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RESULTS OF AGE-GROUPING WORK ON SOME
ANOPHELINE MOSQUITOS IN SOUTHERN GHANA

by

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1. INTRODUCTION

Following the directions of the Plan of Operations for the pre-eradication survey in the Volta Region of Ghana, various attempts were made at age-grouping the local anopheline population. Starting in December 1958, the project attempted to assess natural daily mortalities by a combination of the techniques of Davidson (1955) and Gillies (1958). Although several thousand dissections were performed, it proved impossible to analyse the data so produced in any worth-while way without a knowledge of the frequency distribution of the ampullae measurements of local anophelines. During the attempt to measure ampullae from mosquitos of proven parity, the technique of Detinova (1959) was tried out and a simple form of this technique perfected, similar to that of Lewis (1958). The data so provided made it possible to analyse all the previous dissections, but it became apparent that the results were less reliable and not as interesting as those provided by Detinova's method of dissections. Accordingly, this latter technique was extended and the following work represents the results of some 1700 such dissections performed between 17 February 1960 and 1 September 1960.

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The villages mentioned in the report come from the following rough topographical areas:

Open grassland savannah, Accra Plains	- Okwenya, Dendo
Semi-closed bush savannah	- Kpetoe, Duvanu, Ho
Coastal lagoon strip	- Viepe, Denu, Salakope
Forest (a cocoa area)	- Matse

2. METHODS

Dissections were performed according to the techniques described in the unpublished WHO document "Course in Advanced Entomological Techniques applied to Malaria Eradication - WHO/Mal/238". With the exception of the dissections of night-catches, all material used was collected in flit-catches. Generally, mosquitos were caught early in the morning and dissected the same day. Occasionally, mosquitos were dissected 24 hours after capture, after being kept overnight in a refrigerator. On these occasions, only those mosquitos which showed no apparent disintegration of the fine structure of the ovarioles were dissected. Both unfed and fed mosquitos were dissected.

The following procedure was adopted: If the ovaries were between stage I and mid-stage II, one of them was removed entire and placed in a drop of distilled water on a separate slide (suitably numbered) and allowed to dry. If, during the dissection, it appeared that the tracheoles were in skeins, the specimen was provisionally recorded as nulliparous and the presence of the skeins checked later from the dried-out ovary. It was found easiest to see skeins in the dried ovaries by placing a small drop of water on them immediately before examining. Ovarioles were dissected out only if the ovaries were mid-stage II or later; it was found too difficult to see relics in ovaries later than very early stage IV.

Mosquitos were recorded as parous if a relic was visible on the broken end of the pedicel of at least three ovarioles. They were recorded as nulliparous if no relics were apparent on at least ten ovarioles and if no pigmented lumps were visible when the membranes of the ovary were stretched out (mid-stage II to late stage III), or if skeins were present (stage I to mid-stage II).

A chain of separate dilatations, as described by Detinova (1959) and as photographed by Bertram & Samarawickrema (1958) for Mansonioides, was never found in the five anopheline species dissected. The largest species examined and the one, therefore, in which it would seem most likely to find such dilatations was A. pharoensis, but unfortunately only five specimens of this species were found at the time and these were all nulliparous. Very occasionally, it was possible to determine separate but coalescing swellings on a pedicel which might have been the relics of distinct previous follicles, but in the vast majority of dissections the relics appeared to break more or less through the middle and seemed to be very close to the posterior end of the pedicel if not actually coalesced with the sphincter and calyx wall.

3. RESULTS

3.1 Detailed results of dissections of A. gambiae from various localities in the area are shown in Table 1.

TABLE 1. DISSECTIONS OF A. GAMBIAE

Date	Place	Number nulliparous	Number parous	Total dissected	Proportion parous
9 March to 17 March	Viepe	119	254	373	0.681
9 March to 17 March	Denu	7	24	31	0.774
17 February to 14 March	Bush savannah	12	39	51	0.766
26 July to 26 August	Kpetoe	38	139	177	0.786
22 July to 10 August	Duvanu	26	98	124	0.790
5 August to 27 August	Matse	30	72	102	0.706
26 July to 27 August	Salakope	11	32	43	0.744
21 July to 1 September	Okwenya	20	64	84	0.762
16 August to 30 August	Dendo	4	2	6	?

- Notes:
- (a) "Bush savannah" refers to villages near Ho; range = 5/9 to 20/21 parous.
 - (b) For difference between Matse and Duvanu $X^2 = 1.71$.
 - (c) It is probable that there was a certain proportion of A. gambiae melas in the population at Salakope, Viepe and Denu.

With the exception of the March figures from Viepe, therefore, which were taken during the first influx of newly-emerged females after the start of the rains, the A. gambiae population seems to be fairly closely distributed about the 13% daily mortality mark (see Table 3).

3.2 In the same way as Table 1, the following table presents the A. funestus dissection.

TABLE 2. DISSECTIONS OF A. FUNESTUS

Date	Place	Number nulliparous	Number parous	Total dissected	Proportion parous
9 March to 17 March	Viepe	6	25	31	0.807
17 February to 14 March	Bush savannah	12	73	85	0.859
26 July to 26 August	Kpetoe	4	25	29	0.863
22 July to 10 August	Duvanu	2	23	25	0.920
21 July to 1 September	Okwenya	95	217	312	0.696
16 August to 30 August	Dendo	12	21	33	0.636

Note: "Bush savannah" refers to villages near Ho; range = 59/67 to 8/11 parous.

In the case of this species the results fall clearly into two groups, and it is particularly interesting, therefore, that Okwenya and Dendo should be so similar topographically (see the Introduction above). The comparison of these figures is as follows:

	<u>Total</u>	<u>Proportion parous</u>	<u>X² for difference</u>
Okwenya and Dendo	345	0.690)	16.26
Rest	170	0.859)	

The low mortality of this species, which is repeated in both time and space, except in the grassland, is striking and unexpected.

3.3 Totals for all species: the following table presents the total results of dissections on all species, caught either in spray or in night-biting catches.

TABLE 3. DISSECTIONS OF ALL SPECIES OF ANOPHELES

Species	Locality	Month	Total dissected	Proportion parous	Standard error	"p"	Daily mortality %
<u>A. gambiae</u>	Coast	March	404	0.688	0.023	0.83	17 ± 1
	Bush savannah	Feb., March July, Aug.	587	0.760	0.018	0.87	13 ± 1
<u>A. funestus</u>	Bush savannah	Feb., March July, Aug.	170	0.859	0.027	0.93	7 ± 2
	Grass savannah	July & Aug.	345	0.690	0.025	0.83	17 ± 1
<u>A. nili</u>	Kpetoe	July & Aug.	90	0.644	0.051	0.80	20 ± 3
<u>A. hargreavesi</u>	Kpetoe	July & Aug.	79	0.304	0.052	0.55	45 ± 5

Notes: (a) The A. hargreavesi figures include two nulliparous found at Dendo.
(b) The five A. pharoensis dissected were all nulliparous.

4. DISCUSSION

There are some interesting features illustrated by these dissections which deserve mention.

4.1 Comparison of catch method: as mentioned above, only two sources of material were used, namely flit-catches inside houses and night-biting catches both inside and outside houses. As Hamon et al. (1959) found a difference between the proportion parous in outside shelters and that from-night catches, it was thought worth comparing our flit and night catch results.

Table 4 compares the A. gambiae figures from night-catches (1800 to 0600 hours) and flit catches for the period 26.7 to 26.8 from the three villages where both types of catching were performed (Kpetoe, Salakope, Okwenya).

TABLE 4. COMPARISON BETWEEN A. GAMBIAE COLLECTED BY DIFFERENT METHODS

Method	Number nulliparous	Number parous	Total dissected	Proportion parous	Standard error
Flit	53	182	235	0.774	0.027
Night	15	42	57	0.737	0.058

The sample from night-catches is unfortunately small, but from what figures there are it can be said that there is no statistical difference ($X^2 = 0.18$).

The night-catch figures are obviously much too small to analyse in any greater detail.

4.2 Comparison of fed and unfed mosquitos: Table 5 shows the difference between the proportions parous of A. gambiae and A. funestus caught in a fed and in an unfed condition. The figures are all taken from flit-catches, as night-catch material obviously contains a large proportion of unfeds, and these will presumably be the same age as the total catch (see 4.1 above). The period covered is 22 July to 1 September 1960.

TABLE 5. DIFFERENCE BETWEEN THE PROPORTION OF PAROUS A. GAMBIAE AND A. FUNESTUS IN FED OR UNFED CONDITION

Species	Fed or unfed	Number nulliparous	Number parous	Total dissected	Proportion parous	Standard error
<u>A. gambiae</u>	Fed	102	335	437	0.768	0.020
	Unfed	3	3	6	0.500	0.205
<u>A. funestus</u>	Fed	72	233	305	0.764	0.024
	Unfed	35	48	83	0.578	0.054

The number of unfed A. gambiae found in flit-catches has been so small that it is impossible to analyse the difference in any worth-while way ($X^2 = 1.58$). For A. funestus, however, the difference is highly significant ($X^2 = 10.35$). This is exactly what one would expect, as it is one's general experience that unfeds resting inside houses usually have very young ovaries (stage I or early II) and are presumably newly-emerged females which have failed to feed on their first night.

4.3 Age make-up of different ovary stages: Table 6 shows the proportion parous found in the first five ovary stages (Christopher's stage II subdivided into 3).

TABLE 6. PROPORTION OF PAROUS ANOPHELES
IN THE FIRST FIVE OVARY STAGES

Species	Ovary Stage									
	I		Early II		Mid-II		Late II		III	
	Number dis- sected	Propor- tion parous	Number dis- sected	Propor- tion parous	Number dis- sected	Propor- tion parous	Number dis- sected	Propor- tion parous	Number dis- sected	Propor- tion parous
<u>A. gambiae</u>	17	0	53	0.038	77	0.208	171	0.743	648	0.862
<u>A. funestus</u>	4	0	23	0.087	77	0.441	144	0.688	266	0.929
<u>A. nili</u>	0	-	0	-	17	0.177	45	0.667	26	0.962
<u>A. hargreavesi</u>	0	-	7	0	35	0.372	28	0.322	7	0.286

Notes: (a) These figures represent the total of all material dissected.

(b) Only two A. gambiae and two A. funestus in early stage II were found parous, and the ovaries of both the A. funestus specimens and one of the A. gambiae were choked with what appeared to be the sporangia of Coelomomyces sp.

The most probable interpretation of these figures is as follows:

By the morning after oviposition parous females: (a) most often have ovaries developed as far as late stage II or stage III; (b) occasionally have ovaries only developed as far as mid-stage II, usually because they have not fed again or have fed

very late; (c) very rarely indeed (1 out of 703 parous A. gambiae) have only reached early stage II, and only when unfed; whereas for nulliparous females roughly the converse is true, presumably because they start off with less developed ovaries and possibly feed later.

The difference between the proportion parous for stage III and late stage II is explainable on the same basis, i.e. that it is more likely that nulliparous females will only get as far as late stage II than to stage III, and that parous females are more likely to have got as far as stage III than only to late stage II.

4.4 The problem of deriving "p" and the length of the nulliparous period

4.4.1 Length of gonotrophic cycle: calculations of "p" (as in Tables 3 and 7) are based on the assumption that the gonotrophic cycle at the time of dissection lasts for two days and that females therefore lay their first eggs four days after emerging. In other words, using the formula:

$$p = \sqrt{\text{proportion parous}} \quad (\text{Davidson, 1955})$$

The figures for the mean temperatures at Ho (in the bush savannah area) for the relevant months were:

March	-	83 ^o F		
July	-	77 ^o F	(being $\frac{\text{mean max.} + \text{mean min.}}{2}$)	(Gillies, 1953)
August	-	76 ^o F		

For all the A. gambiae and most of the A. funestus populations it would therefore seem, on the basis of Gillies' figures, that the gonotrophic cycle was, in fact, lasting two days. For A. nili and A. hargreavesi there is no information available, but it was extremely rare to find "half-gravids" (i.e. stage IV ovaries) in morning catches and it seems likely that their respective cycles must also have been lasting for two days.

4.4.2 Presence and absence of "pre-gravids": calculation of "p" from the formula depends on the assumption that nulliparous females are all less than, and parous females all more than, four days old. Unfortunately, when a species exhibits the pre-gravid phase in only a part of its nulliparous population, this is not always the

case, and Gillies has shown that this occurs in A. gambiae (1958) and A. funestus (1955) at least, though there seems to be no information available for the other species.

The situation is complicated, in that by not dissecting the unfeds, one misses a certain proportion of the parous population (e.g. in Table 5, 48 out of 281 parous A. funestus were unfed). It is clearly necessary, therefore, to dissect the unfeds along with the feds. This being so, it will no longer be possible to recognize Gillies' (1958) "Group 1" and adjust the figures by that method.

The effect of including or excluding Gillies' "Group 1" on the calculation of daily mortalities will evidently be great. The following example to illustrate these relative effects is taken from the Kpetoe dissections, 26 July to 26 August:

TABLE 7. CALCULATION OF DAILY MORTALITIES OF ANOPHELES

Number in Group 1	Number in Group 2	Number in Group 3		Total dissected	Proportion parous	"p"	Daily mortality %
25	13	139	Group 1 included	177	0.786	0.89	11 ± 2
			Group 1 excluded	152	0.914	0.96	4 ± 1

One's interpretation of the mean survival probability will vary accordingly, and, until a way of distinguishing the parous less than four days old from the rest of the parous population has been discovered for a species exhibiting the pre-gravid phase partially, or it is known that the species does or does not exhibit it, one would seem to run the risk of being misleading by deriving figures for "p". For this reason, even though it is begging the question to do so, the example of Hamon et al. (1959) has been followed in this paper and in most of the tables only the proportion parous has been quoted.

4.5 Variations of population age make-up with time

Superficially, the results as presented in Tables 1 and 2 seem to show very little difference between different places except, of course, for differences between some topographical areas already noted in sections 3.1 and 3.2. Thus, for example, there is the case of the difference between the A. gambiae figures for Matse and Duvanu, the X^2 for which was only 1.71. However, this is only true if one totals the dissections over a period of weeks. If, instead, one analyses the differences between sets of dissections performed close together over periods of only a few days one finds highly significant changes in the proportions parous. Thus, for A. gambiae, the figures for the Coast (Viepe and Denu totalled) from March, the figures for Matse from August and the figures for Okwenya from July and August show a wide variation over a brief space of time.

TABLE 8. VARIATIONS OF A. GAMBIAE POPULATION AGE MAKE-UP WITH TIME

Place	Period	Number nulliparous	Number parous	Total dissected	Proportion parous	Standard error	X^2 for difference
Coast	9 March to 12 March	29	143	172	0.831	0.029	27.50
	16 March to 17 March	97	135	232	0.582	0.032	
Matse	5 Aug. to 20 Aug.	2	27	29	0.931	0.047	8.41
	27 August	28	45	73	0.616	0.057	
Okwenya	21 July and 5 Aug.	13	27	40	0.675	0.073	2.33
	18 Aug. to 1 Sept.	7	37	44	0.841	0.055	

Admittedly, the figures from Okwenya are not statistically significant, but had the sample been slightly larger it seems not unlikely that they, too, would have shown a real difference. It appears, therefore, that the population of this species, and presumably many others as well, is in a freely fluctuating state with regard to its age make-up.

This particular method of age-grouping is based on the ratio between the very young and the entire rest of the population; it must therefore be particularly susceptible to the influence of the arrival in the population of newly-emerged females. It is to be presumed that this is just what the figures in Table 8 illustrate, namely, that with the production of suitable breeding sites at the Coast and Matse with the advent of the rains in March and August there was a significant increase in the proportion of the population that was very young, i.e. less than a few days old. This is exactly what one would expect and the decrease in the proportion parous at these two places presumably does not necessarily indicate that there was also an increase in the daily mortality.

5. CONCLUSIONS

From the discussion above, four important conclusions emerge:

1. that it may be misleading to derive figures for "p" without an exact knowledge of the population structure younger than four days old;
2. that, by the very nature of the calculation, the figure for "daily mortality" will be heavily influenced by the influx of newly-emerged females into a previously stable population;
3. that it is dangerous to perform dissections over a short period (less than a week) and assume that the results so obtained reflect the over-all long-term picture;
4. that, conversely, it is dangerous to assume that, because dissections performed over a long period (several weeks) appear fairly constant, there is no fluctuation in population age make-up.

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