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Partial exophily of *Anopheles gambiae* species B in the Khashm Elgirba area in eastern Sudan

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Many Anopheles gambiae *were found resting outdoors in an area that had been sprayed with DDT, the numbers being particularly large 4 months after the first annual spraying cycle. A high proportion of the resting females had consumed a blood meal. Window-trap studies showed that a marked exodus from sprayed huts occurred in the morning. It is concluded that the outside resting of* An. gambiae *is either a natural habit or the result of the irritant action of DDT. This behaviour of the vector is regarded as one of the factors responsible for the marked increase of malaria in the area.*

Anopheles gambiae is the principal malaria vector in the Sudan and is generally known to be endophilic and anthropophilic. Observations made in the Khashm Elgirba area in eastern Sudan during July–November 1967 showed that *An. gambiae* was resting in large numbers in natural outdoor shelters. In this paper these observations are described and the factors favouring the exophilic pattern of behaviour in the area discussed.

The Khashm Elgirba area, which has an agricultural settlement scheme, is situated in the eastern part of the Sudan between latitudes 14°N and 16°N and at longitude 35°E. Geographically, the area is classified as dry savanna with an average annual rainfall of 300 mm. The rainy season is short, starting at the end of June and lasting until early October. The relative humidity reaches 75% in the rainy season and drops to 15% in the dry season. Winters are dry and the temperature ranges from 16°C to 35°C; summers are dry and hot, and temperatures reach a maximum of 45°C.

The agricultural scheme at Khashm Elgirba was designed to resettle the Nubian population of Wadi Halfa after their original land in northern Sudan was submerged by the reservoir formed by the Aswan high dam in the Arab Republic of Egypt. Agriculture then became the main occupation of the resettled population. Cultivation is mainly dependent on the canal irrigation scheme that was started in 1964 after the completion of the Khashm Elgirba dam. Before the resettlement began, the area was inhabited by nomadic tribes, which were concentrated along the Atbara river.

A special malaria control project was initiated in the area at the beginning of the irrigation scheme, the object of the project being to protect the non-immune Nubian population coming from a vector-free zone, *An. gambiae* having been eradicated from southern Egypt and northern Sudan in 1948 (Lewis, 1949). The control project was divided into two operational areas—namely, the riverine area, which includes 60 villages situated along the Atbara river and which is occupied by the indigenous nomadic population of approximately 32 000 people, and the resettlement area, which consists of 33 villages and Halfa town, accommodating the 50 000 Nubian immigrants from northern Sudan. The observations presented here were made mainly in four localities—namely, Kurraj and Andala, which are riverine villages situated about 0.8 km from the bank of the Atbara river, the research farm, and the eucalyptus forest in the resettlement area; the research farm and the forest lie about 13 km from the Atbara river (Fig. 1).

The malaria control project began in 1964 and applications of DDT were started in the same year. Previously, only the riverine villages were sprayed annually with gamma-HCH. In the first round of spraying, DDT was applied at a level of 2 g/m^2 in June 1964. Subsequently, two rounds of DDT at the same dosage level were applied annually, one in June and the other in mid-October. The entomo-

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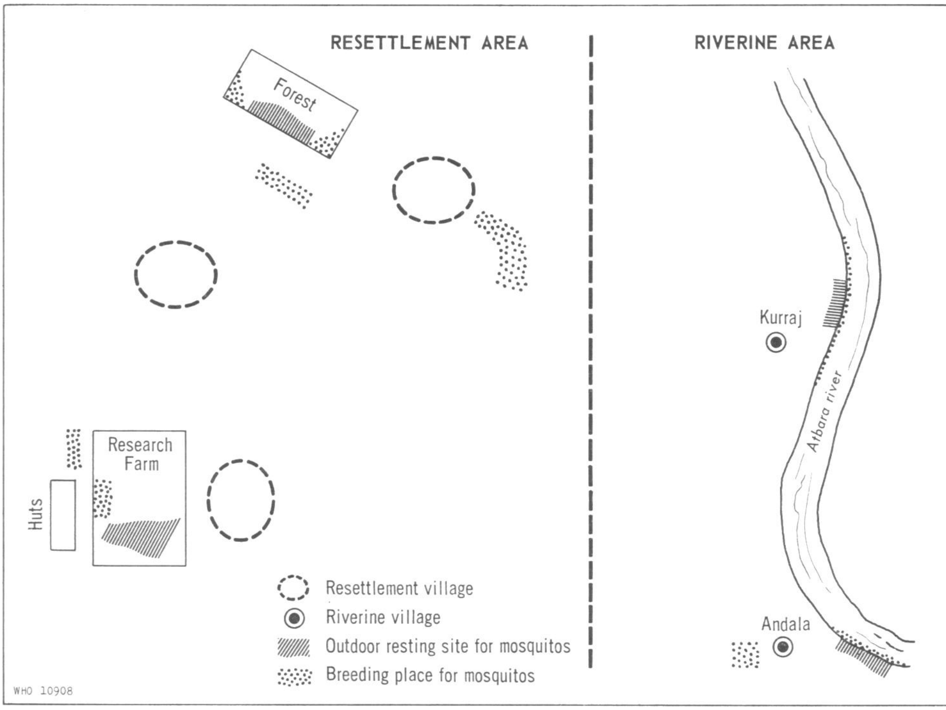

Fig. 1. Localities in the Khashm Elgirba area where observations were made on *Anopheles gambiae*.

logical observations reported here commenced in June and ended in October 1967, i.e., before the second annual spraying round was applied. In addition to the house spraying with DDT, endrin and organophosphorus compounds were regularly applied to cotton crops to control insect pests. Before control measures were undertaken, the area was considered mesoendemic, and an average annual parasite rate of 28.8% was recorded among the riverine population, with a seasonal malaria peak in October. *Plasmodium falciparum* and *P. vivax* were both prevalent.

METHODS

Indoor spray capture

From June 1967 routine spray-sheet collections, made with a solution of 0.2% pyrethrins in kerosine, were carried out at 2-weekly intervals in 10 fixed stations in the research farm and Kurraj. During October, additional collections were made in Andala village. All *An. gambiae* females collected were classified according to their abdominal condition and some were dissected to determine the parous rate and the presence of salivary gland infections.

Outlet window trap observations

An outlet window trap was installed in each of five sprayed huts in the locality of the research farm during August. The traps were operated in the early morning between 05.00 and 06.00 hours four times a week at 2-weekly intervals; during October, collections were made weekly. Female mosquitos collected in the traps were identified and classified according to their abdominal condition. Those collected alive in the traps were kept for 24 hours and the delayed mortality was recorded.

Outdoor collections

From June 1967, outdoor searches by hand capture were carried out routinely at 2-weekly intervals in and around Kurraj and the research farm. Additional searches were made during October in Andala. All collections were made in the morning between 08.00 and 10.00 hours and natural shelters were searched. Female mosquitos were identified and the abdominal condition was classified about 2 hours

Table 1. Outlet window trap collections of *An. gambiae* from DDT-sprayed huts at Khashm Elgirba, 1967. The first round of spraying was in mid-June 1967; the second round was applied in mid-October 1967

Month	No. of trap days	*An. gambiae* females collected	Morning trap collection						No. of females held for 24 hours	Percentage of 24-hour survival
			unfed		fed		gravid			
			alive	dead	alive	dead	alive	dead		
August	16	2	0	0	2	0	0	0		
September	40	253	101	2	121	0	29	0	251	94
October [a]	80	111	31	1	65	0	14	0	50	88
November	40	4	1	0	3	0	0	0		

[a] Observations made before the second round of spraying was applied.

after capture. Samples of unfed and recently fed female *An. gambiae* were dissected to determine parous rates according to the method of Detinova (1962) and Polovodova's simplified technique.[1] Some of the females were dissected for an examination of the salivary glands and blood-meal smears were taken from the recently fed females for precipitin testing.

No observations could be made in an unsprayed area at the same time as those in the Khashm Elgirba area.

RESULTS

As shown in Table 1, very poor catches were obtained in outlet window traps during August but the traps were quite productive during the period September–October. The density of catches was high during September and 48% of the females collected were blood-fed. Of 251 females in different abdominal stages, 94% survived the 24-hour holding period. The density dropped in October and about 59% of the mosquitos collected were blood-fed females. Of 50 blood-fed females, 88% survived the 24-hour holding period. As shown in Table 1, the number of mosquitos found dead in the traps was negligible. Bioassays performed in three of the trap huts showed that DDT deposits were still effective, and a susceptibility test carried out on part of the trap collections showed that *An. gambiae* was susceptible to DDT, with the reservation that no replicate control was kept. However, this result was confirmed by further tests made in April 1968, which gave complete mortality (75 mosquitos tested) on exposure to 4% DDT for 1 hour, with no mortality in the controls.

When outdoor searches were first attempted in June 1967, *An. gambiae* were found resting at the bases of trees in the eucalyptus forest. Subsequently, outdoor searches were arranged regularly in all the possible resting sites (Gillies, 1954). All sites that harboured *An. gambiae* were shaded, and some were heavily shaded. The natural outdoor shelters in the irrigated area were as follows: the openings of canal water pipes, the undersides of bridges, the bases of trees, and dense piles of fallen leaves. Along the river bank, mosquitos were found resting in small ridges under rocks and overhanging edges. The habitat was always found to be shady and humid. Although the natural shelters in the riverine villages were more than 0.8 km from the villages, there were no animal shelters or human habitations between the river and the village. It would therefore appear that *An. gambiae* can fly a distance of 0.8 km, even when fully fed, to seek a suitable outdoor shelter.

A summary of the numbers of female *An. gambiae* collected from the various outdoor resting places is given in Table 2. Following the application of DDT to houses in June, outdoor searches were unproductive for 2 months. Successful collections resumed in September, a peak density being reached in October, prior to the application of the second round of DDT.

During September, 37 *An. gambiae* were collected from three localities; 35% were blood-fed, 38% were unfed, and the remainder were in advanced stages. In October, 395 females were collected from four localities and 59% were blood-fed, 14% unfed, and 27% comprised both half-gravid and gravid females. The blood-fed females constituted a high

[1] World Health Organization (1963) *Practical entomology in malaria eradication*, pt. 1, Geneva (unpublished document MHO/PA/62.63).

Table 2. Female *An. gambiae* collected from natural outdoor shelters in the Khashm Elgirba area, 1967. Observations were completed before the second round of spraying was applied in mid-October 1967

Month	Locality	Abdominal stages of *An. gambiae*					Average density per man/hour	Females dissected for parity determination	No. parous	Percentage parous	Females examined for salivary gland infection	Females with salivary glands positive for sporozoites	Blood-meal smears examined by precipitin test	Percentage of smears giving positive reaction for man
		unfed	fed	half gravid	gravid	total collected								
July	forest	0	0	0	1	1								
	Kurraj	0	0	0	0									
August	forest	0	0	0	0	0	0							
	Kurraj	0	0	0	0	0	0							
September	forest	7	7	2	2	18	3							
	Kurraj	0	5	0	2	7	2.3							
	research farm	7	1	0	4	12	4							
total		14	13	2	8	37	3.1							
October	forest	5	12	4	4	25	2.8						20	50
	Kurraj	14	44	4	30	92	6.4	24	8	33.3	18	0	37	46
	research farm	8	17	1	4	30	5						13	7
	Andala	28	160	12	48	248	20.6	30	23	76.6	38	2	55	4
total		55	233	21	86	395	8.7	54	31	57.4	56	2	125	26

Table 3. Indoor resting density of *An. gambiae* in DDT-sprayed huts, July–October 1967

Month	Locality	No. of huts searched	Abdominal stages of *An. gambiae* collected			Density per hut
			empty	blood-fed	gravid	
July	research farm	20	0	0	0	0
	Kurraj	10	0	0	0	0
August	research farm	20	0	0	0	0
	Kurraj	10	0	0	0	0
September	research farm	20	6	25	7	1.9
	Kurraj	10	0	10	1	1.1
October	research farm	19	1	14	5	1
	Kurraj	10	0	139	37	17.6
	Andala	5	0	66	19	17

proportion of the total catch, which is close to the findings of Service (1963) in Northern Nigeria, where the proportion of blood-fed females varied from 30.9% to 50.6% of the total outdoor catch.

Precipitin tests carried out on 125 blood meals from females caught from all outside resting sites combined showed that 26% had fed on man, about 70% had fed on cattle, and the rest had fed on other vertebrate hosts. The proportion of smears giving a positive reaction for human blood varied among the four localities according to the man : animal ratio and the position of the animal shelters relative to the outdoor resting sites. However, the sample sizes of blood-meal smears taken from the research farm, 13 and 20, respectively (see Table 2), does not allow inferences to be made. While Kurraj and Andala smears had nearly the same man : animal ratio, those taken from Andala showed that only a very small proportion of mosquitos had fed on man. This could be explained by the fact that the outdoor resting shelters were near the animal shelters.

The salivary glands of a small sample of the outdoor resting population of *An. gambiae* females were examined for the presence of sporozoites in order to determine whether or not they were responsible for malaria transmission. While no positives were found in a sample of 18 females dissected from Kurraj, there were 2 positives among 38 females from Andala.

Indoor collections were performed simultaneously with outdoor searches in the localities under observation; indoor searches during July and August were practically negative (Table 3). Indoor resting densities increased markedly in September, and reached a maximum in October. During October, the fed : gravid ratios in the research farm, Kurraj, and Andala were nearly the same—namely, 3 : 1, 3.8 : 1, and 3.5 : 1, respectively. One of 11 parous females dissected for gland infection taken from the indoor shelters at Andala was found to be positive.

Samples from both outdoor and indoor resting *An. gambiae* populations were examined for parous rate determination in October. Altogether, 54 females collected outdoors were dissected, and 57.4% were found to be parous (confidence limits at 95% probability level: 43.21–70.77). A total of 72 specimens from the indoor collections were dissected and 65.3% were found to be parous (confidence limits at 95% probability level: 53.14–76.12). Assuming that the duration of the gonotrophic cycle of both indoor and outdoor populations was 2 days, the probability of daily survival, as inferred from both parous rates, is 0.758 and 0.808, respectively.

Malaria incidence

Case detection in the area was dependent on the personnel available; both active and passive case detection operated in the two areas. House visits were made at 2-weekly intervals in the resettlement

Table 4. Numbers of malaria cases in the Khashm Elgirba area in July–October 1967

Month	No. of malaria cases in: resettlement area	No. of malaria cases in: riverine area	Kurraj	Andala
July	39	35	4	0
August	28	19	1	2
September	50	48	6	3
October	441	588	42	41

area and at monthly intervals in the riverine villages. There was a dispensary in every village in the resettlement area, which acted as a passive case-detection post. No classification of cases was made. However, a very high malaria incidence appeared during the period July–October 1967, when 1 347 cases were detected in the whole area (Table 4).

DISCUSSION

Information on outdoor resting habits of *An. gambiae* in Africa has been available for a long time, and through the successful genetical identification of members of the *An. gambiae* complex more information is being gained on the behaviour of each species. Suspected behaviouristic changes in *An. gambiae* resulting from insecticide spraying in the southern part of Africa were found to be connected with species C of the *An. gambiae* complex (Patterson, 1963; Davidson, 1964).

From available identifications of the *An. gambiae* species complex made on samples taken from outdoor natural or artificial shelters (Davidson, unpublished report to WHO), species A was recorded in Upper Volta, Ghana, and Togo, and species B in Mauritius and Zanzibar. In Zanzibar, species C was also identified from samples taken from outdoor shelters. The salt-water species *An. merus* in East Africa and *An. melas* in West Africa were identified in samples taken from outdoor resting shelters.

Outdoor resting of *An. gambiae* in the Sudan was first reported from the Gezira area by Lewis (1956). However, since the abdominal condition of females collected resting outside was not shown, it is difficult to consider this as an indication of exophilic behaviour. In the present investigation, specimens taken from indoor and outdoor shelters in the same area were identified as species B (Davidson, personal communication). The results obtained from the present investigation in the Khashm Elgirba area, which has been subjected to DDT spraying for the last 3 years, have clearly shown that the considerable number of species B so far identified were resting outdoors. Most of the outdoor collections were made in places more than 0.8 km from the village, which indicates that *An. gambiae* can fly that distance in search of a suitable outdoor shelter. Adults were collected in large numbers from these places, and a large proportion of them were blood-fed. The total fed : gravid ratio was about 2 : 1. The presence of a proportion of the population resting outdoors coincided with a large exodus of blood-fed females from sprayed huts.

The observed exodus may have been partly the result of a natural behaviour pattern of *An. gambiae* species B in this part of Sudan, but no observations could be carried out simultaneously in an unsprayed area to support this suggestion. The presence of large numbers of cattle outdoors was an important factor, as shown by the results of precipitin tests. Nevertheless, 26% of the sample tested gave positive reactions for human blood. This raises the question whether this proportion of females found positive for human blood represents mosquitos escaping from human habitations or mosquitos that had actually fed outside (some people were sleeping outside at the time of the investigation). The answer to this question remains unknown, notwithstanding the fact that a marked exodus from the sprayed huts has been demonstrated.

The high survival rate among the escaping females reported in the present investigation could also be a result of the irritant effect of DDT on *An. gambiae*, which has been reported by several authors, including Muirhead Thomson (1951), Davidson (1953), and Hamon (1963). As assumed by Hamon (op. cit.), the survival of the vector is favoured by the insufficiency of the amounts of DDT in aging deposits of insecticide. Thus it may be postulated that, owing to the partial exophilic habit of the vector and to the irritant effect of DDT, only active deposits of the insecticide would be effective in producing a high mortality among female *An. gambiae* visiting sprayed houses. Nevertheless, the possibility of poor quality spraying and the presence of unsprayable objects cannot be excluded.

Most of the information collected previously on outdoor resting populations of *An. gambiae* has not clearly determined their role in malaria transmission, although Service (1963) found infective females among outdoor resting mosquitos. In the present study their role has been clearly demonstrated by the presence of infective females among the small number dissected. We have assumed that the presence of sporozoites in the salivary glands is indicative of human malaria since other primates were absent from the area.

The ability of *An. gambiae* to survive outside is clearly indicated by the high parous rate (57.4%) of the outdoor resting mosquitos. Considering the fairly high probability of daily survival derived from this rate, and despite a relatively low human blood index, the epidemiological significance of the outdoor resting population should not be ignored.

Together with the rise of vector density both indoors and outdoors, there was a steady rise in malaria incidence in the area. The outdoor resting habit of the vector discussed here should be considered to be one of the factors responsible for the persistence of malaria transmission. However, further observations should be undertaken in areas where the vector is still susceptible to DDT and where total coverage of DDT has been applied and well maintained.

It is worth noting that outdoor searches attempted in the Sennar malaria pilot project were always negative, although house spraying with DDT has been carried out in Sennar for more than 8 years. This might suggest a difference in vector behaviour in ecologically different areas and calls for more observations to be undertaken with the aim of defining the types of ecological conditions favoured by outdoor resting vector populations.

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RÉSUMÉ

EXOPHILIE PARTIELLE D'*ANOPHELES GAMBIAE* B DANS LA RÉGION DE KHASHM ELGIRBA, AU SOUDAN ORIENTAL

Les observations dont il est rendu compte ont été faites dans une zone traitée (Khashm Elgirba) de la partie orientale du Soudan, pendant les mois d'août à octobre 1967. On a constaté une forte densité d'*Anopheles gambiae* au repos dans des abris naturels extérieurs, en particulier à partir du 4ᵉ mois suivant la date de la première tournée de pulvérisations annuelles. Une grande proportion des femelles trouvées au repos à l'extérieur étaient gorgées de sang. D'après les épreuves aux précipitines effectuées sur des échantillons de repas de sang prélevés sur ces femelles, environ 26% d'entre elles s'étaient gorgées sur l'homme. Deux femelles capturées à l'extérieur étaient porteuses de sporozoïtes.

Les collectes faites dans les pièges posés aux fenêtres de cases traitées ont révélé d'importantes sorties de *A. gambiae* aux premières heures du matin. Près de la moitié des insectes ainsi capturés étaient des femelles gorgées; leur taux de survie après 24 heures était très élevé. D'autre part, des densités d'insectes importantes ont été observées dans des locaux apparemment traités, avec prédominance de femelles gorgées et une proportion plus faible de femelles gravides. Cela pourrait s'expliquer par un défaut de couverture des pulvérisations qui n'avaient pas été convenablement contrôlées. Cependant, le fait que dans les conditions écologiques de la région étudiée des spécimens d'*A. gambiae* aient été trouvés au repos à l'extérieur peut être attribué au comportement naturel de l'insecte, ou à l'effet irritant du DDT, ou à ces deux facteurs à la fois. Dans cette région et dans d'autres secteurs de la partie orientale du Soudan, *A. gambiae* a été génétiquement identifié comme appartenant à l'espèce B.

Parallèlement à l'augmentation saisonnière de la densité du vecteur, l'incidence du paludisme augmente considérablement dans la région. Le comportement du vecteur semble être l'un des facteurs de cette augmentation marquée de la densité des populations vectrices et de l'incidence du paludisme.

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