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Observations on the Determination of the Age Composition and Epidemiological Importance of Populations of *Anopheles gambiae* Giles and *Anopheles funestus* Giles in Tanganyika

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Following a request from the World Health Organization an investigation was undertaken to determine whether the method developed by Polovodova in the USSR for determining the physiological age of female anophelines could be applied to the two main vectors of malaria in Africa, Anopheles gambiae *and* Anopheles funestus.

It was demonstrated that Polovodova's method was fully applicable to these African species. The greater difficulties encountered in determining the age of these species, as compared with A. maculipennis, *are due to the smaller size of* A. gambiae *and* A. funestus *and to the paucity of ovarioles in which follicular degeneration has occurred.*

Among the mosquitos examined during a short-term investigation were individuals that had completed up to nine ovipositions. Among the mosquitos infected with sporozoites were females that had completed between three and seven ovipositions. Observations on the numbers of eggs developing in gravid females showed that, in these African species, there was no clear-cut fall in fecundity with age, such as occurs in A. maculipennis.

Polovodova's (1949) method has been widely used in the Soviet Union for the study of various aspects of the biology of mosquitos and subsequently of other blood-sucking Diptera, for determining the age composition of the insect populations concerned, and for assessing the efficacy of the control measures carried out. The method has proved its worth in every case (Detinova, 1962).

In tropical countries the method has so far been comparatively little used. For Africa, in particular, there are no published data, beyond the generalized report of Lebied (1959), to indicate the possibility of using Polovodova's method to study the very important vectors of malaria, *Anopheles gambiae* and *Anopheles funestus*.

This paper embodies the results of short-term observations on the age composition of the two main malaria vectors in Tanganyika. The studies, conducted following a request from, and with the assistance of, the World Health Organization, were made from 3 to 21 October and from 4 to 11 November 1962 in the Muheza district near the East African coast at Tanga, and from 23 to 29 October 1962 at Gonja in South Pare district further inland.

At Muheza no mosquito control measures were in force. There are virtually no domestic animals in the area and both *A. gambiae* and *A. funestus* are known to be highly anthropophilic. In the villages round Gonja in the period 1955-59, i.e., three-and-a-half years before the start of the present investigations, house-spraying with dieldrin had been carried out. As a result of these measures *A. funestus* had apparently disappeared. In this district there are many cattle, which are kept at night-time in the immediate vicinity of the houses.

At Muheza mosquitos were caught for dissection on 13 days and at Gonja on six days. During the intervening days the females caught on the previous occasions were examined. Altogether, 847 *A. funestus*, 556 *A. gambiae*, 25 *A. machardyi* and 19 *A. coustani* (*s.l.*) and 4 *A. pharoensis* were dissected.

The mosquitos were caught in huts and, in the Pare area only, occasionally in cattle sheds. Space-

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spraying with pyrethrum was employed. The paralysed mosquitos were kept in the refrigerator and remained easy to dissect for two, and sometimes even three, days after capture.

At the same time as the physiological age was determined the number of eggs maturing in individuals in Sella's stages 4-7 (gravids) was also counted, so as to determine the fertility in a single oviposition. In females in the pregravid stage (Gillies, 1954), the presence or absence of mating plugs or of sperms in the spermatheca was recorded. In females that had completed three or more ovipositions the salivary glands were examined for the presence of sporozoites.

RESULTS OF DISSECTION

In determining the physiological age of *A. gambiae* and *A. funestus* we encountered the same difficulties as those usually met with in examining mosquitos of other species. The greatest difficulties, of course, occur in determining the age of females that have just oviposited or in those with fuily developed ovaries. In the first case the ovarioles from which the mature eggs have been passed are still in the form of sacs, and it is impossible therefore to judge the age of the females from them. Even when dilatations are beginning to be formed, the stalks of the ovarioles easily rupture when stretched and part of the sac that has begun to contract comes away with the next follicle. In such a case an inexperienced observer may make a false diagnosis and place the specimen in the uniparous group.

In the second case, when the ovarioles are stretched, the tunica investing the mature egg usually ruptures and it is difficult to determine the number of dilatations on the ovariolar stalk.

The difficulty of determining the physiological age of female *A. gambiae* and *A. funestus*, compared with *A. maculipennis* and also with some African species such as *A. coustani* (*s. l.*) and *A. machardyi*, is in many cases heightened by the fact that, in a high proportion of individuals of the first two species, the ovaries contain few or no ovarioles in which degeneration has occurred of follicles that had already begun to develop. Such ovarioles are, of course, of great value in determining the physiological age of females at all stages of ovarian development. It is of interest that, in those *A. gambiae* and *A. funestus* in which degeneration has occurred, individuals may be found with many degenerating follicles, even in extreme cases up to 25% or 50% of ovarioles being in this condition.

Physiological age is most easily determined in females of *A. gambiae* and *A. funestus* with follicles in Christophers' stage IV. By that time the process of formation of dilatations in the terminal part of the ovarioles after the passage of the mature eggs from the previous gonotrophic cycle is nearing completion. However, in view of the need to obtain as complete data as possible on the age composition of whole populations, it is essential to dissect females collected at all stages of blood digestion and ovarian development.

Since the total number of mosquitos examined was small and dissections were carried out during a very short period, it cannot be claimed from our results that the true age composition of the *A. gambiae* and *A. funestus* populations was determined. We can merely judge the age at which individuals were found in the populations during our work. The results of dissections are shown in Table 1.

As will be seen from Table 1, of the female *A. gambiae* dissected after capture, both at Muheza and Gonja, the oldest were individuals that had completed six ovipositions, i.e., that were in their seventh gonotrophic cycle. Among the considerably greater number of *A. funestus* dissected were some that had completed a larger number of ovipositions. The oldest were females in their tenth gonotrophic cycle. It proved impossible to establish the age of some

TABLE 1

PHYSIOLOGICAL AGE OF FEMALE *ANOPHELES GAMBIAE* AND *ANOPHELES FUNESTUS* CAPTURED INDOORS

Species	District	Total No. of females dissected	Unfed females (Sella 1)	Pre-gravid	Nulli-parous	No. of dilatations among parous females									
						1	2	3	4	5	6	7	8	9	?
A. funestus	Muheza	847	33	109	166	118	168	99	78	23	7	5	1	3	37
A. gambiae	Muheza	142	4	23	43	26	22	6	5	2	1	0	0	0	10
A. gambiae	Gonja	414	0	59	117	95	94	34	7	0	1	0	0	0	7

females, there being a predominance in this group of individuals with developed ovaries in which no ovarioles with degenerating follicles were found.

The absence of physiologically very old females among both species can, in our opinion, be explained in two different ways: being due either to the small number of dissections, among which there was little probability of encountering females of a higher physiological age, or to a high mortality among parous females. The proportion of nullipars in *A. gambiae* was higher than in *A. funestus*. The data we obtained on the percentage of nulliparous *A. gambiae* in the Gonja district during this short period of observation were in full agreement with data one of us (M.T.G.) had obtained during the six months preceding our joint observations (Table 2). The high percentage of nulliparous females in the population points not only to continuous large-scale breeding of mosquitos but also to a high mortality among those that have emerged.

We also determined the fertilization rate in females in the pregravid stage. To establish this, the common oviduct was examined for the presence of mating plugs (Gillies, 1956) and the spermatheca for the presence of sperms. As a result we were able to determine:

(1) the time of copulation in newly emerged females, and

(2) the link between copulation and biting activity in females of the species concerned.

TABLE 2

PERCENTAGE OF NULLIPAROUS FEMALES AMONG ALL *ANOPHELES GAMBIAE* EXAMINED IN GONJA DISTRICT BETWEEN MAY AND OCTOBER 1962

Month	Total No. dissected	Percentage nulliparous [a]
May	126	41.5
June	131	43.9
July	100	38.5
August	137	48.3
September	310	34.9
1-18 October	123	35.7
23-29 October [b]	414	32.8

[a] Excluding pregravid females.
[b] Present series.

TABLE 3

RATIO BETWEEN FERTILIZED AND UNFERTILIZED FEMALES IN THE PREGRAVID STAGE

Species	No. examined	No. fertilized	No. unfertilized
A. gambiae	59	25	34
A. funestus	96	61	35

Our data for *A. funestus* fully agreed with those obtained earlier (Gillies, 1955), but in the case of *A. gambiae* from Gonja they differed considerably from earlier work at Muheza. It is probable that the figure under consideration may fluctuate considerably under the influence of various internal and external factors. The data obtained are given in Table 3 and show that biting activity in *A. gambiae* and *A. funestus* begins independently of copulation.

In examining parous females in Sella's stages 2 and 3 (fed females) we noted the condition of the ovariolar tubes and on that basis assessed the time that had elapsed between oviposition and a new blood meal. This method was devised by Detinova (1953) and used for determining the length of the gonotrophic cycle in *A. maculipennis*. The data obtained for *A. gambiae* and *A. funestus* are given in Table 4. Hamon et al. (1961) found varying degrees of contraction of the ovariolar tubes of these species. Gillies & Wilkes (1963), in a study of the condition of the ovarioles in *A. funestus* at Muheza, came to the conclusion that in this species the gonotrophic cycle lasted three days throughout

TABLE 4

CONDITION OF THE DISTAL PORTION OF THE OVARIOLES IN UNFED FEMALES AND FRESHLY FED FEMALES OF *A. GAMBIAE* AND *A. FUNESTUS*

Species	Sella stage	Total No. of females examined	Condition of ovarioles		
			Sacs	Beginning of constriction	Dilatations formed
A. funestus	1	6	3	0	3
A. gambiae	1	1	0	0	1
A. funestus	2	41	9	12	20
A. gambiae	2	13	0	2	11
A. funestus	3	126	32	27	67
A. gambiae	3	56	3	14	39

TABLE 5
NUMBER OF DEVELOPING EGGS IN RELATION TO PHYSIOLOGICAL AGE

Species	Physiological age	No. of females examined	Mean No. of developing eggs	Standard error	Range of individual variation in the No. of developing eggs
A. funestus	Nullipars	42	114.2	±4.5	54-163
	1-par	48	126.6	±2.9	88-171
	2-par	56	114.2	±2.7	71-166
	3-par	20	117.0	±3.9	84-152
	Over 3-par	16	116.1	±5.6	77-163
A. gambiae	Nullipars	51	159.7	±6.2	66-275
	1-par	21	152.9	±6.6	107-210
	2- and 3-par	11	140.7	±7.8	101-190

the year. At Gonja unpublished observations have indicated that *A. gambiae* there has a three-day cycle. Data for this species at Muheza are as yet incomplete.

The data given in Table 4 show that *A. gambiae* and *A. funestus* may take a new blood meal either soon after oviposition or after a longer period. These findings are of importance in determining the calendar age of females found with sporozoites in their salivary glands.

RELATIONSHIP BETWEEN AGE AND FECUNDITY

As remarked above, the ovaries in the majority of *A. gambiae* and *A. funestus*, even in physiologically old individuals, contained only a very small number of ovarioles with degenerating follicles. In those with ovaries in stages IV and V of development we counted the number of developing eggs at the same time as the physiological age was determined, so as to establish the relationship between fecundity and age. The results are given in Table 5. Although further material must be collected before final conclusions can be drawn, it appears from the table that there is no clear-cut fall in fecundity with age in *A. gambiae* and *A. funestus* such as was observed in *A. maculipennis* (Detinova, 1955).

Considerable differences in the number of developing eggs were noted in different individuals of the same age. As will be seen from Tables 6 and 7, the differences are mainly dependent on the dimensions of the insect body. The data show that *A. gambiae* females produce more eggs than *A. funestus*. This is due to the larger size of the former species.

RELATIONSHIP BETWEEN PHYSIOLOGICAL AGE AND INFECTION WITH SPOROZOITES

As stated above, in all females found to have completed three or more gonotrophic cycles, the salivary glands were examined for the presence of sporozoites. The results of such examinations of *A. funestus* are given in Table 8. Although the total number of mosquitos examined was small, we discovered one infected female that had completed three gonotrophic cycles, and the majority of the infected insects had completed four or five ovipositions. Since females of these ages were encountered quite frequently among those dissected, it can be assumed that these age-groups are epidemiologically the most important in relation to malaria transmission.

Among the group under consideration, two individuals were found that had completed five ovipositions each and were infected with fully developed

TABLE 6
RELATION OF FECUNDITY TO WING SIZE AND PHYSIOLOGICAL AGE IN *A. FUNESTUS*

Length of wing (mm)	No. of females examined	Mean No. of developing eggs			
		Nullipars	1-par	2-par	3-par and over
2.56-2.75	34	139.1	144.2	130.0	128.7
2.36-2.55	71	115.4	120.9	114.9	116.4
2.11-2.35	23	83.6	116.8	92.4	94.7

TABLE 7

RELATION OF FECUNDITY TO WING SIZE AND PHYSIOLOGICAL AGE IN *A. GAMBIAE*

Length of wing (mm)	No. of females examined	Mean No. of developing eggs		
		Nullipars	1-par	2-par and over
3.31-3.60	12	198.5	166.0	190.0
3.11-3.30	44	153.3	158.3	137.3
2.81-3.10	22	133.5	138.7	132.3

filarial larvae. Two other females, that had completed three and five ovipositions respectively, contained developing filarial larvae (" sausage " stage).

Among 14 *A. gambiae* in the age-groups under consideration and captured in the Muheza district, two were found to be infected with sporozoites. One had completed three and the other six ovipositions. No infected females were found in the Gonja district.

TABLE 8

RESULTS OF EXAMINATION OF *A. FUNESTUS* FEMALES FOR SPOROZOITE INFECTION

	No. of dilatations in the ovarioles						
	3	4	5	6	7	8	9
No. of females dissected	95	64	24	7	5	1	3
No. infected with sporozoites	1	4	2[a]	1	1	0	0
Percentage of infected individuals	1	6	9	—	—	—	—

[a] In addition, one positive female from special gland dissections was found to have 5 dilatations.

Theoretical calculations also confirm that individuals that have completed three ovipositions should be included in the epidemiologically important group. In view of the length of the gonotrophic cycle and of the pregravid stage, it will be seen that the calendar age of females in the fourth cycle is 10-13 days, while the duration of the cycle of sporogony in *Plasmodium falciparum* is about 12 days.

Dissection disclosed females found to have taken a second blood meal in the course of one gonotrophic cycle (16 *A. funestus* and 5 *A. gambiae*). The epidemiological importance of such females is naturally greater.

CONCLUSIONS

1. It was established that Polovodova's method of determining the physiological age of mosquitos is fully applicable to *A. gambiae* and *A. funestus*. Age determination in these two species proved to be somewhat more difficult than in the case of *A. maculipennis* and some other anopheline species.

2. The difficulties in determining the physiological age of female *A. gambiae* and *A. funestus* are due in the first place to the small size of the body and ovaries, and in the second place to the absence or paucity in many females of ovarioles with degenerating follicles.

3. Of the *A. gambiae* dissected the oldest physiologically were individuals that had completed six ovipositions, and among *A. funestus* nine ovipositions.

4. Among mosquitos infected with sporozoites were individuals that had completed between three and seven gonotrophic cycles.

5. Observations on the number of eggs developing in gravid females showed that, in these African species, there was no clear-cut fall in fecundity with age, such as occurs in *A. maculipennis*.

RÉSUMÉ

La méthode de Polovodova pour la détermination de l'âge physiologique des anophèles est largement utilisée en Union soviétique. L'on sait maintenant de façon certaine que cette méthode est pleinement applicable aux moustiques africains, *Anopheles gambiae* et *A. funestus*.

Les difficultés de détermination de l'âge physiologique de *A. gambiae* et *A. funestus* femelles sont dues tout d'abord à la petitesse du corps et des ovaires, ensuite à l'absence ou au petit nombre, chez de nombreuses femelles, d'ovarioles présentant une dégénérescence folliculaire.

Parmi les *A. gambiae* disséqués, les femelles physiologiquement les plus âgées avaient effectué 6 pontes; parmi les *A. funestus* elles avaient effectué 9 pontes.

Parmi les moustiques infectés par les sporozoïtes certains avaient accompli 3 à 7 cycles trophogoniques.

Une étude portant sur le nombre d'œufs formés chez la femelle gravide a montré que dans ces espèces africaines l'âge n'entraîne pas, comme chez *A. maculipennis*, une chute nette de la fécondité.

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