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METHODS OF DETERMINING THE PHYSIOLOGICAL AGE OF FEMALE *ANOPHELES*

by

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Determination of the physiological age of female anophelines is important both for the epidemiological study of transmission and for evaluation of the efficacy of insecticide-spraying operations.

Different methods have been proposed by Mer, Polovodova, Detinova, Gillies & Lewis for the purpose of distinguishing parous from nulliparous females, including one method which even makes it possible to determine the exact number of gonotrophic cycles.

For use in the usual conditions in a malaria control pilot zone, a method for the determination of physiological age must be simple, fairly rapid and capable of producing the same results when the observations are made by different workers. It must also permit dissection of salivary glands, and possibly ovaries, in order to establish the rate of infection on the same material.

Determination of the exact number of gonotrophic cycles by counting the number of dilatations on the funiculi of the ovarioles takes too much time and requires too much dexterity to be employed as routine practice in the field. In the Bobo Dioulasso pilot zone we tried simultaneously - as a means of detecting parous females - the qualitative change in the ampullae and in the common oviducts (Detinova-Polovodova-Gillies), the structure of the funiculi of the ovarioles after "teasing out"[1] the ovary (Lewis), and the shape of the tracheoles inside the ovary

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[1] "Teasing out" appears to be the nearest English equivalent to "dilacération" as used by the authors.

(Detinova-Polovodova). The ovaries were extracted very carefully by one of us (Chauvet), and identified as parous or nulliparous; one of them was placed in distilled water and the tracheoles were examined (dry) by another member of our team (Thélin); the other ovary was teased out in saline solution and examined, fresh, by the third member of the team (Hamon). Each female was given a number so that the results could be compared after each dissection session. The results of the comparison may be summarized as follows:

There was not very much difference in the percentages obtained for parous females but if the diagnoses are examined one by one it is noted that those females which are classified as parous on the basis of the form of the tracheoles are nearly always also considered as parous on the basis of the structure of the funiculi of the ovarioles, and that those classified as nulliparous on the basis of the form of the tracheoles are also classified as nulliparous on the basis of the structure of the oviarole funiculi. On the other hand, 10% of the diagnoses based on the appearance of the ampullae and common oviducts do not tally with those based on the tracheoles and ovariole funiculi. On several occasions it was found that ovaries with all the characteristics of those of a parous female were, in fact - on the basis of the tracheoles and ovariole funiculi - nulliparous in one specimen and parous in another. As a means of distinguishing between parous and nulliparous females, therefore, the morphology of the ampullae and oviducts does not seem to be very reliable. The other two techniques seem to be about equally reliable but the tracheole technique cannot be applied beyond ovarian stage II, whereas examination of the funiculi can still be effected in fairly good conditions in stage III. Furthermore, by the method of teasing out ovaries for immediate examination of the funiculi, only 12-15 anophelines can be examined in an hour, particularly if there are numerous nulliparous specimens, whereas 50-60 specimens can be subjected to the tracheole test in an hour (not including extraction of ovaries).

On the basis of this study, the technique adopted in the Bobo Dioulasso pilot zone is as follows: the female anophelines are identified, the salivary glands are extracted for the detection of sporozoites, and the ovaries are also removed and passed to the entomologist in a drop of normal saline. After brief examination under the binocular lens, ovaries at stages I, IIj, IIm and part of stage IIa[1]

[1] The authors subdivide the stage II of the ovary into II young (jeune), II middle (moyen) and II late (agé). (Editor's remark)

are placed in a drop of distilled water for examination after desiccation. Most of the stage II and all of the stage III ovaries are examined by teasing out under the binocular lens, and the structure of the funiculi of the ovarioles are studied under the microscope. With this technique, 150-300 females can be examined in a morning (4-1/2 hours); the tracheoles can be examined in the afternoon in about an hour. The difficult point is to distinguish between the ovaries to be teased out and those to be left to dry. In some parous females containing a few eggs retained from the last oviposition, the tracheoles still show signs of the "skeins" that are characteristic of nulliparous females. It is therefore advisable to determine, with respect to each specimen, up to what stage of ovarian development the tracheoles of a nulliparous female can be clearly differentiated from those of a parous female. Interpretation of the tracheoles generally becomes doubtful in the middle of stage IIa.

In view of the fact that it takes longer and is more difficult to carry out and read the "teasing out" than the "tracheole" test, we established, over a long series of dissections, the percentage of parous females when the tracheole test alone was applied (females with ovaries in stages I, IIj, IIm and part of IIa), and when the "tracheole" and "teasing out" tests were both applied (females in stages III and part of IIa). It is quite clear (Table 1) that the teasing out method gives a far higher percentage of parous females than does the tracheole method, but the percentage of females at an advanced ovarian stage being low in our material (female anophelines taken on human bait during the night), the tracheole test alone gave a percentage of parous females very similar to that for the total population.

Our estimates of the percentage of parous females were made with females taken during the night because this provided us with a good sample of the anopheline population in contact with man and with females with ovaries at stages which lend themselves to the technique for the determination of physiological age. Night capture is, however, a costly and often unproductive proceeding, and we therefore tried to use the same method with females from temporary shelters in the zone treated with DDT. With this method, unfed or freshly-engorged females have to be used because in the others the ovaries are at too advanced a stage (IV or V). Here, however, there is a sampling problem: are the unfed and freshly-engorged specimens representative of the total population? Table 2 indicates, with respect to A. funestus and A. flavicosta, the percentages of parous females obtained in

October 1959 with, respectively, females captured during the night, and unfed or freshly-engorged females taken in temporary shelters, all the specimens being obtained from the same villages in the DDT-treated zone. There is a definite discrepancy in the results with regard to A. funestus, the females from the temporary shelters giving a much larger proportion of nulliparous specimens than the females captured during the night. This factor is probably not due only to non-examination of pregnant females, because the sporozoite rates for A. funestus females captured during the night are higher than for A. funestus captured in the temporary shelters, whereas with A. gambiae this factor is practically absent - (Table 3). The percentages of parous females cannot therefore be established without any regard to the manner in which the females are captured and it follows that the sporozoite rates may sometimes vary according to the method of capture - which is of the greatest importance in the organization of entomological control of malaria eradication campaigns.

In some night captures, the number of females for examination being very small, they were all teased out in order to ascertain exactly what ovarian stage the females took their blood meal and, with respect to parous females, in order to discover whether they had deposited their eggs during the night of the meal (presence of a residual sac) or one of the preceding nights (presence of a dilatation). The results of this study are given in Table 4. It will be seen that although there is no strict rule, a large proportion of A. gambiae and A. funestus take their blood meal on the night of the oviposition, whereas the majority of A. nili and A. coustani do not take a blood meal on the night of the oviposition. It will also be noted that a considerable proportion of females bite at the IIa stage or an even more advanced one. However, the ovaries of females which lay as soon as their eggs are ripe are nearly always at the IIm stage, whereas a large proportion of those which lay 24 hours after their eggs are ripe have ovaries at the IIa stage. It is therefore probable that, in nature, a certain number of females do not lay until 24 or 48 hours after their eggs have matured. These two phenomena combined mean that in nature the lapse of time between two successive ovipositions is certainly on an average considerably longer than the duration of the gonotrophic cycle. All the "night-capture" females were studied for the purpose of ascertaining the percentage of parous females in relation to the hour of attack. Table 5 summarizes our observations with respect to A. funestus, A. nili and A. coustani. There are some very marked variations according to the hour, due in many cases to the small number of specimens examined in each period. Grouping of the catches by three-hour periods from sunset to sunrise, makes the variations less marked. Although the deviations are not generally significant (except in the case of A. coustani) it seems fairly clear that parous females are more numerous in the second than in the first half of the night. It is therefore possible that catches made during a part of the night do not constitute a satisfactory sample of the anopheline population as a whole.

All the females caught at night were also studied to obtain the percentage of parous females in relation to the zone of capture (pilot villages or DDT-treated zone). The results, summarized in Table 6, show a marked drop in the proportion of parous females in the DDT-treated zone as compared with those in the demonstration area, the deviations noted being nearly always significant. Unfortunately, the percentages of A. coustani parous females show almost as marked a difference between demonstration area and DDT-treated zones as do A. funestus and A. nili, although A. coustani practically never enters the houses in the region in question and it is difficult to imagine that its death-rate is affected to such an extent by the DDT-spraying of the houses. This again shows that the question of sampling is of paramount importance and that it must be clarified by thorough investigation before full confidence can be placed in this technique for the evaluation of the effect of insecticide spraying on the vector anopheline population.

The various studies referred to above are not completed, and the results are given merely as an indication of the many problems that arise. The conclusions are therefore only tentative.

TABLE 1. PERCENTAGE OF PAROUS FEMALES OBSERVED AMONG NIGHT CAPTURES IN RELATION TO STAGE OF OVARIAN DEVELOPMENT

Species and zone	Exam. technique and ovary stage	No. females examined	No. parous females	% parous females	95% confidence interval
A. gambiae Demonstration villages	Teasing out St. IIa & +	15	12	80	58-100
	Tracheoles St. I-IIa	58	49	84.5	75-94
	Teasing out and tracheoles	73	61	83.6	75-92
A. funestus Demonstration villages	Teasing out St. IIa & +	42	38	90.5	75-100
	Tracheoles St. I-IIa	196	119	60.7	54-68
	Teasing out and tracheoles	238	157	66.0	60-72
A. nili DDT-treated villages	Teasing out St. IIa & +	221	97	44	37-51
	Tracheoles St. I-IIa	446	147	33	29-37
	Teasing out and tracheoles	667	244	37	35-41
A. coustani DDT-treated villages	Teasing out St. IIa & +	56	43	77	66-88
	Tracheoles St. I-IIa	411	201	49	44-54
	Teasing out and tracheoles	467	244	52	47-57

TABLE II. PERCENTAGE OF PAROUS FEMALES OBSERVED IN DDT-TREATED VILLAGES IN THE PILOT ZONE OF BOBO DIOULASSO, IN RELATION TO THE METHOD OF CAPTURE (OCTOBER 1959)

Species	Mode of capture and nutritional state	No. females examined	No. parous females	% parous females	95% confidence interval
A. funestus	Night captures Fasting females	30	14	47	28.4-65,6
	Temporary shelters Fasting females	162	24	14.8	9.2-20,4
	Temporary shelters Engorged females	21	11	52	30-74
	Temporary shelters All non-pregnant females	183	35	19.1	13.3-24,9
A. flavicosta	Night captures Fasting females	41	31	75.6	62.0-89,2
	Temporary shelters Fasting females	37	23	62	46-78
	Temporary shelters Engorged females	16	13	81	61-100
	Temporary shelters All non-pregnant females	53	36	67.9	54.9-80,9

TABLE III. COMPARATIVE SPOROZOITE RATES FOR FEMALES CAPTURED DURING THE NIGHT AND FEMALES CAPTURED IN TEMPORARY SHELTERS IN DDT-TREATED VILLAGES OF THE BOBO DIOULASSO PILOT ZONE FROM JUNE 1958 TO OCTOBER 1959

Species	Mode of capture	No. females dissected	No. females infected	Sporozoite rate	95% confidence interval
A. gambiae	Temporary shelters	1 186	3	0.252	0.052-0.739
	Night captures*	922	4	0.434	0.118-1.111
A. funestus	Temporary shelters	16 721	9	0.054	0.024-0.102
	Night captures	1 055	7	0.664	0.266-1.366

*The village of Sinorosso is excluded as it is too close to the dieldrin-treated villages where infected *A. gambiae* are common.

TABLE IV. DISTRIBUTION OF OVARIAN STAGES AND OF "SACS" AND "DILATATIONS" IN FEMALE ANOPHELINES CAPTURED ON HUMAN BAIT DURING THE NIGHT, FROM JUNE TO SEPTEMBER 1959

Ovarian stages, parous or nulliparous, etc.	No. females with these characteristics			
	A. gambiae	A. funestus	A. nili	A. coustani
Parous with dilatations				
III	3	1	14	3
IIa	4	5	7	13
IIm	0	0	5	12
Parous with sacs				
III	4	5	5	1
IIa	11	13	7	8
IIm	9	11	6	11
IIj	1	0	0	2
Nulliparous				
III	3	5	15	4
IIa	6	2	16	5
IIm	8	5	40	19
IIj	5	4	3	12
I	2	3	3	2
Total parous females with sacs	25	29	16	22
Total parous females with dilatations	7	6	26	28
TOTAL PAROUS FEMALES	32	35	42	50
TOTAL NULLIPAROUS FEMALES	24	19	77	42

TABLE V, DISTRIBUTION OF PAROUS FEMALES OVER VARIOUS PERIODS OF THE NIGHT DURING CAPTURE ON HUMAN BAIT FROM JUNE TO OCTOBER 1959 IN THE BOBO DIOULASSO PILOT ZONE

Period of Time (hour)	Number of females examined and percentage parous					
	A. funestus		A. nili		A. coustani	
	No.	% parous	No.	% parous	No.	% parous
18 - 19	3	0	14	0	51	47
19 - 20	3	67	26	31	55	51
20 - 21	3	67	68	38	36	50
21 - 22	10	30	95	34	68	54
22 - 23	6	83	99	33	78	47
23 - 24	17	65	94	40	56	54
00 - 01	18	72	95	34	44	55
01 - 02	45	73	97	53	75	57
02 - 03	41	66	55	35	51	67
03 - 04	50	70	81	42	50	68
04 - 05	70	70	79	43	39	72
05 - 06	33	58	54	39	32	63
18 - 21	-	-	108	31.5	142	49.3
standard deviation				4.5		4.2
21 - 24	-	-	288	35.8	202	51.5
standard deviation				2.8		3.5
18 - 24	42	54.8	-	-	-	-
standard deviation		7.8				
00 - 03	104	70.2	247	41.3	170	59.4
standard deviation		4.5		3.1		3.6
03 - 06	153	67.3	214	41.6	121	67.8
standard deviation		3.8		3.4		4.3
18 - 06	299	66.6	857	38.3	635	56.2
standard deviation		2.7		1.7		2.0

N.B. A. funestus: demonstration villages; A. nili and A. coustani: DDT zone

TABLE VI. PERCENTAGES OF PAROUS FEMALES OBSERVED IN THE DDT-TREATED ZONES AND DEMONSTRATION VILLAGES OF THE BOBO DIOULASSO PILOT ZONE FROM JUNE TO OCTOBER 1959

Species	Zone of capture	No. females dissected	Percentage parous females	95% confidence interval
A. gambiae	Demonstration villages	155	74.1	67.1-81.1
	DDT zone	70	41.4	29.6-53.2
A. funestus	Demonstration villages	300	66.7	61.3-72.1
	DDT zone	89	48.3	37.7-58.9
A. nili	Demonstration villages	135	53.3	44.7-61.9
	DDT zone	858	38.3	34.7-41.9
A. coustani	Demonstration villages	26	73.1	55.3-90.9
	DDT zone	637	56.2	54.2-58.2