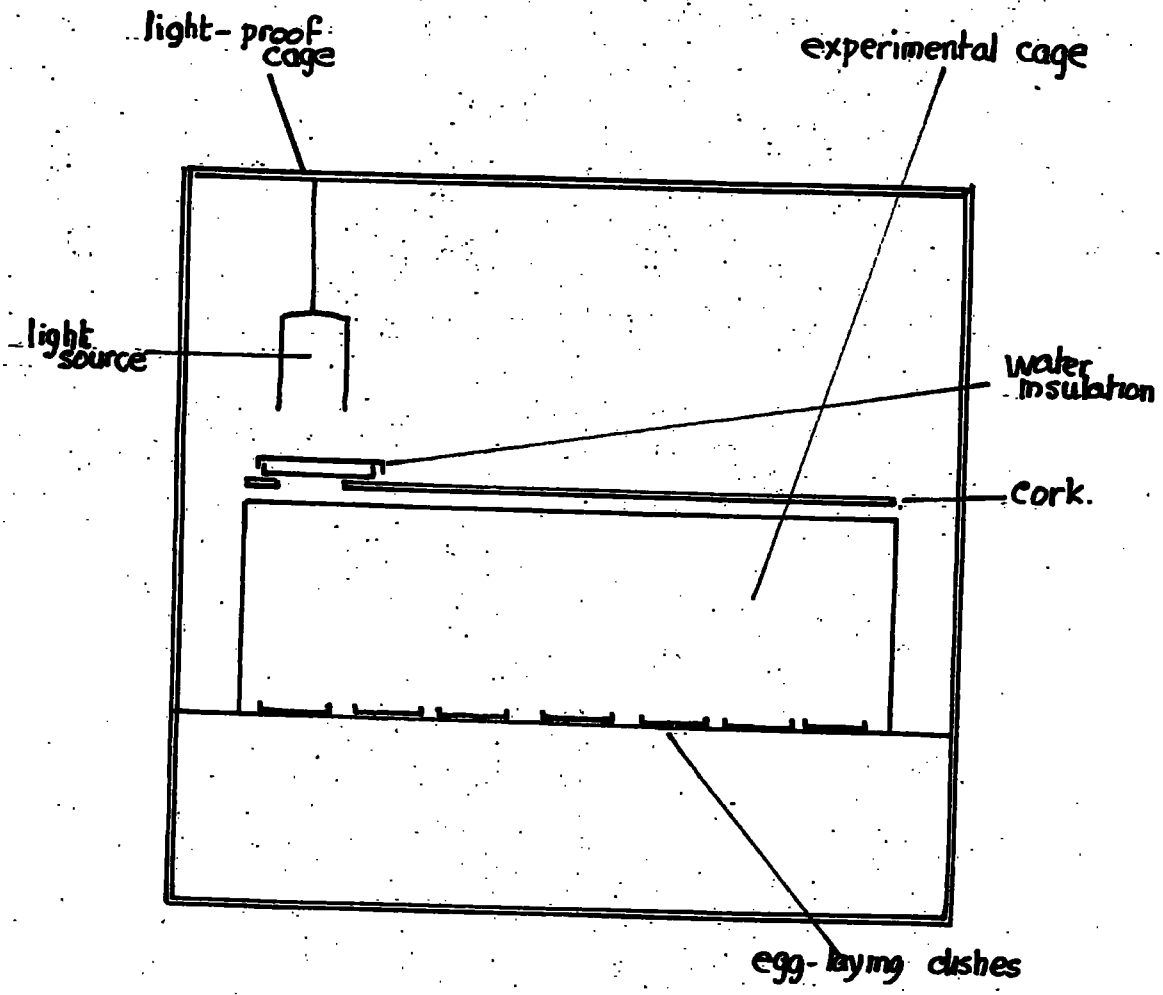
Apparatus used to produce temperature gradients.



Temperature choice-tube



Apparatus used to produce light-intensity gradient



APPENDIX III

Note on the routine methods used for maintaining *Aedes aegypti* colonies.

Cages;

These are made of wood and mosquito-gauze, measuring 3 feet x 3 feet x 3 feet. These are kept in a screened insectory outside of the main laboratory. Each cage is fitted with two sleeve openings for the purpose of removing mosquitoe or placing in food etc.

Feeding;

The adult females are allowed to feed on anethetised white mice twice weekly. The males are provided with a 10 per cent. glucose solution at all times.
The larvae are fed on finely crushed food prepared in the following way.

Whole meal flour	45%
Ground oats	40%
Fish meal	8%
Dried skimmed milk	3%
Cod-liver oil	2%
Dried yeast	1%
Sodium Chloride	1%
	100%

Egg-laying;

100 cc. beakers are left in the cages at all times, these containing about 50 ccs. of tap water and filter paper is placed around the insides. Thes papers, with eggs on them are removed daily and stored in air-tight jars until required. The larvae are hatched at room temperature and bred up in enamel bowls. The pupae are extracted daily and placed in beakers in the cages.

APPENDIX IV

Photographic Plates

Plate I.

The eastern edge of the village, showing the scrub-zone and beginning of the forest.

Plate II.

Late secondary forest on the eastern side of the village.

Plate III.

View of cocoa plantation, showing patches of cocoa-yam and thick floor litter.

Plate IV.

Early secondary forest and mixed farmland to the south of Ilobi village.

Plate V.

A typical patch of farmland, showing cassava and some cocoa-yams with a banana plant top right.

Plate VI.

Banana plant, showing the leaves grouped around the main stem, their petioles forming water retaining axils.

Plate VII.

Water retaining axil of banana plant.

Plate IX.

Detail of ground litter in cocoa plantation, showing water retaining cocoa husks and dried leaves.

Plate X.

Tree-hole in cocoa tree.

Plate XI.

Water retaining axil of cocoa-yam.

Plate XII.

Sampling a bamboo pot. The contents are poured into a white enamel tray from which the larvae can be taken with a pipette. The pot is then washed out several times to obtain all the larvae.