OBSERVATIONS ON A SPECIES OF THE *ANOPHELES FUNESTUS* SUBGROUP, A SUSPECTED EXOPHILIC VECTOR OF MALARIA PARASITES IN NORTHEASTERN TRANSVAAL, SOUTH AFRICA

BOTHA DE MEILLON, G. J. VAN EEDEN, L. COETZEE, M. COETZEE, R. MEISWINKEL, C. L. N. DU TOIT AND C. F. HANSFORD

Siegfried Annecke Institute, Tzaneen, South Africa

ABSTRACT. Low grade malaria transmission persists sporadically in the northeastern Transvaal in the absence or near absence of the usual house-frequenting vectors. The possibility of exophilic transmission was considered and attention focused on a hitherto unrecognized outdoor biting member of the *Anopheles funestus* subgroup closely resembling *An. aruni* and *An. funestus*. This species called *aruni*? here was found to bite man readily out of doors and to be fully receptive to *Plasmodium falciparum*. In the adult stage *aruni*? can usually be distinguished from *funestus* and a list of these characters is given and illustrated graphically. Workers in Rhodesia have found that the polytene chromosomes and spermatogenesis of hybrids show *aruni*? and *funestus* to be separate species. The whole *funestus* subgroup requires investigation by modern cytotaxonomic, chemical and cross-breeding techniques especially as their immatures have so far defied separation.

INTRODUCTION

Investigations to assess the prevalence of malaria and the vectors responsible for transmission were initiated in 1928 (Swellengrebel et al. 1931). The principal vectors were found to be *Anopheles gambiae* Giles and *An. funestus* Giles and subsequently to be extremely endophilic and anthropophilic. The discovery of this behavior led to the development of indoor spraying with insecticides as a rural malaria control measure. Pyrethrum was first used in the 1930's with very encouraging results in Natal and subsequently DDT and BHC in both Natal and Transvaal. By 1950 the number of malaria infections had fallen to a few hundred annually and ceased to be of public health importance. In the continued absence of indoor resting vectors and malaria the area sprayed was gradually reduced and confined to certain high risk areas. By 1970 small scattered foci of infection were detected and such small foci, not always in the same locality, have continued to occur ever since. Intensive entomological investigations through the years failed to reveal significant numbers of *An. gambiae* or *An. funestus* biting man either indoors or outdoors. It gradually became apparent, however, that a previously unrecognized member of the *funestus* subgroup, called *An. aruni*? in this paper, was often present in outdoor catches biting man and rarely appeared indoors. In view of this the existence of a possible exophilic vector was postulated and we decided to investigate the matter during 1974–75. In 1934 de Meillon, basing his identifications on the larval stage, reported the presence of *funestus* larvae above a certain altitude but that adults no longer entered houses. We now feel certain that this non-house frequenting form was *aruni*? whose larva is not distinguishable from *funestus*. *Anopheles aruni*? is therefore not an introduction but an old member of the fauna misdiagnosed as *funestus* in the past.

IDENTIFICATION OF THE PRESUMED EXOPHILIC VECTOR. Members of the *funestus* subgroup are not as yet distinguishable in the larval and pupal stages with any certainty (Gillies and de Meillon 1968) and the recognition of outdoor biting adults became of paramount importance. At first sight the adults so caught were typical of members of the *funestus* group and especially the subgroup. It was noticed, however, that often the wing was paler, the pale bands on the female palp and hind tarsi more pronounced and somewhat broader as in *An. aruni* Sobti (Sobti 1968). Since considerable variation was obvious among the adults caught biting outside, a program was initiated to rear progeny from such blood-fed adults to determine the extent of the variation and to exclude the other members of the group whose larvae, and in some cases pupae, were identifiable. In short it was proposed to attempt differentiation between adults of our *aruni*? and *funestus*.

In order to facilitate recording of the adult characteristics of *aruni*? and *funestus*, all the pale and dark spots on an ideal wing were numbered (Fig. 1) and their absence in individual specimens recorded on punch cards. In addition we recorded certain other characters such as wing length, absence or presence of a pale spot at the base of the male palpal club, the prominence of the pale tarsal banding and other features that we thought might be useful. It eventually became apparent that the relative lengths of certain pale areas on the costa and palp were also worth recording.

For comparison, specimens of *funestus* were solicited and obtained from many sources. During the investigations there occurred small but sharp outbreaks of malaria near Tzaneen and at Uitspanning in the northwest Transvaal which yielded the first indoor biting *funestus* seen since about 1950. In addition we received some *funestus* from Lusese, a malarious area in the Caprivi, where they were taken indoors as a result of space spraying with pyrethrum. In all 212 apparent *funestus*, many with individal larval and pupal skins, and the progeny from 54 isolated females of *aruni*? numbering 330, were examined. In the case of the latter the investigation continued for 1 yr so that all seasons were covered.

Progeny rearing enabled us to evaluate not only variations within a family but also between families and sexes. Appropriate data were subjected to statistical analysis using the "t" test.

RESULTS

TAXONOMIC AND BEHAVIORAL. The only useful characters for separating female *aruni*? from *funestus*, after our analysis of the mass of data gathered are summarized below. When characters 2 and 3 above are plotted on a graph (Fig. 2) it is seen that the exophilic species we call *aruni*? falls outside a square approximately limited by 1.0 units, whilst the endophilic anthropophilic fraction of the population, namely *funestus*, falls within these 2 parameters. It must be emphasized that these parameters will not always identify a single specimen, no matter where it is caught.

We are informed by our colleague in Rhodesia (C. A. Green, personal communication) that he has evidence from a

Character	*aruni*? (n =330)	*funestus* (n = 120)
1. Adult behavior.	Mainly exophilic; zoophilic and anthropophilic	Mainly endophilic, anthropophilic.
2. Wing, ratio spots 8 + 10/9	More than 1.	Less than 1.
3. Palp, ratio spots 3 + 5/4	More than 1.	Less than 1.
4. Wing spot No. 4 (See Fig. 1).	Absent in 3% of females.	Absent in 79% of females.
5. Wing spot No. 53 (See Fig. 1).	Absent in 1% of females.	Absent in 50% of females.
6. Marked pale tarsal banding.	When present, diagnostic.	Never present.

Fig. 1a

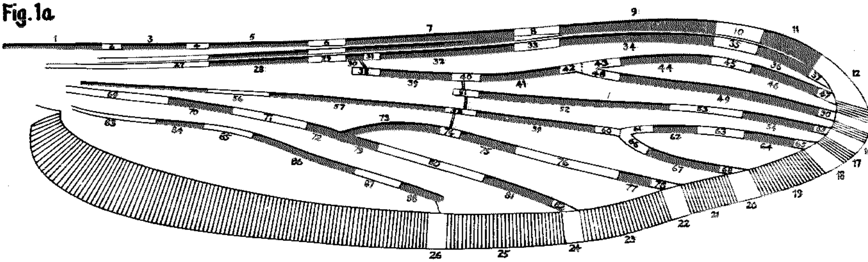

Fig. 1b

Fig. 1a Idealized wing of a female of the An. funestus group showing the system of numbering of the spots used.

1b Palp of a female of the An. funestus group showing the numbering of the dark and pale bands.

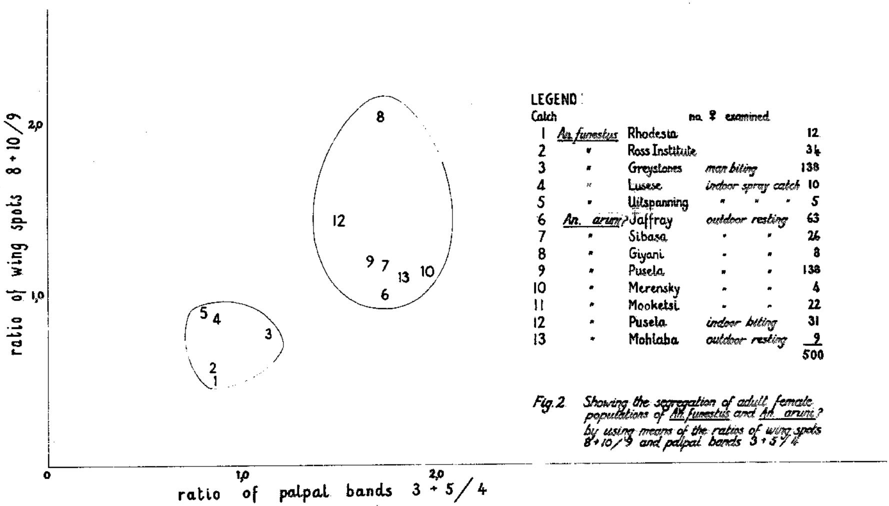

Fig. 2. Showing the segregation of adult female populations of *Anopheles funestus* and *Anopheles aruni*? by using means of the ratios of wing spots 8 + 10/9 and palpal band 3 + 5/4.

study of the polytene chromosomes and spermatogenesis in hybrids that a sample of the insects we call *aruni*? and *funestus* are distinct species. We were fortunate to be able to examine paratype *aruni* from the type locality and because of the extensively pale wing and presence of a marked pale spot at the base of the male palpal club we believe it to be distinct from our *aruni*? in which the base of the club is dark in nearly all specimens or only a few pale scales are present.

We are fully aware of the fact that the situation regarding the *funestus* group of species will not be resolved until more modern taxonomic techniques are applied. Quite apart from difficulties experienced in identifying the known members, complications arise from other species which show variations causing them to be mistaken for members of the group. *An. demeilloni* Evans, for instance, in which a dark upper branch of the 5th vein is not uncommon, is one of these. We have therefore taken great care to avoid such errors by depending on specimens with associated larval and pupal skins. Nevertheless, as is well known, the immatures of the *funestus* subgroup are not distinctive, and hitherto unrecognized species are to be expected. That this is not so unlikely is shown by some cross-breeding experiments performed by us in which apparent normal hybrids resulted from one mating and males with undeveloped testes from another. These experiments were performed with specimens identified by us as *aruni*? and *funestus*.

OUTSIDE RESTING AND BITING. An occupied experimental hut at the site of an *aruni*? breeding place yielded only a single blood-fed female caught in a window trap over the course of 1 yr. None was ever found resting or biting indoors. The blood-meal proved to have been taken from a bovid. During the same period of catching on human bait 325 female *aruni*? were taken biting outside near the experimental hut; this constituted 23% of all mosquitoes of 13 different species. In nearby pit shelters 78% of 1,371 mosquitoes, represented by 10 species, were *aruni*?. It should be noted that in Fig. 2 catches 1, 3, 4 and 5 were associated either with sharp outbreaks of malaria (3 and 5) or with stable malaria. The rest of the catches which are clearly grouped and separated from the above all represent specimens which fit into our conception of *aruni*?. These specimens were all caught either resting or biting out of doors which is usual for *aruni*?. Catch 12, however, was made in a lighted room. Since this episode we have begun special catches to explore this unexpected behavior. So far the findings are of a preliminary nature but nevertheless show that more than twice as many attack man under a fluorescent light indoors as do in the dark outside.

RECEPTIVITY TO PLASMODIUM FALCIPARUM. Laboratory bred *aruni*? from nearby localities were fed on a volunteer who had a mild *P. falciparum* infection with gametocytes. Altogether 48 females were fed on this carrier and subsequently examined for stomach and gland infections as they died. Of 28 examined for oocysts and 14 for sporozoites, 5 and 3 respectively were positive. It is thus shown that *aruni*? is fully receptive to the parasite and that it could transmit it to man after an infecting feed.

Very few dissections of wild caught females of *aruni*? have been made. During the outbreak of malaria near Tzaneen mentioned above, of 92 female *funestus* caught indoors 6 showed sporozoites while 53 *aruni*? from outside haunts were negative. It is known that *aruni*? feeds readily on bovids and of 82 caught outdoors and tested for source of blood meal only one was of human origin. It is therefore to be expected that, as happens elsewhere in Africa with incidental vectors, natural infections, when they do occur, will be of a very low order but yet sufficient to prevent interruption of transmission of malaria until the regular vectors appear on the scene.

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THE NIGHT-TIME FLIGHT ACTIVITY AND RELATIVE ABUNDANCE OF FIFTEEN SPECIES OF LOUISIANA MOSQUITOES

MICHAEL K. CARROLL AND JAMES A. BOURG

New Orleans Mosquito Control Board, 6601 Lakeshore Drive, New Orleans, Louisiana 70126

ABSTRACT. Fifteen species of Louisiana mosquitoes were sampled by use of a truck-mounted funnel trap. Collections were made hourly for a 12-hr period through the night. It was shown that for most species, including *Aedes sollicitans* and *Culex salinarius*, the greatest activity occurred at dusk and dawn.

For various reasons, a knowledge of the flight activity of mosquitoes is essential to a mosquito control program. Comparative analysis of adult surveillance data should be contingent upon information concerning the periods of adult activity and inactivity. To be most effective, a ULV space spray should be applied at the peak of flight activity of a species. Therefore, flight activity data are of paramount importance in the scheduling of ULV treatments.

Migration, appetential dispersion, circadian rhythm, moonlight, brood age, season of the year, and species characteristics are factors which determine overall flight activity.

Migration probably occurs only on the night of initial departure from the breeding site, with twilight departure resulting in a longer migration than departures later in the night (Provost 1957). Using p^{32} labeled *Aedes taeniorhynchus* adult males and females, Provost showed that appetential flights expand the distribution of the brood well beyond the migrational range. Females were recovered up to 25 miles away through the 24th night after emergence. However, the few males which were recovered were within 3 miles of the depature point.

Circadian rhythm of mosquito flight activity has been documented by several workers (Haddow et al. 1961, Jones et al. 1967, Nayar and Sauerman 1971). Simply stated, circadian rhythm refers to biologi-