



AGE DETERMINATION OF SOME ANOPHELINE MOSQUITOS BY
DAILY GROWTH LAYERS OF SKELETAL APODEMES

by

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Introduction

A method for the age determination of certain Diptera including Culex and Aedes mosquitos by the counting of the number of growth layers on the skeletal apodemes was described by Schlein and Gratz (1972). In view of the epidemiological importance of age-grouping in anopheline mosquitos, several malaria vector species of Anopheles were examined to determine the applicability of this method to that genus.

The age assessed by counting the daily layers is the actual calendar age, whereas the previous methods, summarized by Detinova 1962, rely mainly on observations of changes in the female reproductive system which indicate the physiological age.

The daily rhythm of cuticle deposition, first described by Neville in 1963, has since been studied in many insect species. In Diptera, the growth of the cuticle seems to be limited and the skeletal apodemes are apparently the only parts of the skeleton in which daily growth layers can be observed. The thoracic apodemes are the sites of attachment of the thoracic muscles and their length is directly related to the size of these muscles. Thus the length of the apodemes and the amount of daily growth reflect the amount of growth of the thoracic muscles under different environmental conditions.

Material and methods

The specimens examined were a series of laboratory reared adults of A. gambiae, A. stephensi and A. albimanus killed at different known ages and field collected A. gambiae and A. funestus. All the specimens were dry stored since the cuticle is damaged and its staining properties deteriorate if the material is preserved under humid conditions after collection.³ The apodeme showing daily growth layers in anophelines is the thoracic phragma (Figs 1A and 1B). After the mosquitos had been macerated in potassium hydroxide (KOH) it was excised with the adjacent sclerites and treated as follows. A 7% solution of KOH containing the mosquitos was brought to boiling point, removed from the flame and left standing for a few minutes. The mosquitos were then dissected in water using watchmakers' forceps; the abdomen was first

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³ In order to ensure that the specimens are sufficiently dry, the mosquitos may be kept for two hours in the sun or alternatively in front of an electric heater for one or two hours. They should be preserved in sealed tubes having a small layer of calcium chloride or silicagel. They should not be exposed to naphthalene or paradichlorobenzene.

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separated from the thorax and then the thorax was cut transversely between the second and third pairs of coxae and up to the scutellum. The separated rear part of the thorax (Fig. 1A) was rinsed in water, cleaned of the tracheae and its ventral part (metasternum and coxae) was cut off. These preparations were stained employing the following procedure:

- (1) Oxidation in 1% potassium permanganate for five minutes.
- (2) Rinsing in water.
- (3) Mordant, 1% iron alum, 15 minutes.
- (4) Rinsing in water, 10 minutes.
- (5) Changing of water and rinsing for further 10 minutes.
- (6) Staining in a ripe solution of 2% haematoxylin in 70% ethyl alcohol for 1-2 minutes (under microscopic control to avoid over-staining; about 10 preparations can be stained and observed at a time).
- (7) Rinsing in water.
- (8) Counterstaining for 20 minutes in 0.2% Congo red in water.
- (9) Rinsing in water and flattening of preparation.
- (10) Dehydration in absolute ethyl alcohol.
- (11) Clearing in xylol and mounting in Canada balsam.

Preparations of A. funestus, which is smaller than the other species and has a thinner cuticle, were treated and stained as above, except that they were left in potassium permanganate for 10 minutes and for five minutes in iron alum.

Results

The duration, degree and form of cuticle deposition varies between different areas for the same mosquito species. Growth layers can be observed only on the mesosternal apodeme and the thoracic phragma. The striation on the mesosternal apodemes stains faintly and no more than five to six growth layers can be counted, whereas up to 13 daily layers can be observed on the thoracic phragma.

A distinct line on the thoracic phragma marks the extent of the area of the phragma at eclosion. This is followed by a wide layer (nearly band-like) of the first day's growth and the narrower layers of the subsequent days (Fig. 1B). The daily layers can be distinguished from one another through staining as they appear in alternating light and dark colours. However, this picture may sometimes be less clear when the daily layers do not stain deeply enough and, in fact, they can then be confused with other striations present within the first day's growth. When this first day striation is confluent with the daily layers, it becomes impossible to count those layers. In most of the field collected mosquitos, however, the beginning of the second day growth was fairly distinct and the age could be determined in more than 85% of the batches of field collected A. gambiae (Fig. 2).

It was of considerable interest to find that, unlike the field collected mosquitos, the daily layers failed to stain in most of the laboratory reared anophelines of all the above-mentioned species and the age could be determined in only approximately 25% of the specimens examined. The appearance of the layers varied from one batch to another in the same species and in many specimens the daily layers were visible only as interruptions on the longitudinal ridges of the thoracic phragma.

These mosquitos had been kept in constant temperature of 27°C and light and dark alternating every 12 hours. Better distinction of the stained growth bands was achieved in mosquitos that had been reared in room temperature and exposed to temperature fluctuations, but even in these, the visibility of the daily layers was far less distinct in appearance than in the field collected material.

Some measurements were taken to estimate the rate of growth and to try to account for the variation in visibility of the daily layers. The width of the cuticle and the length of the growth of the thoracic phragma were measured in both field collected and laboratory bred A. gambiae; 15 females of the same size and of different known ages of laboratory bred mosquitos were compared with 15 selected field collected females of the same size and corresponding age. The thickness of the cuticle was measured in the anterior midline of the mesonotum and the anterior upper part of the fore femur after the mosquitos had been macerated in KOH. The average thickness of the femur cuticle in the field collected mosquitos was 4 μ compared with 5.25 μ in the laboratory mosquitos. The thickness of the cuticle of the mesonotum of the field collected mosquitos was 4.7 μ in average compared to an average of 5.4 μ in the laboratory mosquitos.

The range of variation of cuticle thickness of the mesonotum and femur in mosquitos of the same age and batch reared in the laboratory was the same as the range measured in the field collected mosquitos (all mosquitos were more than one day old). The growth in length of the thoracic phragma in the first day in laboratory reared mosquitos was 38 μ in average compared to 36 μ in the field collected mosquitos.

It appears that there is little or no apparent relation between the amount of growth in the first day and that of subsequent days. The first day's growth in a given mosquito might be 42 μ and then 20 μ in the following nine days or, in another specimen, 22.5 μ in the first day and 20 μ in the subsequent nine days. The first day's growth is apparently independent of the feeding of the adult since the cuticular band of the first day was deposited to its normal size even in mosquitos that were not allowed to feed after eclosion. The variation measured in the growth between the second and tenth day was much smaller than that of the first day and ranged between 20 μ to 27 μ . In contrast the first day's growth may range as shown above from 22.5 μ to 42 μ .

Discussion

The number of growth layers in the Anopheles spp. bred in the controlled conditions of the laboratory was found to correspond with the age of the mosquitos in days whenever the layers were distinct enough to allow counting. The same correlation was observed in mosquitos when reared under variable conditions in the insectary; it therefore may be assumed that the layers of growth on the apodemes in the field collected material are also daily, i.e. each layer representing the amount of growth in one day. This makes it possible to assess the age of field collected anophelines and relate the chronological age to the physiological changes which take place up to a period of 10 to 13 days. In most of the mosquitos of all the species examined, it was possible to count up to 10 growth layers, i.e. corresponding up to 10 days of calendar age. In a small number of specimens, up to 13 layers could be determined. In less than 15% of the field collected specimens the growth layers could not be counted either because the preparations failed to stain or due to lacking distinction of the daily layers; therefore, while success is not certain for every individual mosquito, up to 85% of the specimens collected can be age graded if properly prepared.

As the thoracic phragma is the attachment area of the longitudinal flight muscles, its growth in length would be directly related to the growth of these muscles. It could apparently be used to estimate the degree of muscle development, the daily layers indicating that a certain amount of growth occurred in each day. Estimation of the degree of muscle

development by weighing the residual dry weight had been suggested by Bursell (1961), as a method for age determination in Glossina. This method was later used (Bursell & Kuwengwa, 1972) to establish the differences in the rate of growth between field and laboratory populations of Glossina.

It appears that in the anophelines the amount of growth in the first day is determined by the nutritional condition of the larva while the rest of the growth is dependent on the environment of the adult, but this must be ascertained through further study.

The appearance of the daily layers differs markedly between mosquitos reared in the laboratory and those collected in the field. The cuticle in A. gambiae reared in the laboratory was found to be thicker than that of field collected mosquitos of the same species. It does not seem likely that the thickness of the cuticle could account for difficulty in defining the absence of the daily growth layers in most of the laboratory material. Alternations of thickness of cuticle within the apodeme would suggest a partial answer because if the apodemes are stained without oxidation, the daily layers show but faintly in field collected mosquitos. The oxidation with potassium permanganate selectively intensifies the demarcation lines of the daily layers, without having an effect on the intensity of staining of the rest of the cuticle; this being the case, it does not seem that these differences in the visibility of the daily layers are only a result of variation in the amount of cuticle deposited, but in certain properties of the cuticle itself, dependent upon physiological differences between the anophelines in the field and those reared in the laboratory.

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Summary

An earlier study showed that it was possible to determine the calendar age of Culex pipiens and Aedes aegypti by counting the number of daily growth layers on the skeletal thoracic apodemes. The present investigation was carried out to determine the applicability of the method for anopheline mosquitos.

The method was applied utilizing laboratory-reared adults of Anopheles gambiae, A. funestus and A. albimanus killed at different known ages and field collected A. gambiae and A. funestus. Using special staining procedures daily growth layers could be observed on the thoracic phragma. The appearance of these layers was less visible in the laboratory-reared material than with field collected material. With field collected A. gambiae, the calendar age could be determined in about 85% of mosquitos examined, while in laboratory-reared specimens the age of only 25% of mosquitos examined could be determined. Sometimes the daily layers did not stain sufficiently and were confused with another type of striation which in some specimens was present within the first day's growth. In most of the field collected mosquitos examined it was possible to count up to 10 growth layers corresponding to 10 days of calendar age and in a small number of specimens up to 13 layers could be determined. It is hoped that following sufficient trials of this method in the field, it can be introduced in epidemiological studies involving the longevity of the vector.

RESUME

Une étude précédente avait montré que l'on pouvait déterminer l'âge effectif de Culex pipiens et d'Aedes aegypti en comptant le nombre des couches de croissance quotidienne sur les apodèmes du squelette thoracique. La présente investigation visait à déterminer si la méthode était applicable aux anophèles.

On s'est servi d'une part d'Anopheles gambiae, d'A. funestus et d'A. albimanus adultes élevés en laboratoire et tués à différents âges connus, d'autre part d'A. gambiae et d'A. funestus collectés dans la nature. Au moyen de techniques de coloration spéciales, on a pu observer les couches de croissance quotidiennes sur le phragma thoracique. Ces couches étaient moins nettement décelables chez les insectes élevés en laboratoire que chez ceux que l'on avait capturés dans la nature. Dans le cas des A. gambiae récoltés dans la nature, l'âge effectif a pu être déterminé chez environ 85 % des individus examinés, contre 25 % seulement pour les spécimens élevés en laboratoire. Parfois, les couches quotidiennes ne prenaient pas suffisamment la coloration, de sorte qu'on les confondait avec un autre type de striure qui, chez certains spécimens, apparaissait dès le premier jour de la croissance. Chez la plupart des moustiques recueillis dans la nature, on a pu compter jusqu'à dix couches de croissance correspondant à dix journées d'âge, et, chez un petit nombre d'individus, on a pu dénombrer jusqu'à treize couches. On espère qu'après avoir été suffisamment éprouvée sur le terrain cette méthode pourra être introduite dans des études épidémiologiques portant sur la longévité du vecteur.

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FIG. 1A. A. GAMBIAE POSTERIOR VIEW OF THORACIC SKELETON

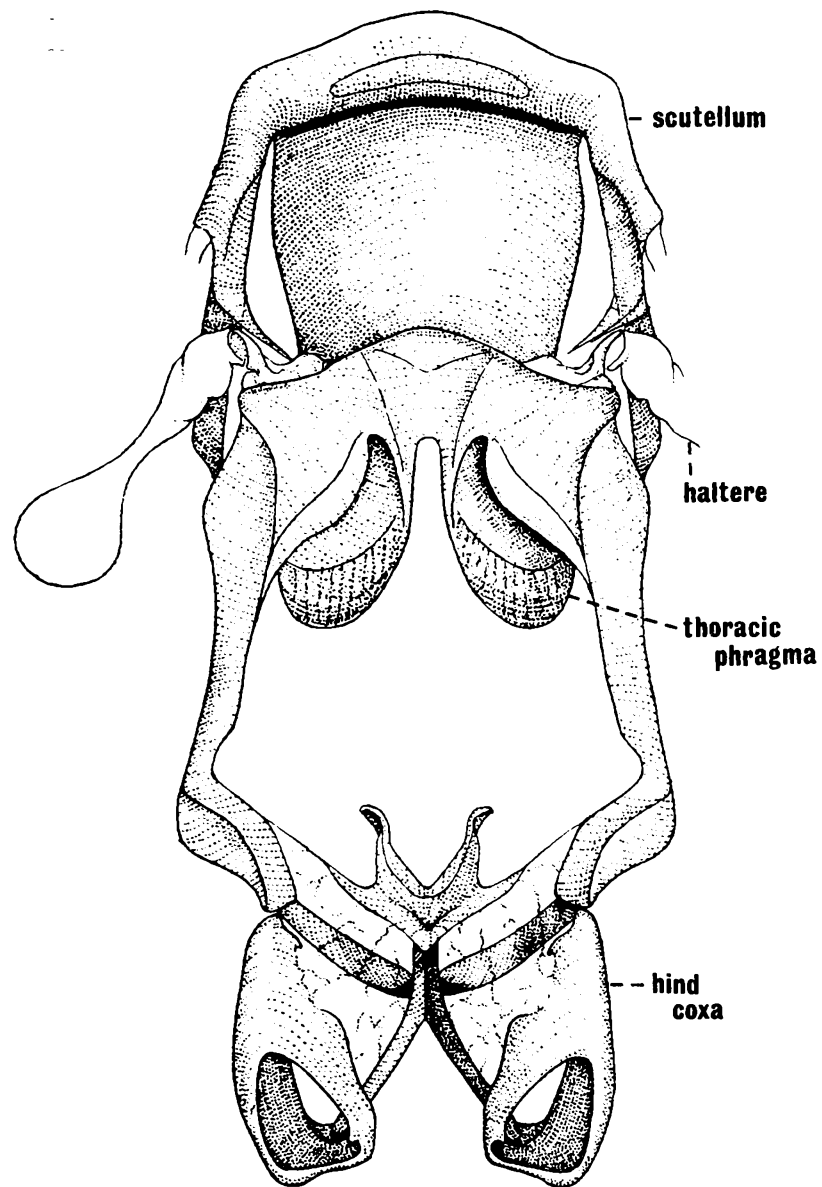


FIG. 1B. THE DAILY LINES OF GROWTH
ON THE THORACIC PHRAGMA OF FIELD
COLLECTED A. GAMBIAE

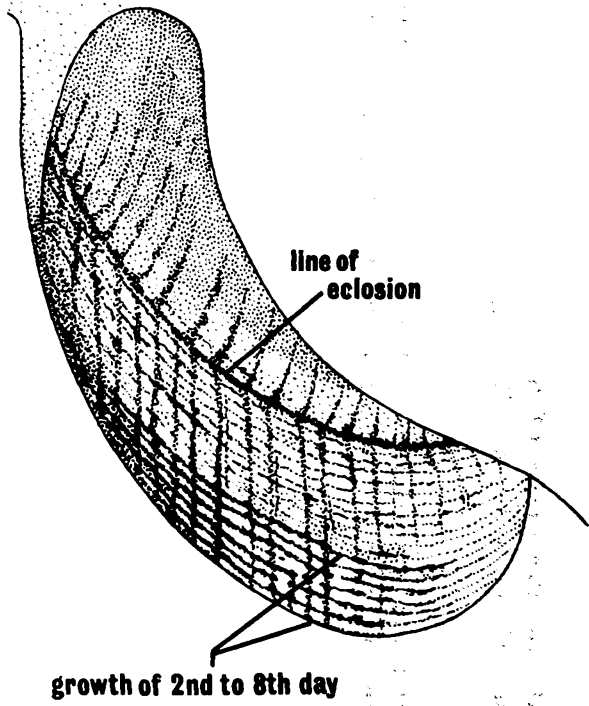
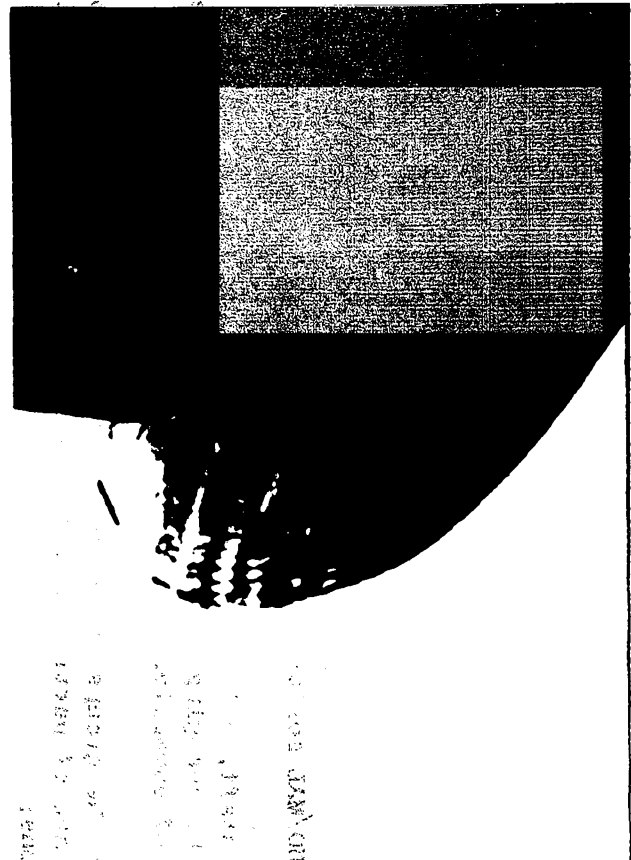


FIG. 2. THORACIC PHRAGMA OF 5 DAYS
OLD A. GAMBIAE FROM NORTH NIGERIA



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