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The Chief of the Malaria Section
has the honour to communicate hereunder the
following note:

THE PROBLEM OF EXOPHILY IN
ANOPHELES GAMBIAE

by

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Summary

Analysis of outside population of *Anopheles* should be made primarily in terms of the relative opportunities of resting indoors or out of doors, and secondly according to the degree of ovarian development. Thus exophily may be described as obligatory in uninhabited regions, facultative in partly built-up areas where feeding takes place outside, or as deliberate where feeding occurs indoors but where the mosquitos afterwards adopt outside resting sites.

Studies in a humid coastal, and in an arid inland, region of Tanganyika gave very different results. In the former well vegetated area, catches of *A. gambiae* were scanty and largely composed of unfed and gravid females. In the latter area, where suitable resting sites were limited, large numbers of mosquitos were caught outside and a great many of them were recently fed females. The differences are attributed primarily to the presence outside of large herds of cattle in the inland region, and to their absence near the coast.

The reports of exophily from other parts of Africa are analysed in a similar manner. This information, together with the new data from East Africa, shows that there is much variation in the behaviour of *A. gambiae* in different regions. Some of the variation

can be explained in terms of environmental differences, particularly in the availability at night of different hosts. But some of it may be genetically determined. It is suggested that the main task in this field is twofold. Firstly to establish the existence and nature of the behaviour differences described. Secondly to study in detail the mosquitos that survive as exophilic populations in areas where systematic house spraying is in operation.

In recent years increasing attention has been paid to the occurrence of outside resting populations of *Anopheles* in many parts of the world. It is the object of this communication to report on recent work on the subject in East Africa and to discuss the problem in connexion with *Anopheles gambiae* throughout Africa.

Types of Exophily

As defined by Senior-White,¹⁵ exophily implies a tendency for a mosquito to rest outside, rather than in man-made shelters, during the daytime. This may come about through a variety of circumstances, depending on the condition of the ovaries of the mosquitos, and the whereabouts of the host at night. If feeding takes place outside, then the relative availability of houses or natural sites is important; and all these factors may be quite independent of any inherent behaviour characteristics. What one wants to know therefore is, at what stage in the gonotrophic cycle are the mosquitos found outside? Is the preferred host under shelter at the time when most of the mosquitos are feeding? Are the houses or animal shelters suitable as resting sites in the daytime and, if feeding takes place outside, are they adjacent to the nocturnal habitat of the host?

The concept of opportunity as a factor in influencing the mosquito's choice of resting site is an obvious one. But, in the author's opinion, it is one that needs to be emphasized, particularly when one is concerned with possible inherent behaviour differences between separate population groups of the same species. Thus the sets of conditions that may lead to exophily can be classified into three groups:

(i) Obligatory

Where no houses exist in an area, it is obvious that all mosquitos are exophilic. The occurrence of A. culicifacies in uninhabited jungle in Ceylon¹⁴ may be quoted as an example.

(ii) Facultative

This is the situation found when one of the preferred hosts is outside but where human or animal shelters are plentiful in the vicinity. An instance of this has been observed in parts of the South Pare District of Tanganyika, where the cattle are kept on the edge of villages in open enclosures. After feeding, mosquitos such as A. gambiae appear to fly to the nearest convenient resting site, which may be either a natural one or man-made. Thus the preference shown is primarily influenced by ecological factors.

(iii) Deliberate - Type A. (Endophagic of Senior-White)

This is perhaps the most important aspect of the subject and probably represents the idea in many people's minds when referring to exophily in malaria vectors. Essentially it describes the position where feeding takes place inside the house or animal shelter, where apparently suitable resting sites within the house are available, yet where the mosquitos leave the shelter for natural harbourages before daylight. A. maculatus in Malaya and A. aquasalis in the West Indies are well known examples of this type of behaviour.

Type B. (Exophagic of Senior-White)

A second type of deliberate exophily is shown by those mosquitos that feed mainly out of doors and tend to avoid using houses as resting sites even when they are available. A. coustani (s.l.) and A. pharoensis fall into this category.

One should also take into account the different stages of gonotrophic development of the mosquitos found outside. These stages may be divided up as follows:

(i) Unfed females. Those females with neither blood nor eggs will either be newly emerged or else older females resting outside after the act of oviposition. In neither case will their previous activity have had any necessary connexion with houses and their presence outside is of minor interest.

(ii) Fed females. Those that have recently fed are, epidemiologically speaking, of much greater interest. The presence of such females, gorged with human blood, belonging to a species that bites mainly after the inhabitants have retired for the night, is a sure indication that deliberate exophily is occurring. In making comparisons between one area and another in search of differences within a species, it is on the behaviour of fed females that attention should be mainly directed.

(iii) Gravid females. Those with fully, or nearly fully, developed ovaries may be derived from any of three sources: (a) They may be those that, earlier in the cycle, have fed outside without ever having entered a house. (b) They may be those that fed indoors but left again immediately afterwards; where the gonotrophic cycle lasts 48 hours, they will already have spent some 36 hours resting outside. (c) They may be those that fed and rested indoors and have only left the houses half-way through the gonotrophic cycle. In this last instance the presence of gravid females outside may have quite different implications from the similar finding of recently fed mosquitos. And one may be unable to determine their origin without additional data on the exodus of half-gravid females from huts. Thus it is evident that caution should be shown in interpreting the finding of gravid females outside.

(iv) Males. It has been stated from time to time that the presence of males in outside shelters can be regarded as the criterion of an exophilic species. Such a conclusion is hard to accept. If the reactions of females towards houses vary according to the stage of ovarian development, it would appear most unsound to assume that there will be no differences between the sexes. It seems to be true, at least in the author's experience, that one does not normally find in houses males of species, the females of which are largely exophilic at all stages of the gonotrophic cycle. But conversely, the presence of males in outside shelters in no way indicates that one will find females. The responses of males towards houses as resting sites may in some instances coincide with those of the females. But in general, their choice will be dictated by the site of their previous activity, which will either have been emergence, swarming or possibly nectar feeding. If these

activities take place near houses, such as the swarming of A. gambiae that occurs in the middle of villages on the East African coast, then some may tend to rest indoors. But if they are at some distance from houses at daybreak, it is obvious that the adoption of natural shelters has no significance as such.

A further point should be noted. The composition of the outside resting population, in terms of ovarian stages, will vary according to the location of the resting sites. Mosquitos caught near houses, or near the host, may contain relatively large numbers of fed females. Those found at some distance from houses or near the breeding sites will be largely composed of unfed and gravid females, as shown by work already reported,⁶ and by the results recorded here. Therefore an attempt should always be made to sample the outside resting population in as great a number of resting sites as possible so as to obtain a reliable picture of its overall composition. It must be confessed that this condition may be a difficult one to satisfy. For instance, mosquitos resting near houses or animal shelters may be relatively concentrated and easy to find, while those nearer the breeding sites are often scattered over a wider area. Collections from the former source may therefore tend to predominate although the latter may be numerically more important.

Recent work in East Africa

Studies on this problem have been carried out in two separate areas of Tanganyika. The findings in the two places have been very different. The first district lay in the humid sub-coastal belt with a rainfall of 40-50 inches. The country is well vegetated and tall grasses cover the uncultivated areas for most of the year and render walking in any direction except along native paths a slow and uncomfortable affair. These are difficult conditions under which to look for natural resting sites, and it is not surprising perhaps that very meagre results were obtained. This led to the adoption of other methods than direct searching, and after some experimentation a type of artificial box-shelter was devised, which gave moderately successful results.

The essential characteristics of the outside resting populations of both A. gambiae and A. funestus in this sub-coastal region was found to be the predominance of unfed and gravid females in the catches.⁶ Fed females were only caught in very small numbers and then only in the vicinity of houses. Nearly all of them had fed on man. By the concerted use of observations on the mosquitos resting indoors, those resting out of doors, and on the proportions of them attempting to leave experimental huts through window traps at different stages in the gonotrophic cycle, it was possible to show that all except about 5 per cent. of recently fed mosquitos of both species were resting inside houses. On the other hand, it was concluded that about half the A. gambiae females, and a rather smaller proportion of the A. funestus, left the houses half way through the cycle and by this means came to constitute the major group in the outside resting population. Thus deliberate exophily was found to be a regular feature in the life of A. gambiae in the area, although this behaviour was only exhibited during the second half of the gonotrophic cycle. It should be noted that no evidence was obtained of the existence of any wild mammalian hosts in the district. The mosquitos had therefore to enter houses to feed, and any exophily occurring would of necessity fall into the third category described.

One contributory factor to the difficulty of this work on outside resting was the relatively small size of the mosquito population relative to the number of potential resting sites. It was estimated⁸ that at peak seasons the density of female mosquitos resting outside did not amount to more than 15-20 A. gambiae per acre, or less than one per 200 square yards, and for much of the year it would have been far less.

When the second district was investigated, a very different picture was obtained. This district comprises a belt of arid thorn bush country interspersed with swamps and areas of extensive irrigation at points where the rivers enter the plains from the neighbouring South Pare Mountains. Rainfall varies from slightly over 30 inches per annum to 12 inches or possibly even less. For much of the year there may be no grass at all, apart from the margins of the swamps, and the prevailing hue of the countryside is that of the soil and not of the vegetation. Two other features serve to

distinguish it from the coastal region; firstly the size and concentration of the breeding sites, and secondly the presence of large herds of cattle that are kept at night in open thorn enclosures. The entomological findings were strikingly different. Mosquitos could be caught resting outside in quite large numbers, and their natural resting sites were relatively easy to locate. The use of box shelters in this region was astonishingly successful. In the course of a ten-day catch with 23 boxes at the season of peak mosquito production, well over 3000 female A. gambiae were caught. On one or two occasions over 100 A. gambiae were caught resting inside a single box (3 feet high by 2 feet broad by 3 feet deep).

The other characteristic of the outside resting population in South Pare district was the high incidence of freshly fed females in the catches (see Table I). Their numbers were greatest in collections of adults made within or on the margins of villages, but they were not uncommon in catches made near the fields and breeding sites.

This very marked exophily may be partly explained in the following way. Firstly the actual density of the mosquito populations was certainly far greater than on the coast; secondly, in this arid region suitable resting sites were few and relatively exposed; and thirdly, suitable hosts, in this case cattle, were available in the open throughout the night. Females feeding on them, and subsequently remaining outside, can be regarded as falling into the second category of exophily, that was described as facultative. Their presence outside may have been a question of opportunity secondary to the location of the host, and it does not necessarily imply that their reactions to the house as a daytime habitat are different from the population of A. gambiae studied on the coast.

Two questions remain unanswered from this work in South Pare district. Firstly, does the 10-15 per cent. of fed females in outside catches that was positive for human blood (see Table II) represent those that actually fed outside (in the hot season some of the inhabitants may occasionally sleep in the open), or do they represent those that fed indoors and subsequently left for outside shelters? Do they in other words belong to the third category of exophily that was described as deliberate? Secondly, do females that have fed and rested for 24 hours inside the house then tend to adopt outside harbourages for the remaining 24 hours of the cycle, as was found near the coast?

On the first question insufficient evidence has been obtained, but the impression was gained that the exodus of fed females was certainly not on any very large scale. On the second question, observations made by Dr A. Smith¹⁶ show that fed females greatly outnumber gravid females in house catches. The latter group should therefore be found resting outside in some numbers. In fact the outside resting population of gravid females should contain nearly all those that rested outside when freshly fed as well as a proportion of those that fed inside. Yet it will be seen from Table I that, even in catches made near breeding sites, gravid females only exceeded fed females by 50 per cent. This suggests that either the sampling techniques were inadequate, or that large numbers of females were feeding and subsequently resting outside. These mosquitos should be composed approximately of 50 per cent. each of fed and gravid females. If they were numerous enough, the reinforcements of gravid females arriving from out of the houses on the second day of the cycle would not drastically alter the proportions of the two stages amongst those already outside. For instance, on the coast, where there were very few freshly fed females outside, the exodus of approximately half of the gravid females from houses resulted in a ratio of fed: gravid females outside of between 1:5 and 1:12, which would fit in with the conclusion that fed mosquitos were some 8-20 times as numerous indoors as out of doors. On the other hand, if the feeding population was divided equally between houses and outside shelters, an efflux of half of the gravid females from houses would give a total outside resting population with fed: gravid mosquitos in the proportions of 1:1.5. This is, in fact, the proportion observed in shelters distant from houses in South Pare District. The data from this region are not sufficiently critical for such an exact conclusion to be drawn, but it does indicate that the outside resting population must have represented a very considerable fraction of all the mosquitos in the area. Apart from the data presented here, this line of argument could be used to estimate the relative size and importance of the exophilic and endophilic components of any recently fed mosquito population.

We have, then, fairly detailed observations on the degree of exophily shown by A. gambiae in two contrasted areas of East Africa. The nature of these differences may be illustrated in the form of a grid as follows:

Analysis of exophily in A. Gambia in two
 areas of Tanganyika

<u>Coast (Tengeni)</u>			
	Obligatory	Facultative	Deliberate
Unfed	+	+	
Fed	-	-	-
Gravid	-	-	++

S. Pare

Unfed	+	+	
Fed	-	+++	?
Gravid	-	+++	++

Exophily in other parts of Africa

Information on outside resting of A. gambiae in other regions is scattered and not always easy to analyse. The present state of our knowledge can perhaps best be assessed by making use of the three categories of exophily described at the beginning of this paper, and by attempting to fit existing records into the same pattern.

Outside resting in the absence of houses probably occurs in certain arid, uninhabited regions, as in parts of the Sudan and Somaliland for instance, and it must certainly occur in the wet, uninhabited forest of Bwamba in Uganda.⁹ This exophily must obviously be obligatory, and may be without any great biological significance as such; although it indicates, of course, the impossibility of species eradication in such areas by existing methods of control.

As has just been shown in describing conditions in the Pare district of Tanganyika, A. gambiae may rest outside in considerable numbers when large herds of cattle are kept out of doors at night. It is likely that this type of facultative exophily, in which distribution of the resting mosquitos is apparently dependent on the availability of houses or natural shelters, occurs in many parts of Africa where cattle are kept in the open. One should mention in particular those parts of the Upper Volta and the French Sudan reported on by Holstein¹² (p.134) and Bernet (in Holstein¹²).

Reports of a definite exodus of female A. gambiae from houses are quite numerous, but these mainly refer to half gravid mosquitos. It is only in a dry region of the Transvaal,⁴ and possibly the Gambia,² that any marked departure of freshly fed females from houses appears to take place. In North West Nigeria Bruce-Chwatt¹ has reported the finding of fed females in some numbers outside, 60 per cent. of which had fed on man. Similarly Holstein¹² has reported the capture of many fed females resting outside in other parts of West Africa, but it is not possible yet to determine the origin of these mosquitos and hence to assess the type of exophily shown. On the other hand, there is fairly good evidence from Southern Rhodesia,¹³ Southern Nigeria,¹⁷ Uganda,³ Tanganyika,⁷ and perhaps the Upper Volta, Holstein¹² (p.104), that partial exophily in the second half of the gonotrophic cycle is of regular occurrence. In fact it looks as if this may be regarded as a definite characteristic of the species over much of its range. It should be noted however that in Mauritius, where the behaviour of fresh water A. gambiae is atypical in several respects, Halcrow¹⁰ found only unfed females outside. It is also known, thanks mainly to the work of Muirhead Thomson,^{17,18} that salt water forms of A. gambiae, both males and the East Coast form, show deliberate exophily to a considerable extent. This is true of freshly fed females and even more so of females in the second half of the cycle.

Practical significance of exophily

This subject will be discussed from two points of view, firstly exophily under natural conditions, and secondly in the presence of residual insecticides.

(i) Natural conditions

(a) Survival rates

The importance of the microclimate of the resting site is at present unknown. It has been suggested that those resting in houses may tend to survive longer owing to the more favourable microclimate indoors, and possibly to reduced predation. The collection of convincing evidence on this subject will involve much painstaking and difficult work. But it may be pointed out that on the East African coast evidence has been produced⁵ of a higher survival rate for A. gambiae than for A. funestus, although the former species shows a higher degree of exophily.⁷

(b) Measurement of infectivity

Infection rates in exo- and endophilic populations of the same species may not necessarily be the same. In fact since the composition of the two is nearly always different in terms of ovarian development, one would hardly expect it to be so. For instance 33 per cent. of the females resting outside on the East African coast were found to be unfed, and the great majority of these were virgin females.⁶ Indoors only four per cent. were unfed.⁷ Obviously comparison of the sporozoite rates in the two populations without allowing for this would be unsound.

(c) Exophilic and Endophilic races

The possible existence of such races as postulated by Holstein,¹¹ is another important question. But in the absence of critical morphological differences between two such populations, and it is my opinion that the maxillary index is not a precise enough character for this purpose, demonstration of their existence becomes a matter of considerable difficulty. Naturally the first step in studying the problem would be the definition of the exact type of exophily that might be shown by the particular mosquito population under investigation. The presence of unfed females outside would be of no significance in this context, nor would the finding of gravid mosquitos, since one would be ignorant of the stage in the gonotrophic cycle at which they had adopted outside shelters. So one would be reduced to considering the behaviour of recently fed females alone. The next step would be

to eliminate the possibility that their exophily was simply a question of opportunity, secondary to the presence of the host outside or to the absence of suitable houses in the vicinity, and to show that it was an inherent biological characteristic of that particular mosquito group. In fact, the only exophilic population that could justify investigation as a possible distinct race would be one composed of recently fed females, that were either caught leaving houses after feeding or were found resting outside when the host was known to be indoors throughout the night. Other types of exophilic races might exist, of course, but they would have to be studied from the point of view of host preferences or from some other aspect of their behaviour, since one would be unable to demonstrate that the choice of resting site was biologically determined.

The search for morphological differences should therefore be concentrated on freshly fed females; and the best technique would probably be the use of experimental huts with window traps. Experience in East Africa gave no support to the view that an exophilic race might exist in the areas studied there.⁷ On the other hand, Campbell's² work in West Africa suggested that the exodus of females (presumably mainly fed ones) was on a larger scale and might be indicative of behaviour differences between separate population groups. But as this author points out, a difference in the tendency to leave houses after feeding might simply be due to a fatigue factor, depending on the distance of the breeding sites from villages. In fact investigation of this subject is fraught with difficulties, and it may very well be that clarification of the problem will not come from studies of resting preferences but only through work on other aspects of behaviour.

(ii) The influence of insecticides

The last subject to be discussed is perhaps the most important of all namely, what significance, if any, does exophily have when residual insecticides are being systematically applied to houses? It may be the view of some malariologists that, provided all the mosquitos coming into houses are killed, those resting outside can be safely ignored. With a species such as A. funestus which appears to be highly

susceptible to residuals, this argument may be valid. But with other mosquitos this may be far from true. From Malaya for example, there is clear evidence that the exophilic habits at all stages of the gonotrophic cycle of A. maculatus enable it to maintain a reduced rate of malaria transmission even in the presence of efficiently applied insecticides.

Turning to A. gambiae, it cannot be said at the present juncture that complete interruption of transmission will inevitably follow the application of residuals, regardless of local conditions or of the degree of malarial endemicity. The results of future control programmes may make such a statement possible. But until we have amassed a comfortable body of evidence to show that we can safely ignore the mosquitos that rest outside, time and money will have to be spent in studying this exophilic group and in assessing the impact of residual sprays upon them. Nothing would be more disastrous than to discover, in the later stages of a large scale control programme, that low grade transmission was continuing and that we were unable to discover the reason why. Without preliminary observations it might be exceedingly difficult to determine the mechanism by which transmission was being maintained; and one would certainly be unable to decide whether this represented a change in the behaviour of the mosquitos or, perhaps even more important, whether such altered responses would become progressive. During the present exploratory phase of malaria control in Africa, it is essential that every major control scheme in areas of high endemicity should incorporate preliminary studies on mosquitos wherever they rest, and these should be continued for as long as indicated.

Since the inception of residual sprays, a new outlook has been developing in entomology, which has led to more critical studies of the changes in feeding and resting habits and mortality rates in those species of *Anopheles* capable of surviving in areas sprayed with insecticide. It should be possible to refine the techniques used so that much more precise data on the effects of residuals on mosquito populations can be obtained. The study of mortality rates, for instance, is only just beginning, yet curtailment of the mosquitos' span of life is usually the sole object of modern control methods. The opportunities for progress along these lines, and

progress that is of great practical significance, are probably greater with A. gambiae than with any other mosquito. And it is my belief that such knowledge will come to be regarded as an essential basis for successful malaria control where A. gambiae is the vector.

TABLE I. CATCHES OF A. GAMBIAE MADE IN OUTSIDE SHELTERS IN THE SOUTH PARE DISTRICT OF TANGANYIKA

	Total numbers caught	Percentage		
		Unfed	Fed	Gravid
Shelters near houses	6512	28.3	44.9	26.8
Shelters distant from houses	1049	57.4	16.9	25.7

TABLE II. RESULTS OF PRECIPITIN TESTS ON OUTSIDE CATCHES OF A. GAMBIAE IN THE SOUTH PARE DISTRICT OF TANGANYIKA

Percentage of smears positive						
	Man	Ox	Sheep or Goat	Dog	Others (includ. negatives)	Number of smears
Near houses	14.9	70.7	3.6	3.9	6.9	2039
Distant from houses	8.3	75.9	2.1	3.4	10.3	145

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