Chapter Four

ENTOMOLOGY

This chapter deals with the entomological observations and their interpretation, both in themselves and in their relationship with some meteorological variables and with the insecticidal control operations. The entomological sampling scheme and the methods of collection and of examination were described in Chapter 2. The relationships between the entomological variables and their epidemiological consequences are dealt with in subsequent chapters, in particular in Chapters 5 and 10. The entomological findings have been the object of WHO reports (175, 177, 184).

Anopheline Fauna and Observations regarding Minor Species

Eleven species were identified: ***A. gambiae*** sensu stricto ***(A. gambiae*** species A), ***A. arabiensis (A. gambiae*** species B), ***A. funestus,A. rufipes, A. pharoensis, A. wellcomei, A. squamosus, A. coustani, A. maculipalpis, A. nili*** and ***A.preforiensis.*** Only the first 6 species were found in appreciable numbers. These numbers increased markedly during the wet season, the time lag between rainfall and mosquito density depending on the species. The ***2*** species of ***A. gambiae*** sensu lato along with ***A.funestus*** are the main vectors, and are the subject of the remaining sections of this chapter. The results with ***A. gambiae*** s.l. and ***A. funestus*** are presented in the next 5 sections. Findings on the relative abundance and characteristics of ***A.gambiae*** (S.S.) and ***A. arabiensis*** are presented in the section following them. Some of the findings regarding ***A. rufipes, A.pharoensis*** and ***A. wellcomei*** are given in the present section.

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a This chapter is based on the aork carried out by, or under the direct supervision of Mr G.R. Shidrawi, Mr J.L. Clarke, Mr J.R. Boulzaguet, and Mr T. S. Ashkar. The application of the Polovodova method was done by Dr N. Detinova, and the intraspecific chromosomal studies of the *A.gambiae* complex were done by Dr M. Coluzzi, both being WHO consultants in this research. Mr R.F. Fritz, Mr C. Garrett-Jones, Mr J. Hamon, and Dr K.S. Hockingassistedin the design of the entomological study.

Before intervention ***A. rufipes*** constituted only 0.06% of the night-bite collections (NBC), but as much as 3.5% of the pyrethrum spray collections (PSC) and 30% of the outdoor-resting collections (ORC). It was less affected by propoxur than the main vectors, and during the intervention period it formed 43.1 % of the PSC (but still only 0.2% of the NBC) in the sprayed villages. In 1974-1975, in the previously sprayed villages, it still formed a somewhat larger fraction of the PSC than before intervention.

A.pharoensis was collected mainly by NBC, more commonly outdoors than indoors; it represented 4.9% of the NBC before intervention, but only 0.1 % (36 individuals in 3070 collections) of the PSC. One specimen was found positive for sporozoites. The species was found in all village clusters; the highest recorded man-biting rates were 4.5 bites/man/night indoors and 25.8 bites/man/night outdoors, both in the wet season in Sugungum. This species also was less affected by propoxur than the main vectors, and during the intervention period it formed 18% of the NBC in the sprayed villages. In 1974-1975 in the previously sprayed villages, its relative abundance was back to its baseline level.

A. wellcomei was collected almost exclusively by NBC, again more commonly outdoors than indoors, and almost exclusively in Sugungum; the highest measured man-biting rates were 8.5 indoors and 17.3 outdoors. This species almost completely disappeared from the collections during the intervention period.

Collections as Indices of Density

The numbers of female mosquitos caught per collection, by means of standardized sampling methods, are used as density indices. Such indices may obviously be affected by factors other than density itself; in the case of the pyrethrum spray collection (PSC), the numbers collected might be affected by factors such as the presence of a fire, or the size of the hut, or the number of sleepers in the hut. The presence of a fire in the hut had, in fact, little or no effect on the indoor-resting density (IRD); the fires were indeed small and produced little smoke. The size of the huts in the area varied only a little (see p. 41), and could therefore not be an important factor. The relationship between the number of sleepers in a hut on a given night and the number of ***A. gambiae*** s.l. females caught by PSC the next morning (the indoor-resting density, or IRD, i.e., number of females per hut) was systematically investigated (Table 3). Because the IRD varies widely between times and places (see below), and as this may mask an association with the number of sleepers, the PSCs were first stratified

Table 3

Mean indoor-resting density of *A. gambiae* s.l. (No. females/hut, by PSC) as a function of the number of persons sleeping in the hut on the night preceding the PSC made in the morning; wet season of 1971 (pre-intervention), all villages combined

Density stratum [a]	Number of sleepers [b]				
	0	1	2	3	⩾4
0.1-4	0.8	1.7	1.5	2.9	2.4
	(15)	(40)	(64)	(36)	(12)
4.1-16	3.7	8.7	10.1	10.9	13.6
	(20)	(59)	(84)	(48)	(18)
16.1-64	21.6	28.1	27.2	41.1	36.6
	(18)	(52)	(77)	(56)	(20)
64.1-256	203.4	94.0	87.6	122.3	86.3
	(51)	(27)	(37)	(23)	(8)

[a] PSCs were stratified according to the mean density at the same time and place (village cluster).
[b] In parentheses: number of collections with the given number of sleepers in the given density stratum.

according to the average IRD for the given village cluster and fortnightly cycle of entomological collections. At low to medium densities (density strata 0.1-4 and 4.1-16), the IRD increased clearly between zero and 1 sleeper, but only slightly (much less than proportionally) with an increasing number of sleepers; there was a relatively large variation around the means. At high vector densities, there was no demonstrable effect of the number of sleepers. This investigation was repeated taking into account in the PSC only those females which had presumably entered the hut the night before the collection, i.e., appearing as empty, partly fed, fully fed or late fed (see pp. 66-67); this led to the same conclusions as in Table 3 where all females were included. The number of sleepers thus has a limited effect on the IRD; in addition, the number of sleepers per hut varied relatively little, either in the course of time or between village clusters, and should therefore not affect comparisons in time or space. The number of sleepers was nevertheless used to compute alternative indices of the IRD, for comparison. Three indices of indoor-resting densities were computed for A. gambiue s.l. and for *A. junestus: (1)* the total number of female mosquitos per hut; (2) the number of partly or fully-fed females per sleeper; (3) the number of partly, fully- or late-fed females per sleeper. The second and third indices are estimates of the number that have fed on 1 sleeper in 1 night, and are sometimes used for

estimating the man-biting rate (MBR). All 3 indices were used before intervention, to describe a time trend, i.e., the change in IRD in the course of the wet season, and to study geographical variation, i.e., to compare the IRDs of the 8 village clusters. The 3 indices behaved in parallel, i.e., they differed only by a scaling factor. This could be expected in view of what was found regarding the number of sleepers and of the fact that the proportion "fed" (by either of the definitions given above) varies relatively little in the course of the wet season or between village clusters. The 3 indices stood, on the average, in the following relationship: 1.00 : 0.21 : 0.30 for A.gambicae s.l., 1.00 : 0.28 : 0.31 for A.funestus. The first index was adopted in this study to calculate the IRD.

The number of bites per man per night, measured by NBC, was used as man-biting rate (MBR); it was computed separately for the collections made indoors and outdoors and also for the total; the latter constitutes an unweighted average MBR (see below).

The IRD, the average MBR and the exit-trap collection (ETC) (number of females per trap night) were computed, for each of the 8 villages for which the information was available, for ***A. gambiae*** s.l. and for ***A. funestus*** during the wet season of 1971 (baseline phase). The correlations between the 3 indices were quite strong and approximately linear. The simplest interpretation of this finding is that: (1) all 3 measurements are valid indices of variations in density before residual spraying; (2) the sampling was adequate; and (3) the villages are really different. The correlations were, however, not Perfect and there was some variation, in both time and space, in the ratios between indices; this may result from sampling error but may also reflect differences in vector behaviour. The relation between MBR and IRD (see above) is, however, completely upset after residual indoor spraying (see pp. 77, 84).

The sensitivity of the density indices is determined by the sampling design. With 8 man-nights, 10 huts and 5 exit-traps, as normally used during the course of the present work, the lowest measurable indices are: ***(a)*** MBR = 0.125 bite/man/night; ***(b)*** IRD = 0.1 female/hut; (c) ETC = 0.2 female/trap/night.

Baseline Findings

In this section we shall consider not only the observations made during the baseline phase, but also some observations made during the subsequent phases in the untreated comparison villages.

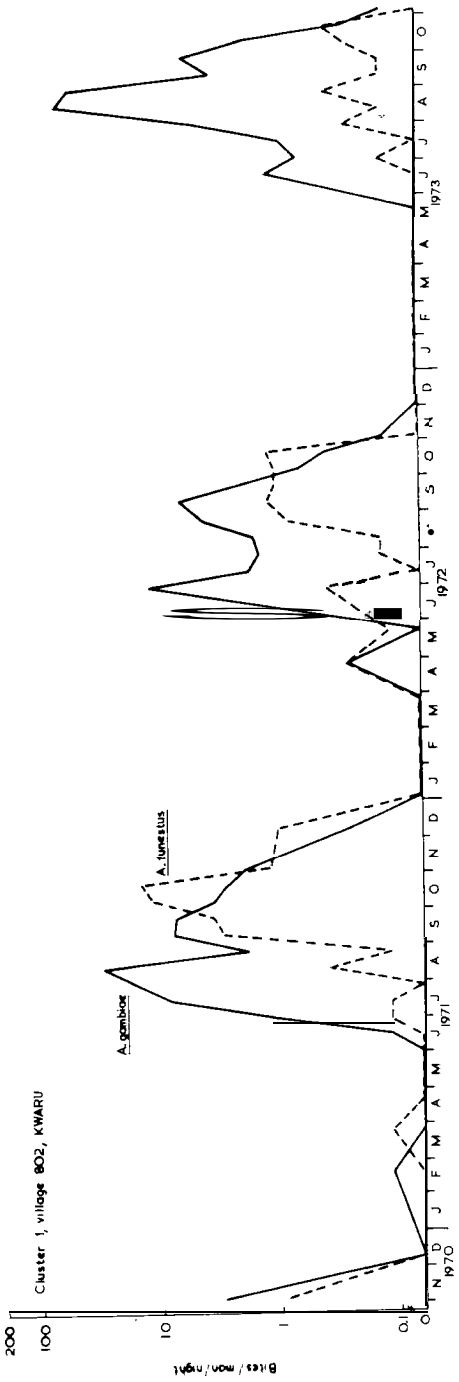


Fig. 7. Man-biting rates of *A. gambiae* s.l. and *A. funestus*, estimated from the night-bite collections in Kwaru, an untreated comparison village, in 1970-1973

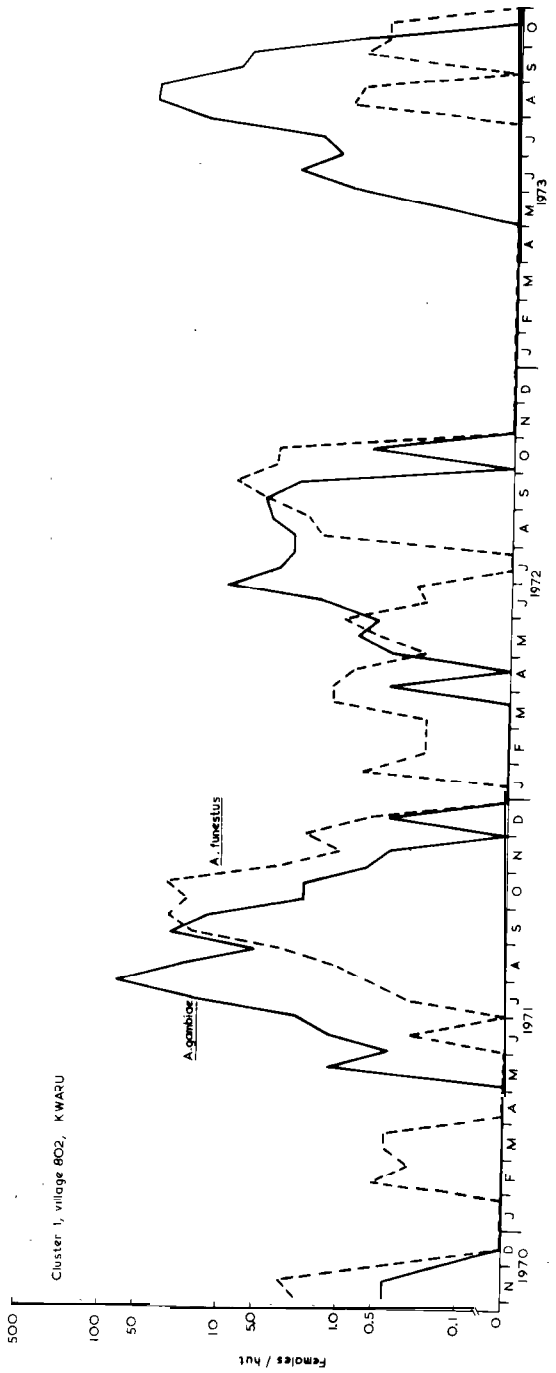


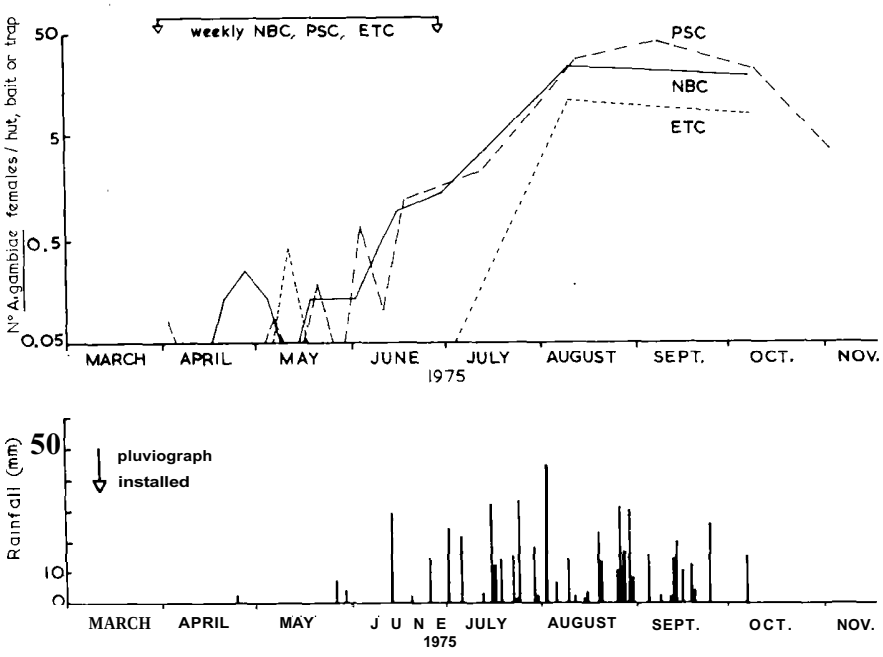
Fig. 8. Indoor-resting densities of *A. gambiae* s.l. and *A. funestus*, estimated from the pyrethrum spray collections in Kwaru, an untreated comparison village, in 1970-1973

Vector density

The average MBR (4 man-nights indoors and 4 outdoors) varied between 0 (or below the measurable threshold) and 174 and 94 bites/man/night, for ***A. gambiae*** s.l. and ***A.funestus*** respectively. Both maxima were observed in Sugungum (cluster No. 3, village 408). The IRD varied between 0 and 223 and 171 females/hut, for ***A. gambiae*** s.l. and ***A.funestus*** respectively (averages of 6 and 7 huts). Both maxima were again observed in Sugungum.

There was a very large seasonal variation in vector density, illustrated in Kwaru, an untreated comparison village, over a period of 3 years, as shown in the MBR (Fig. 7) and the IRD (Fig. 8). The yearly wave of ***A.gambiae*** s.l. regularly precedes that of ***A.funestus.*** The MBR and IRD showed the same trends. The density of ***A. gambiae*** s.l. decreased

Fig. 9. Rainfall and density of A. *gambiae* s.l. by night-bite collection (NBC), pyrethrum spray collection (PSC) and exit-trap collection (ETC) in Ajura, an untreated comparison village, in 1975 a



a Note that the lowest measurable densities were 1/8 or 0.13, 1/12 or 0.08, and 1/5 or 0.2, by NBC, PSC and ETC respectively.

from 1971 to 1972, and increased from 1972 to 1973, while that of ***A.funestus*** decreased from 1971 to 1972 and decreased further from 1972 to 1973. The same trend appeared also in the ETC in Kwaru, and also in the NBC, PSC and ETC in the other untreated comparison village, Ajura. The seasonal increases in density are obviously related to rainfall (see Chapter 2). The density of ***A. gambiae*** s.l. rises very early in the wet season, and in 1975 a special attempt was made to follow the relationship between rainfall and densities recorded through NBC, PSC and ETC. The results obtained in Ajura (Fig. 9) illustrate this relationship, although they are somewhat ambiguous. The first rains were: 2 mm on 25 April, 7 mm on 27 May, 4 mm on 30 May, and 29 mm on 14 June. ***A.gambiae*** s.l. did not disappear during the dry season (see also p. 56). The first clear-tut increase in density, in particular biting density (NBC), was observed on 9 June, i.e., 13 and 10 days respectively after the rains of 27 and 30 May. At the high temperatures prevailing in that season (see Fig. 3) this is a sufficient interval to explain the increase in population by breeding following the rains (see ref. 70), especially if some females reach the end of the dry season in the gravid state (see p. 66 and ref. 130). We have unfortunately no direct proof of successful larval development after the small precipitations of 7 mm and 4 mm on 27 and 30 May. With respect to variation between the years, rainfall decreased from 1971 to 1972 and from 1972 to 1973 (see Chapter 2); this was reflected by the density trend of ***A.funestus,*** but not by that of ***A. gambiae*** s.l.

There was a relatively large and significant variation between villages, for all density indices, and for both ***A. gambiae*** s.l. and ***A. funestus.*** The villages with the lowest and highest vector densities were Rafin Marke (cluster No. 5, village 154) and Sugungum (cluster No. 3, village 408), respectively. Their prespraying MBR is shown in the left half of the graphs for the effect of propoxur (Fig. 10 and 11). The average MBR for the wet season of 1971 varied between villages from 6.1 to 67.2 for ***A. gambiae*** s.l., and from 0.1 to 24.1 for ***A.funestus.*** The latter species had a relatively large MBR only in Sugungum and Kwaru, both located in the south-west of the area. Surface water persists longer in that area; there is in particular a semi-perennial swamp near Sugungum. In the dry season, with the sampling methods adopted (see p. 30), the MBR and IRD dropped to 0 for some time, except in Sugungum where the MBR of ***A. gambiae*** s.l. and the IRD of ***A. gambiae*** s.l. and ***A. funestus*** remained low but regularly measurable. The variation between villages of the biting rate or resting density of either ***A. gambiae*** or of ***A.funestus*** was not significantly correlated with the differences in rainfall between the same villages. In addition, the variation between villages in vector density was proportionately much larger than the variation in rainfall.

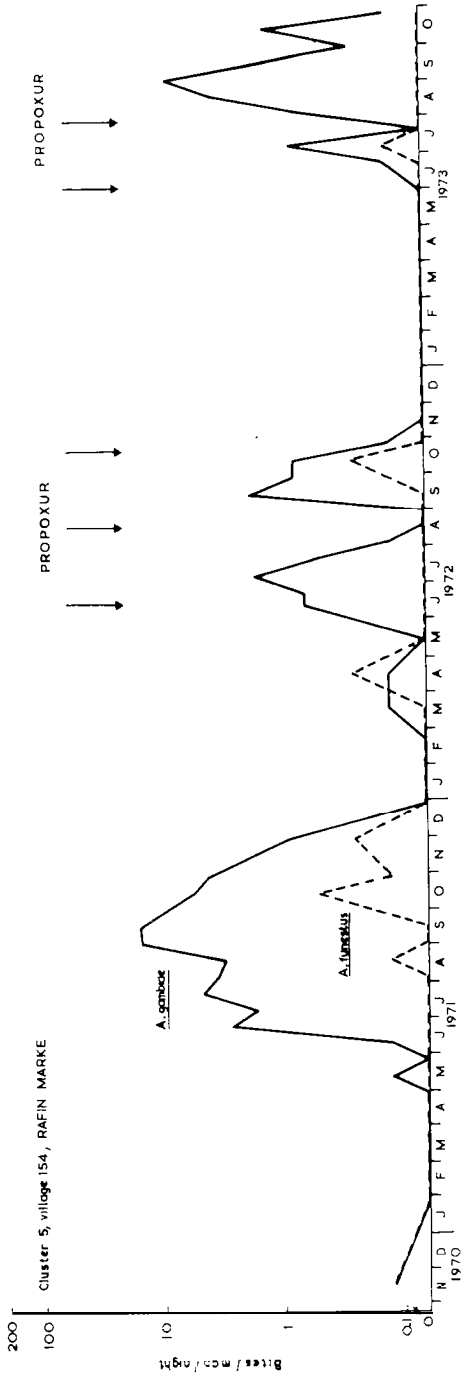


Fig. 10. Effect of propoxur on the man-biting rates of *A. gambiae* s.l. and *A. funestus*, estimated from the night-bite collections in Rafin Marke, the village with the lowest prespraying densities

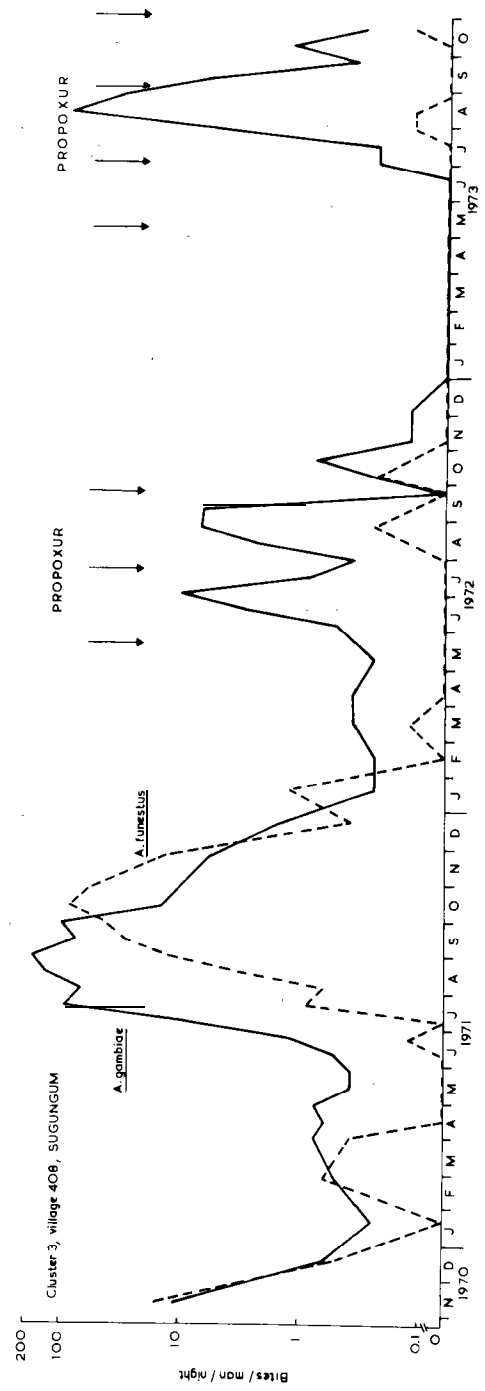


Fig. 11. Effect of propoxur on the man-biting rates of *A. gambiae* s.l. and *A. funestus*, estimated from the night-bite collections in Sugungum, the village with the highest prespraying densities

In some villages there were, not surprisingly, significant differences between different NBC or PSC stations. For the PSC a comparison was also made between the numbers collected in huts always sampled over a period of time and the numbers collected in huts missed at one or more collection cycles; since no significant difference was found, all huts sampled at a given time are included in the results presented.

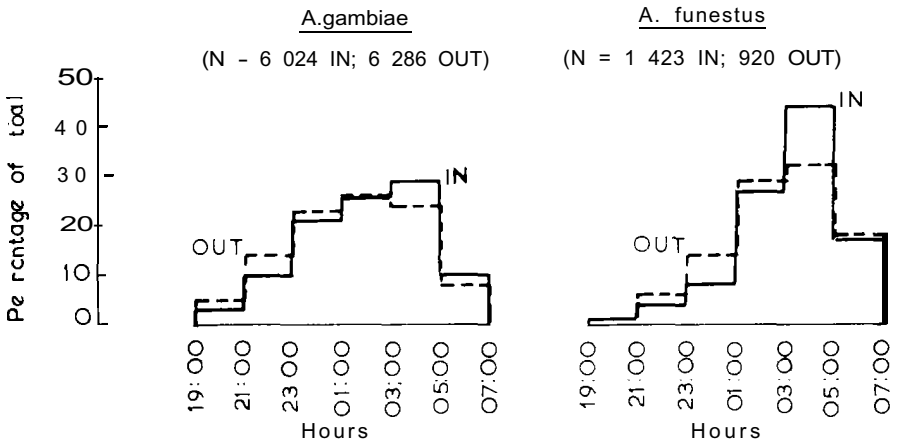
The MBR measured on baits exposed throughout the night was nearly the same indoors and outdoors in the case of A. gambiae s.l., but greater indoors than outdoors in the case of ***A. funestus.*** The ratio between the total numbers collected by NBC indoors and outdoors in the wet season of 1971 was ***0.96*** for ***A. gambiae*** s.l., 1.55 for ***A. funestus;*** the difference is highly significant.

The nocturnal cycle of the MBR of ***A. gambiae*** s.l. and ***A. funestus*** (Fig. 12) was determined in each successive hour of the night indoors and outdoors. It was found that both species bite mainly in the second half of the night, ***A.funestus*** biting later than ***A.gambiae*** s.l. The outdoor biting cycle, as compared to the indoor, is shifted slightly towards the beginning of the night in both species.

The exit-trap collection (ETC), as already mentioned (see pp. 56, 60), was a reflection of the MBR and IRD.

The numbers collected in the artificial outdoor shelters were very small: 199 females of ***A. gambiae*** s.l. and 32 of ***A.funestus*** collected in 3 villages (165 and 30 in Sugungum alone) during the entire baseline

Fig. 12. Biting *cycle* of *A. gambiaes.l.* and *A. funestus,* indoors and outdoors, in thewet season of 1971, before spraying, on human baits available throughout the night a



a **Results from 300 man-nights indoors and 300 man-nights outdoors.**

period. This probably shows that the sampling method is insensitive, rather than that the population resting outdoors is small.

Sporozoite rates and inoculation rates

As expected, the sporozoite rates increased in the course of the wet season. The maxima observed in 8 villages in 1971 in the ***A. gambiae s.l.*** collected by NBC varied between 1.9% and 11.8%. For ***A. funestus*** the maxima were 1.4% in Kwaru, 2.9% in Sugungum.

For both species in 1971 the sporozoite rate was greater in the vectors collected on human bait indoors than in those collected on human bait outdoors. This was very consistent in the individual villages and significant for all villages combined: 3.0% (111/3743) versus 1.9% (66/3451) for ***A; gambiae*** s.l. ($p<0.01$); 1.7% (12/691) versus 0 (0/234) for ***A. funestus*** ($p<0.05$).

The distribution of sporozoite-positive ***A. gambiae*** s.l. by hour of the night was very nearly the same as that of the biting density (Fig. 13). Note that the sampling for dissection, within the NBC (see p. 31), was designed to ensure equal sampling fractions by hour. For ***A. funestus*** the total number of positives was too small to justify an analysis by hour of the night.

The sporozoite rate was determined only for some of the PSCs and then usually on smaller numbers than for the corresponding NBC. Comparison of the NBC and PSC sporozoite rates from the same time and place reveals no systematic difference for either species.

Fig. 13. Distribution by hour of the night of the sporozoite-positive bites and of all the bites by *A.* gambiae s.l., before spraying; all villages combined

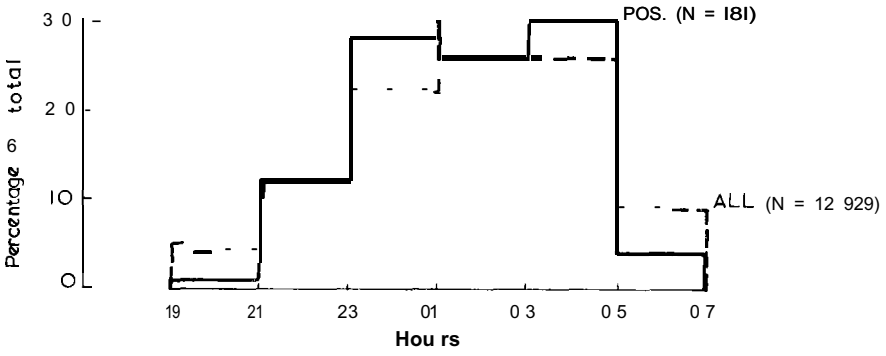


Table 4

Cumulative entomological inoculation rates (total number of sporozoite-positive bites per person) over entire wet seasons, estimated by NBC in 8 village a

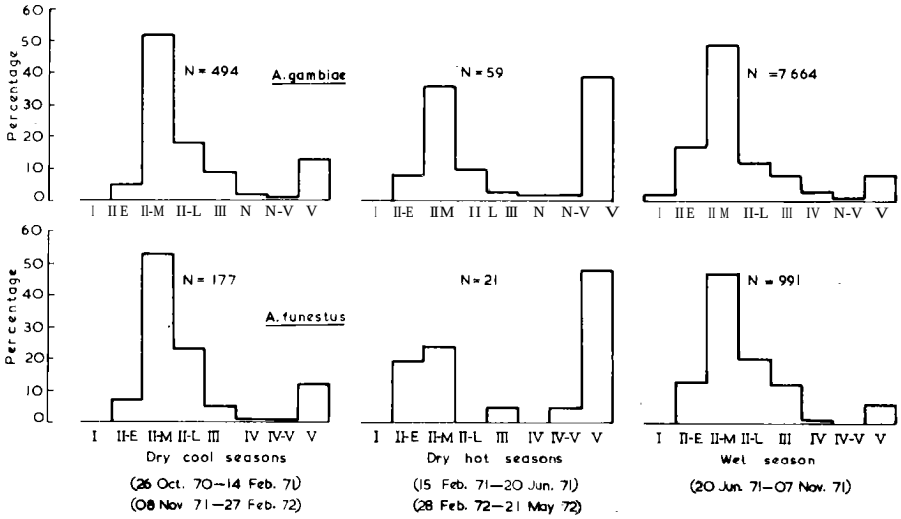
Village	Treatment in 1972-1973	1971 (21 Jun-7 Nov)	1972 (22 May-22 Oct)	1973 (18 Jun-4 Nov)	1974 (29 Jul-15 Dec)	1975 (14 Jul-30 Nov)
1. Kwaru	None	18(4)	17(2)	21(2)	–	–
2. Ajura	None	37(2)	25	28	–	
3. Sugungum	Propoxur	132(56)	0	10	–	–
4. Ungwar Bako	Propoxur	48	3	4		–
6. Matsari	Propoxur and low-frequency MDA	68(2)	4	0	–	–
8. Jaya	Propoxur and low-frequency MDA	64	2	4		–
5. Rafin-Marke	Propoxur and high-frequency MDA	18	5	2	4	6
7. Nasakar	Propoxur and high-frequency MDA	129	0	4	16(4)	24

a In parentheses: the contribution of A. funestus to the total.

The inoculation rate (or entomological inoculation rate, EIR = MBR x SR) was computed as follows: the average MBR (IN and OUT) times the combined sporozoite rate (IN and OUT), for each species and the sum of the two. This is not the only possible method of computing the inoculation rate nor necessarily the best one.

The calculated EIR (*A. gambiae* and A.funestus) was found to range between 0 and a maximum of 2.2/man/night at Sugungum on 15 September 1971. The average for the wet season of 1971 varied between villages from 0.13/man/night (Rafin Marke and Kwaru) to 0.94/man/night (Sugungum). The cumulative inoculation rate in 1971 ranged from 18 in Rafin Marke and Kwaru to 132 in Sugungum (Table 4). The contribution of A.funestus to this total varied from 0 (4 villages) to 56 (Sugungum). In the dry season (late 1971 to mid 1972), the cumulative inoculation rate was 0, except in Sugungum where it was 13 (4 by *A. gambiae* s.l., 9 by A.funestus) In the untreated comparison villages the inoculation rates were relatively stable from one year to the next . The cumulative rates for *A.gambiae* s.l. and *A.funestus* combined, for the wet seasons of 1971, 1972 and 1973, were as follows: 18, 17 and 21 for Kwaru, and 37, 25 and 28 for Ajura. The contributions of *A. funestus* were 4, 2 and 2 in Kwaru, and 2, 0 and 0 in Ajura.

Fig. **14. Distribution, by Christophers' stages, of *A.gambiae* S.l. and *A.funestus*, collected by night-bite collection, according to season, in 1970-1972, before spraying; all villages combined**



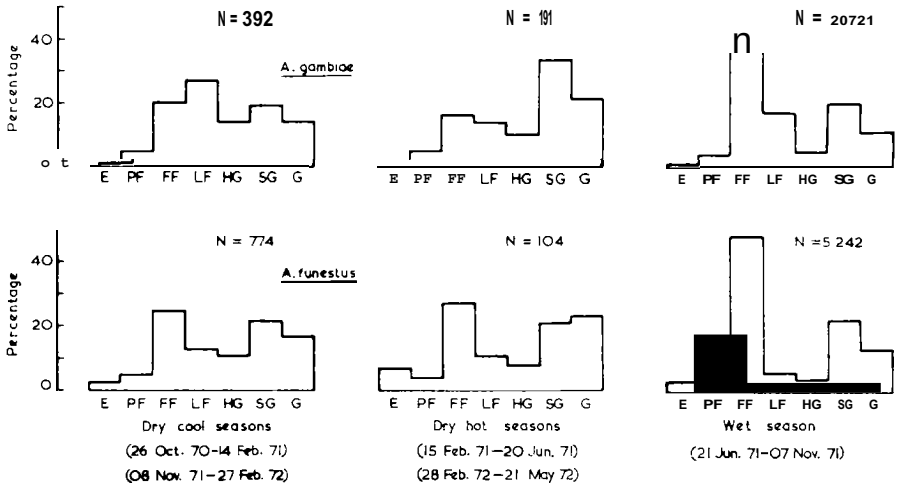
Vector behaviour

Feeding cycle

Figure 14 shows the distribution of ***A. gambiae*** s.l. and A.funestus collected by NBC and classified according to Christophers' stages (176). A proportion of the females feed in the gravid state; these females were collected in the act of probing or feeding and many of them had fresh blood in the stomach on dissection. The proportion feeding in the gravid state is low in the wet season, and increases in the dry season, especially at the end when the air is hottest and the densities are lowest. This is compatible with the hypothesis that in the wet season the vast majority of females take 1 blood-meal per gonotrophic cycle, while in the dry season, an increasing proportion takes more than 1 (see 43, 82, 130).

Figure 15 shows the distribution of ***A.gambiae*** s.l. and ***A.funestus*** collected by PSC according to the modified Sella stages of the abdomen (176). In the wet season the distribution is clearly bimodal, with the second cluster (subgravid and gravid) somewhat smaller than the first (fully and late fed). The first cluster probably fed during the night immediately preceding the PSC, the second cluster on the previous night. These findings combined with those from the ETC (see below) suggest that most vectors leave the hut to oviposit on the second night after feed-

Fig. 15. **Distribution byabdominal appearance (modified Stella stages) of A. gambiaes.l. and A. funestus collected by pyrethrum spray collection, according to season, in 1970-1972, before spraying; all villages combined a**



a E, PF, FF, HG, SG, G = empty, partly fed, fully fed, late fed, half gravid, sub-gravid, gravid, respectively.

ing, but that some leave (or perhaps die) earlier. The proportions between those fed (PF, FF, LF) and those gravid (HG, SG, G) excluding the empty, for the wet season of 1971, were found to be 0.64 and 0.36 for ***A. gambiae*** s.l., 0.59 and 0.41 for ***A. funestus,*** suggesting that a higher proportion of ***A. gambiae*** than ***A. funestus*** leave the huts before their eggs are mature. In the dry season the distribution of the PSC into Sella stages is more uniform, and the proportion gravid is larger: in the dry hot season it ***was 0.65*** for ***A. gambiae*** s.l. and ***0.56*** for ***A. funestus.*** This, together with the corresponding distribution of the NBC by Christophers' stages, suggests that during the dry season the act of oviposition and probably the maturation of the eggs are delayed and that more than 1 blood meal is taken per gonotrophic cycle. Such feeding is, however, of little consequence epidemiologically on account of the greatly reduced densities during that season.

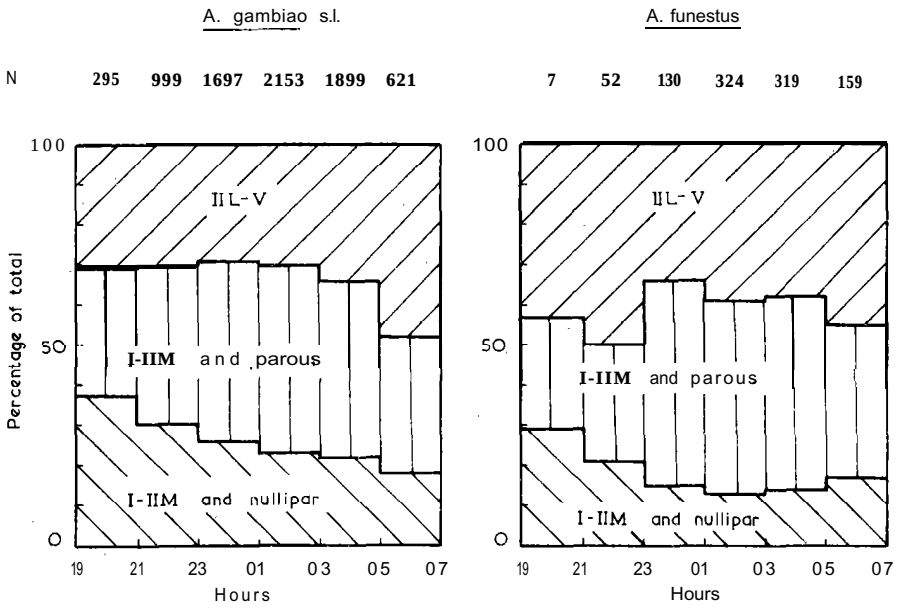
In the exit-trap, during the wet season, the females collected during the early part of the night (before 21hOO) are mostly gravid: 74% of the ***A. gambiae*** s.l. and ***67%*** of the ***A. funestus*** were classified as gravid or subgravid. The females collected during the remainder of the night are mostly empty or partly fed, amounting to ***73%*** of the ***A. gambiae*** s.l. and ***89%*** of the ***A. funestus.*** Most of the first group are probably leaving to

oviposit, while most of the second group are probably leaving in search of blood. In the dry season, the numbers collected by ETC were too small for further analysis.

Only 35 parous A. gambiae s.l., collected by NBC at various times and places in the wet season, were examined for ovariolar sacs; in all of them, the sacs were uncontracted, i.e., they had probably oviposited the same night. Together with the distribution of the PSC into Sella stages (see above), this suggests that A. gambiae s.l. feed every 2 days, at least in the wet season, when most transmission occurs. The information regarding ovariolar sacs is unfortunately net. available for ***A.*** *funestus.*

Given that vector mosquitos oviposit and refeed on the same night, it was interesting to find out how the distribution of the NBC into Christophers' stages and into nullipars and parous varied by hour of the night. The results for the wet season of 1971 were analysed (Fig. 16) by classifying the vector females into 3 groups: (1) 1-IIM *and* nullipar; (2) 1-IIM *and* parous; and (3) IIL-V, the first group being younger than the others

Fig. 16. Distribution of ***A. gambiae*** S.l. and ***A.*** funestus, collected by night-bite collection, into 3 groups according to Christophers and Detinova, by hour of the night; wet season of 1971, before spraying, all villages combined a



a N = numbers dissected. Females were first classified into Christophers' stages; those found in stages I-IIM were further divided into nullipar and parous following Detinova.

(see also p. 72). The proportion of these young mosquitos decreases in the course of the night, in particular for ***A. gambiae*** s.l., for which it decreases from 37% down to 18%. This is compatible with the hypothesis that gravid vectors oviposit and later feed in the course of the same night. However, considering together the biting cycle (see Fig. 12) and the variation in the composition of the NBC in the course of the night (Fig. 16), it is obvious that the biting cycle varies only to a small extent as a function of age. This is also implicit in the similarity of the biting cycle of sporozoite-positive ***A. gambiae*** s.l. to the biting cycle of the whole population (see Fig. 13).

The variation by hour of the night of the age composition of A. ***gambiae*** s.l. and ***A. funestus*** collected by NBC has been investigated by Hamon (77) in the area of Bobo-Dioulasso; he found no significant variation of the proportion parous for ***A. gambiae*** s.l., while for ***A.funestus*** the proportion parous went through a maximum in the middle of the night; the latter result is compatible with the finding in Garki of a minimum of young female ***A. funestus*** in the middle of the night (Fig. 16).

The above distribution of vectors into 3 groups was also used to compare the indoor and outdoor components of the NBC. There was no difference for ***A.gambiae*** s.l., while for ***A.funestus*** the proportion of young females was somewhat smaller outdoors. The difference between indoor and outdoor NBC regarding sporozoite rates therefore cannot be explained by a difference in age composition.

Degree of an thropophily

The source of blood meals was investigated by the precipitin test in the PSC and, to a small extent, in the collections made in the artificial outdoor shelters. In the PSC, the percentage of ***A. gambiae*** s.l. that fed on man was significantly lower in village cluster No. 3 (Sugungum) than in the others: 61 % (49/80) versus 91% (673/738). Among the ***A. funestus*** 97% (229/237) had fed on man, without significant variation between villages. In the artificial outdoor shelters, ***23%*** of the ***A. gambiae*** s.l. were positive for man (11/48, nearly all from Sugungum). Few of the blood meals taken on man were mixed (4/733). Most of the non-human sources of blood were horses or bovids.

Degree of endophagy

The relative magnitude of the indoor and outdoor biting densities of ***A. gambiae*** s.l. and ***A. funestes,*** estimated on human baits made available throughout the night, has been indicated above (see p. 63). This is not sufficient to measure the actual degree of endophagy of vector populations, but it suggests that ***A. gambiae*** s.l. feeds as readily outdoors as

indoors, while ***A. funestus,*** while feeding more freely indoors, also feeds quite readily in the open.

The sporozoite rates were greater in those ***A. gambiae*** s.l. and ***A. funestus*** biting indoors than in those biting outdoors (see p. 64), and the difference could not be explained by a difference in age composition (see above). It is therefore likely that those feeding on man outdoors have taken a larger proportion of their previous blood meals on animals. This suggests that relative exophagy and zoophily are associated with each other, and that both are stable characteristics of individuals among the ***A. gambiae*** s.l. and A.funestus. Such a behavioural difference is likely to have a genetic basis, and indeed Coluzzi found that within either species of the ***A.gambiae*** complex, the females biting man indoors differed from those biting man outdoors in the frequency of certain chromosomal inversions, although there was no significant frequency difference between those biting man outdoors and those biting donkeys (see pp. 98-99 and ref. 32).

Resting behaviour

The small numbers of vectors collected in the artificial outdoor shelters may reflect only the insensitivity of the outdoor-shelter sampling method (see p. 31). Some information about resting behaviour may be deduced from the effect of propoxur spraying (see p. 77), and also from the numerical relationship between the man-biting rate and the indoor resting density (119). The proportion of blood meals taken on man by ***A. gambiae*** s.l. which are followed by rest indoors has been estimated for the wet season of 1971 in the 6 study villages that were followed by NBC and destined to be sprayed the following year, as follows:

Let IRD = the true indoor-resting density
IRD = the indoor-resting density estimated by pyrethrum spray collection
$\widehat{IRD} = b_1 . IRD$
$b_1 = \widehat{IRD}/IRD$ = the bias of the estimated indoor-resting density
MBR = the true man-biting rate
$\widehat{MBR}$ = the man-biting rate estimated by night-bite collection
$\widehat{MBR} = b_2 . MBR$
$b_2 = \widehat{MBR}/MBR$ = the bias of the estimated man-biting rate
HBI = the proportion of blood meals, in the PSC, positive for man (human blood index)
N = the number of persons per hut (the population of the village, divided by the number of huts)

x = the proportion of blood meals followed by rest indoors (at least until the next morning, time of the pyrethrum spray collection)

T = period of rest indoors after feeding, in days

Then:

$$\text{IRD} \cdot \text{HBI} = \text{MBR} \cdot N \cdot x \cdot T$$

or

$$\frac{\text{IRD}}{b_1} \cdot \text{HBI} = \frac{\text{MBR}}{b_2} \cdot N \cdot x \cdot T \qquad [1]$$

$\hat{\text{IRD}}$, $\hat{\text{MBR}}$, HBI, N are measured directly. T is estimated by

$$T = 1 + \frac{G}{F},$$

where G, F are the proportions gravid and fed, respectively, in the PSC (if the maturation time is 2 days, as suggested by the clear-tut bimodal distribution of the PSC by abdominal appearance);

x and $\frac{b_2}{b_1}$ (the relative bias of the 2 sampling methods) are unknown. If we know the one, we can compute the other; from [1]:

$$x = \frac{\hat{\text{IRD}}}{\hat{\text{MBR}}} \cdot \frac{b_2}{b_1} \cdot \frac{\text{HBI}}{N \cdot T} \qquad [2]$$

$$\frac{b_2}{b_1} = \frac{\hat{\text{MBR}}}{\hat{\text{IRD}}} \cdot x \cdot \frac{N-T}{\text{HBI}} \qquad [3]$$

Values for the factors in equations [2] or [3] may be derived from the data for ***A.gambiae*** s.l. and A.funestus in the wet season of 1971 (Table 5). For A.funestus which is generally believed to be very endophilic, setting its x to 1 we can compute its $\frac{b_2}{b_1}$ ratio by formula [3] ; it is equal to 1.16 (i.e., in comparison with the indoor-resting density, the man-biting rate is overestimated by 16%). Taking the same value for ***A. gambiae*** (i.e., assuming that the relative bias of the 2 sampling methods does not vary between the two species) we can compute its x by formula [2]; it is equal to 0.47. Thus, tentatively, only about half of the blood meals taken on man by ***A.gambiae*** s.l. are followed by rest indoors (see Discussion).

Age composition and longevity

Age composition can be used either directly (e.g., to compare it ac-

Table 5

Estimation of the proportion of blood meals followed by rest indoors in the wet season of 1971 (21 June-7 November)

Factor a	A. gambiae s.l.	*A. funestus*
$\hat{MBR}$ (estimated man-biting rate)	*10 837/440 = 24.6*	*2053/440 = 4.67*
$\hat{IRD}$ (estimated indoor-resting density)	10 709/410 = 26.1	4103/410 = 10.0
HBI (human blood index) proportion of blood-meals positive for man, in vectors resting in huts	*5441624 = 0.672*	*125/126 = 0.992*
N (No. of persons/hut)[b]	2209/1507 = 1.47	1.47
T (duration of rest indoors, days)[c]	1 + 7449/13042 = 1.57	1 + *2068/3020 = 1.66*
x (proportion of blood-meals followed by rest indoors)[d]	*0.47*	1
b_2/b_1 (relative bias of the 2 sampling methods)[e]	*1.16*	*1.16*

a Estimated in the 6 villages followed by night-bite collection, and destined to be sprayed the following year, except when otherwise specified.

b Estimated in 5 of the 6 villages.

c Estimated in 22 villages, including the 6, by the formula $\hat{T} = 1 + G/F$, where G and F = No. gravid and fed in the pyrethrum spray collections.

d Set to *1* for *A. funestus,* computed by formula [2] for *A. gambiae.*

e Computed by formula [3] for *A. funestus* and set to the same value for *A. gambiae.*

cording to places, times, interventions) or to compute longevity. Both will be considered for A. gambiae s.l. only, the numbers available for ***A. funestus*** being much lower. Age composition was estimated over a whole wet season to minimize the effect of variable rates of emergence. Two indicators of age composition of the night-bite collection were computed: (1) the proportion parous among those eligible for the method of Detinova (42), i.e., among those found in Christophers' stages 1-IIM; and (2) the proportion that are either 1-IIM and parous, or IIL-V, among all dissected; the vectors included in this second index are not necessarily parous, but they are older than the remainder, i.e., than the 1-IIM and nullipars.

Table 6 shows the proportion 1-ITM, i.e., eligible for the method of Detinova and the 2 indices of age-composition, for the wet seasons of 1971 to 1975, in villages never sprayed (clusters No. 1-8 in 1971, No. l-2 in 1972-1973) currently sprayed (clusters No. 3-8 in 1972-1973),and pre-

Table 6

Age-composition of *A. gambiae* s.l collected by night-bite collection in the wet seasons of 1971 to 1975, in the presence or absence of propoxur

Year	Village clusters	Propoxur	Christophers' stages		Detinova method		No. I-IIM and parous, plus No. IIL-V, as a proportion of the total[b]
			Proportion I-IIM	(No. classified)	Proportion parous	(No. classified)[a]	
1971 (21 Jun.-7 Nov.)	1-8	no	0.68	(7 664)	0.64	(5 175)	0.76
1972 (22 May- Oct.)	1-2	no	0.40	(594)	0.49	(231)	0.80
	3-8	yes	0.56	(442)	0.39	(247)	0.66
1973 (18 Jun.-4 Nov.)	1-2	no	0.46	(1 780)	0.47	(801)	0.76
	3-8	yes	0.55	(1 707)	0.39	(925)	0.67
1974 (29 Jul.-15 Dec.)	5,7	no	0.63	(1 896)	0.62	(1 130)	0.76
1975 (14 Jul.-30 Nov.)	5,7	no	0.86	(1 233)	0.68	(809)	0.78

a Those found in stages I-IIM were examined by the Detinova method; very few of them were not classified.

b Assuming that the few found in stages I-IIM and not classified by the Detinova method were not different from the others; the effect of the assumption is negligible.

viously sprayed (clusters No. 5 and 7 in 1974-1975). The effect and after-effect of propoxur are dealt with later (see pp. 86-88). In the villages that were never sprayed, the proportion eligible for the Detinova method and the proportion parous among them (i.e., the first index of age composition) show a rather large change from 1971 to 1972-1973 which is not explicable by pre-existing differences between clusters No. l-2 and the others. The second index of age composition was more stable, varying only between 0.76 and 0.80 (in the absence of propoxur), and is therefore preferred for further analysie.

The second index may be considered as an estimate of the proportion of the NBC taking their second or a later meal. Given an interval of 2 days between blood meals, p (the probability of surviving 1 day) can be estimated by $\sqrt{P}$, where P = the second index of age composition. In the wet season of 1971, $p = \sqrt{0.76} = 0.87$, and the expectation of life, $1/(-\ln p) = 7.2$ days. An overestimation of the proportion taking their second or a later meal would lead to an overestimation of longevity, and vice versa. îhe result is also affected by the interval between first and second meals. A fraction of the A. gambiae probably take their second

meal, before their first oviposition, one day after their first meal(69). If this applies to half the population, the above estimates become:

$p = \sqrt[1.5]{0.76} = 0.83$ and $1/(-\ln p) = 5.4$ days.

Determination of physiological age by the method of Polovodova (42) i.e., classification of females by the number of ovipositions, has been applied on a small scale in the study area. The results are presented and discussed in a Technical Note (43). The youngest sporozoite-positive A. gambiae s.l. examined had oviposited twice, but it was not possible to relate physiological to chronological age.

Vectorial capacity

The entomological factors of transmission[a] are combined into the vectorial capacity $ma^2p^n/(-\ln p)$, which is the entomological component of MacDonald's basic reproduction rate (97) from which it was extracted by Garrett-Jones (66). The vectorial capacity is a daily rate of potentially infective contact and is the principal input variable for the malaria transmission mode1 (see Chapter 10).

For the baseline period, the vectorial capacity was calculated, for most of the year, on the basis of the following estimates:

- ma = MBR, averaging between the indoor and outdoor result, and smoothing between data points (see pp. 59-63).
- a = HBI/FC, where HBI = the human blood index, by PSC (see p. 69), and FC = the interval between blood meals (see p. 68).
- p = 0.819, corresponding to an expectation of life of 5 days. This value was selected on the basis of the project's findings (see pp. 71-73) and the literature review (20).
- n = 10 days, calculated from the temperature by Moškovskij's formula (42).

The vectorial capacity was thus obtained by multiplying the MBR by the following factors:

	Village clusters	
	No. 3	*Others*
A. gambiae s.l.	0.206	0.308
A. funestus	0.328	0.328

a m = the number of vectors/man; a = the number of meals taken on man/vector/day; p = the probability of surviving 1 day; n = the incubation period in the vector, in days.

Note that, in this computation, ***A. gambiae*** s.l. and ***A. funestus*** differ only by their human blood index. The vectorial capacity at a given time and place is the sum of the corresponding vectorial capacities of the two species.

During the hot dry season, March to June, several factors of the vectorial capacity may be affected, in addition to the obvious decrease in vector density: (1) the incubation period in the vector is shorter at high temperature; (2) the longevity of ***A. gambiae*** s.l. may be increased (82, 130) or shortened to a lesser extent than the incubation period, by analogy with the effect of a geographical temperature gradient on ***A. maculipennis*** (42); (3) feeding continues, in more or less complete gonotrophic dissociation (see p. 66 and refs. 82, 130). It was assumed that, in combination, these factors produced an increase in vectorial capacity. For convenience, this increase was approximated by increasing the expectation of life, without change in the other factors: the expectation of life was set to 7 days (p = 0.867) in the period of March to June. In that period, the vectorial capacity was thus obtained by multiplying the MBR by the following factors:

	Village clusters	
	No. 3	*Others*
A. gambiae s.l.	0.513	0.765
A. funestes	0.814	0.814

The baseline vectorial capacities, calculated as described above for Sugungum and Rafin Marke, the villages with the highest and lowest vector densities, respectively, are shown in Fig. 77 in Chapter 10. The figure shows also the critical vectorial capacity, below which malaria cannot remain endemic (see Chapter 10). In Sugungum, the vectorial capacity stayed well above the critical level throughout the year and reached a peak of about 40, Le., nearly 2000 times the critical value, in the wet season. In Rafin Marke, the vectorial capacity stayed below the critical level for about 5 months of the year and reached a peak of somewhat more than 200 times the critical value in the wet season.

Effect of Intervention, in particular Residual Spraying with Propoxur

It is unlikely that the very limited larviciding operations conducted in the dry season in and around village clusters No. 5 and 7 (area Al) had any significant effect. The comparison between these village clusters and those where only residual spraying with propoxur was applied provided

no evidence that the larviciding operation modified either the "tail" of the current breeding season of A. gambiae s.l. or the onset of the next one. Note that larviciding was not used in those village clusters which had high densities of ***A.funestus.*** The entomological effects of the intervention phase should probably be explained as entirely due to the residual spraying of propoxur. The small-scale preliminary trial of propoxur is considered in the first section, while the subsequent 5 sections are concerned with the intervention period of the main (large-scale) trial.

Results of the preliminary trial of propoxur

This trial and its results have been reported in detail elsewhere (104). The primary objective of the trial was to practise and evaluate the operations (see Chapter 3); the secondary objective was to obtain a preliminary evaluation of the entomological effect of propoxur, although limited by the small scale of the trial. The trial was conducted in 1971 and involved spraying with propoxur (2 g/m^2 of sprayable surface) the villages of Kaya, Gana and Masheme located about 5 km north of Garki (see Fig. 1). The 3 villages were sprayed before the onset of the rains. It was decided a ***priori*** to evaluate a single round of spraying in Masheme and 2 rounds in Kaya; Gana was included in the first round because of its proximity to the 2 other villages. It was arbitrarily decided to respray Kaya 3 months after the first application or earlier if either the IRD reached 1 vector/hut or if the MBR reached 1 bite/man/night, either indoors or outdoors. Some operational conclusions of the trial were mentioned in Chapter 3.

The entomological evaluation, in the absence of baseline data from Kaya and Masheme, was made by comparison with the contemporary baseline data from the villages selected for entomological follow-up in the main study but excluding Sugungum. Kaya and Masheme were followed by NBC, PSC and ETC in exactly the same way as the other villages (see Chapter 2). The effect of propoxur ***on A.funestus*** could not be evaluated because, even in the majority of the untreated comparison villages, the density of ***A.funestus*** barely exceeded the limit of sensitivity of the methods used (see page 56).

A preliminary evaluation of the effect of propoxur on ***A. gambiae*** s.l. was, however, obtained. The first round of spraying took place when vector density was negligible. In the unsprayed villages, density began to increase about 5 weeks later. In the sprayed villages, this increase was partly controlled, but, in both villages the IRD, **MBR(IN)** and **MBR(OUT)** all stayed below 1 for only about 2 months after spraying. Kaya was accordingly resprayed 72 days after the first round. In Masheme, which was not resprayed, both IRD and MBR increased

rapidly to levels within or above the range of the comparison villages, and the total seasonal production of *A. gambiae* s.l. was probably little affected by the single early round of spraying. In Kaya the IRD stayed below 1 for the remainder of the wet season, while in the unsprayed villages it reached peaks of from 7 to 224 according to the village. In Kaya, the rise in MBR was again partially controlled, but the MBR rose above 1 some 3 and 5 weeks after spraying, outdoors and indoors respectively. Peak MBRs of 4 indoors and 17 outdoors were observed 38 days after respraying. Among the comparison villages, the seasonal peaks varied from 26 to 102 indoors, and from 18 to 62 outdoors.

Propoxur thus reduced the IRD more than the MBR, and the MBR (IN) more than the **MBR(OUT).** No significant difference was detected between sprayed and unsprayed villages with respect to the proportion parous in the NBC, or the proportion gravid in the PSC or ETC. Sporozoite rates were also similar: the seasonal averages combining NBC and PSC were 2.9% in the comparison villages (356/12 162), 1.5% in Mesheme (35/2305), 3.2% (10/317) in Kaya. Sporozoite-positive *A. gambiae* s.l. were found 69 and 75 days after the first round, 12 and 40 days after the second (1 positive A.funestus was found 41 days after the first round).

The wall bioassays, conducted in Masheme, showed persistence of a potent insecticidal effect of the sprayed mud walls for periods ranging between 2 and 4 months. The conclusions tentatively drawn from the preliminary tria1 were that propoxur was effective for 2 months, and that the persistence of relatively high man-biting rates especially outdoors, and the lack of detectable effect on the proportion parous and the sporozoite rate, were due to immigration of unexposed vectors.

On the basis of the preliminary trial, 3 rounds of spraying at 2-month intervals per wet season were planned for the main study.

Effect of propoxur on vector density

Effect of propoxur on the man-biting rate

The effect of propoxur on the MBR of *A. gambiae* s.l. and *A. funestus* may be seen in the graphs already presented (see Fig. 10 and 11) for the 2 villages having respectively the lowest and highest baseline densities. In the wet season of 1972 the MBR of *A. gambiae* s.l. is lower than in the wet season of 1971. In the dry season of 1973 it remains undetectable by the methods used (compare in Fig. 11 the dry seasons of 1971, 1972 and 1973). In the wet season of 1973, the MBR of *A. gambiae* is still lower than in 1971, but higher than in 1972. The averages for the wet seasons of 1971, 1972 and 1973 were 6.1, 0.7 and 1.6 in Rafin Marke; and 67.2, 3.1

and 13.7 in Sugungum.

The MBR of ***A. jùnestus,*** on the other hand, decreases from 1971 to 1972 and from 1972 to 1973. The averages for the wet seasons of 1971, 1972 and 1973 were 0.08, 0.04 and 0.01 in Rafin Marke, and 24.1, 0.08 and 0.04 in Sugungum. These changes have to be seen in relation with the contemporary spontaneous changes already mentioned (see p. 60), and illustrated for the untreated village of Kwaru (see Fig. 7). Here the average MBR figures for the wet seasons of 1971,1972 and 1973 were 7.9,2.9 and 13.4 for ***A.*** gambiae and 3.3,0.6 and0.2 for ***A.funestus.*** In the other untreated village, Ajura, the average MBR figures for the wet seasons of 1971, 1972 and 1973 were 10.5,4.9 and 11.0 for ***A.gambiae*** s.l. and 0.3, 0.2 and 0.01 for ***A. funestus.*** Relatively large spontaneous changes were thus occurring in the unsprayed comparison villages, and they were in the same direction as those observed in the sprayed villages.

TO measure the impact of propoxur on vector density in a given village, it is therefore necessary to take into account both the prespraying density in the same village and the concurrent changes in untreated villages. An attempt to measure the impact and to relate it to prespraying variables has been reported on elsewhere (115).

Table 7 shows the **actual** numbers ***of A. gambiae*** s.l. collected by NBC, indoors, outdoors and total, in the wet seasons of 1971, 1972 and 1973 in each of the 8 villages followed by NBC along with the subtotals for the treated and untreated villages. The numbers relating to villages are proportional to the MBR and are directly comparable to each other, because the sampling scheme was the same in the 8 villages and the 3 years. Only a few collections were missed, and only in periods of low to very low density, i.e., they have little effect on the total; this is one reason for using numbers rather than rates, the other being that the actual numbers are more easily amenable to statistical analysis. The numbers actually collected in 1972 and 1973 in a sprayed village were expressed as a fraction of the expected, i.e., the number that would have been collected if propoxur had not been applied. This expected number was calculated on the basis of the baseline (1971) number in the same village and of the relative change observed in the untreated villages between the baseline year (1971) and the intervention year considered (1972 or 1973).

For instance, 61 ***A. garnbiae*** s.l. were collected by indoor NBC in 1972 in Sugungum; in 1971 before spraying the number had been 2439. The corresponding number for the 2 untreated villages combined decreased from 854 in 1971 to 282 in 1972. The "number expected" in Sugungum in 1972 is defined as 2439 x (282/854) = 805, and the "observed" represents 61/805 = 0.076 or 7.6% of the expected. This ratio observed/expected is an adjusted residual density (MBR); a low value indicates a strong impact (a large reduction), and vice versa. An adjusted residual

Table 7

Number[a] of A. gambiaes.I. collected by night-bite collection (NBC) indoors (In) and outdoors (Out) in successivewetseasons, byvillage; and ratio of that number to expected,[b] in the spraved villages

Village	Treatment	1971 (21 Jun.-7 Nov.)			1972 (22 May-22 Oct.)			1973 (18 Jun.-4 Nov.,		
		In	Out	Total	In	Out	Total	In	Out	Total
1. Kwaru	None	359	275	634	95	138	233	386	689	1 075
2. Ajura		495	344	839	187	204	391	438	445	883
Subtotal		854	619	1 473	282	342	624	a24	1 134	1968
3. Sugungum	Propoxur in 1972 and 1973	2439	2 939	5 378	61 (0.076)[c]	186 (0.11,	247 (0.11,	289 (0.12)	804 (0.15)	1 093 (0.15,
4. Ungwar Bako		335	442	777	4 (0.036)	12 (0.049)	16 (0.049)	15 (0.046)	35 (0.043)	50 (0.048)
6. Matsari		943	522	1465	11 (0.035)	42 (0.15)	53 (0.085)	44 (0.048)	153 (0.16)	197 (0.10)
8. Java		670	670	1348	19 (0.086)	37 (0.099)	56 (0.098)	79 (0.12)	239 (0.19,	318 (0.18)
5. Rafin Marke		288	153	441	16 (0.17)	38 (0.45)	54 (0.29)	42 (0.15)	85 (0.30)	127 (0.22)
7. Nasakar		495	933	1 428	5 (0.031)	27 (0.052)	32 (0.053)	10 (0.021)	64 (0.037)	74 (0.039)
Subtotal		5 170	5667	10 837	116 (0.068)	342 (0.11)	458 (0.10)	479 (0.096,	1380 (0.13)	1 859 (0.13)

a The actual numbers collected are shown; the number of collections was nearly, but not exactly, constant for each village and wet season; in 1971, villages l-3 had 10 NBCs, villages 4-8 had 9; in 1972, village4 had 9, the others 10; in 1973, village 4 had 8, the others 10.

b The number expected was computed on the assumption that villages 3-8 would, in the absence of propoxur, have undergone the same natural change as villages l-2; e.g. the expected No. In, in village 3, in 1972 = 2439 x (282/854, = 805. The numbers expected are not shown.

c Ratio of the observed to the expected (i.e., 61/805 = 0.076,.

density of 7.6% means that a 92.4% reduction is attributed to the intervention. If one ignores the changes occurring in the unsprayed villages, one gets, in the example above, an unadjusted residual MBR of 61/2439 = 0.025, or 2.5%, i.e., a 97.5% reduction. The adjustment may decrease the calculated impact, as in 1972, or increase it, as in 1973. Such a measurement allows the impact of propoxur to be compared between villages, between years, between indoor and outdoor NBCs, or between vector species.

There was found to be a relatively large and significant variation between villages: in 1972 for the total NBC the ratio observed/expected varied between 4.9% in Ungwar Bako and 29% in Rafin Marke; in 1973 the ratio varied between 3.9% in Nasakar and 22% in Rafin Marke. There was a significant correlation between the 1972 and 1973 results in the same village, suggesting that the impact is related to some relatively stable characteristic of the village.

When the concurrent changes in the unsprayed villages are taken into account, as is done here, there is probably no significant difference in the effect of propoxur between 1972 and 1973: the second year shows a smaller effect in 3 villages, a larger one in the 3 others. The measured effect is significantly and systematically larger indoors than outdoors, but there is a significant correlation between the effect indoors and outdoors in the same village. With respect to species, the effect of propoxur on the MBR of A.funestus, even after adjustment for spontaneous changes, is greater than the effect on A. ***gambiae*** s.l. The MBR observed in Sugungum, adjusted for concurrent changes in Kwaru, was only 2% of the expected in 1972, 3% of the expected in 1973.

The variation between villages in the impact of propoxur on the MBR of A, ***gambiae*** s.l. was significantly correlated with some of the village's prespraying characteristics, namely, the NBC/PSC ratio and the median biting hour. The variation between villages in their response to propoxur was not clearly associated with variation in the proportions of A. ***gambiae*** and ***A. arabiensis,*** but it was clearly associated with variation in the frequency of certain chromosomal inversions in both of these species of the ***A.gambiae*** complex (see p. 98 and ref. 32). The same variation between villages was, on the other hand, not significantly correlated with several other prespraying characteristics (vector density, the ratios between NBC indoors and NBC outdoors, between ETC and PSC, between fed and gravid in the PSC or ETC), and was also not significantly correlated with variations in recorded spraying coverage, latitude, or distance from unsprayed villages.

The relationship between the prespraying (1971) NBC/PSC ratio of a village and the residual MBR of the same village in 1972 and 1973 was determined after adjustments of the PSC for the human blood index (HBI)

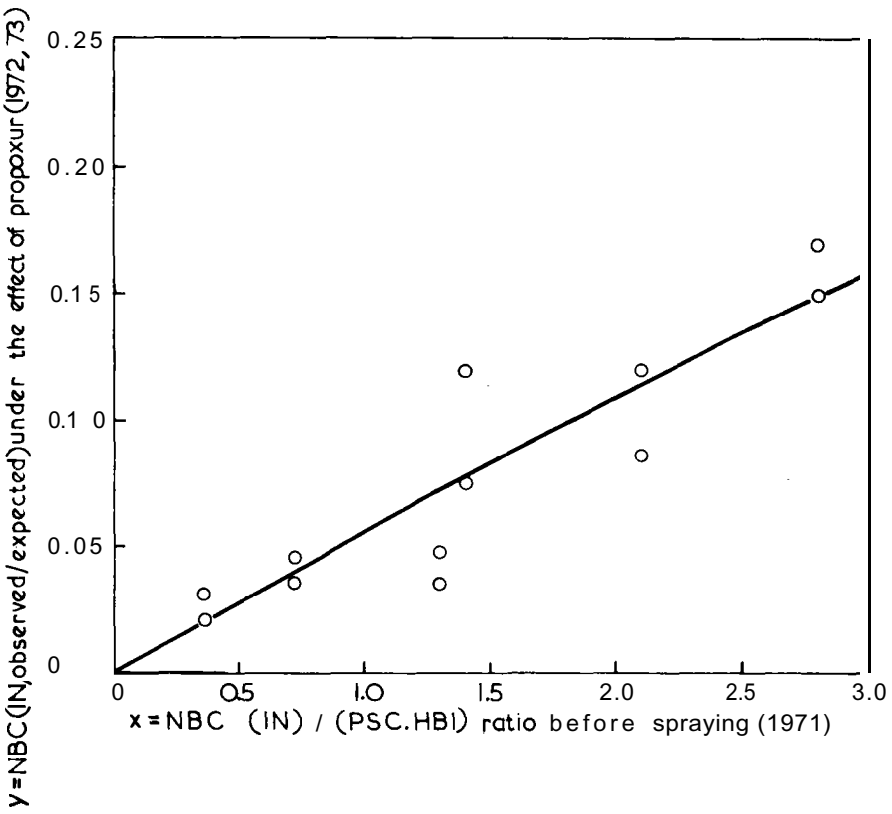
Table 8

Prespraying ratio of the man-biting rate of *A. gambiae* S.l. to its man-fed indoor-resting density, in the wet season of 1971, in various villages, and the residual NBC (observed/expected), under propoxur, in the wet seasons of 1972 and 1973

Village	NBC(In)/man-nights (PSC/huts) (HBI)[a] in 1971		NBC(In), obs./exp.[b] in 1972	in 1973	NBC(In + Out)/man-nights (PSC/huts) (HBI)[a] in 1971		NBC(In + Out) obs./exp.[b] in 1972	in 1973
5. Rafin Marke	288136 / (115/39) (50/51)	= 2.8	0.17	0.15	441 172 / (115/39) (50/51)	= 2.1	0.29	0.22
8. Java	670136 / (810/81) (104/117)	= 2.1	0.086	0.12	1348172 / (810/81) (104/117)	= 2.1	0.098	0.18
3. Suyungum	2430140 / (5066/69) (49/80)	= 1.4	0.076	0.12	5378180 / (5066/69) (49/80)	= 1.5	0.11	0.15
6. Matsari	943/36 / (1422/66) (123/132)	= 1.3	0.035	0.048	1465172 / (1422/66) (123/132)	= 1.0	0.085	0.10
4. Ungwar Bako	[c] 3 3 5 1 3 6 / (1125/79) (119/132)	= 0.72	0.036	0.046	777/72 / (1125/79) (119/132)	= 0.84	0.049	*0.048*
7. Nasakar	495/36 / (2019/47) (99/112)	= 0.36	0.031	0.021	1428/72 / (2019/47) (99/112)	= 0.52	0.053	*0.039*
			[c] r = +0.91 * R = +0.94*				c r = +0.72 R = +0.89*	
			r = +0.90 . R = +0.94*				r = +0.97 ** R = +1.00**	

a HBI = human blood index;
b from Table 7;
[c] r = correlation coefficient; R = Spearman's rank correlation coefficient;
● ,** : significantly different from zero at 5% and 1% levels, respectively.

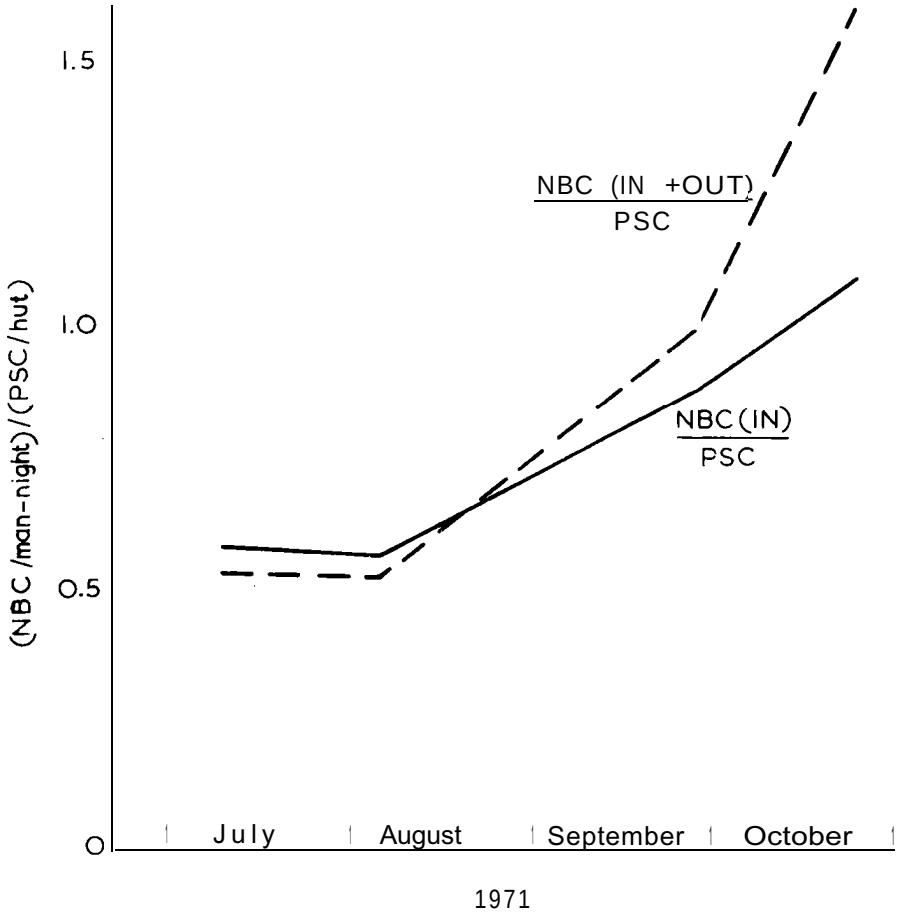
Fig. 17. Man-biting rate of *A. gambiae* s.l., under the effect of propoxur, as a function of the prespraying ratio between man-biting rate and indoor-resting density[a]



[a] Six villages each contributed 1 measurement before spraying (abscissa), 2 measurements after spraying (ordinate); see also Table 8. the curve represents the function $y = 1-e^{-0.058x}$; the value 0.058 was obtained by fitting.

and taking into account either the indoor NBC only (Table 8, left half, and Fig. 17) or the total outdoor and indoor NBC (Table 8, right half). Both the table and the figure show that: (1) the prespraying ratio varies rather widely between villages: NBC(IN)/PSC ranged between 2.8 in Rafin Marke and 0.36 in Nasakar; (2) as already noted (see Table 7), there is a strong positive correlation between the 1972 and 1973 results in the same village; (3) there is a strong and significant positive correlation between the prespraying NBC/PSC ratio and the residual MBR, i.e., the higher the prespraying NBC/PSC ratio, the higher the residual MBR and the poorer the control achieved. These conclusions thus apply equally for the NBC(IN) and for the NBC(IN + OUT). The figure also reveals that

Fig. 18. NBC/PSC ratio of *A. gambiae* s.l., by 4-week periods, during the wet season of 1971 before spraying; all villages combined



an exponential function may be fitted to the 12 data points (6 villages x 2 years), allowing the expected post-spraying MBR to be calculated from the prespraying NBC/PSC ratio.

The 4 species caught biting man in relatively large numbers-namely, the 2 species of ***A. gambiae*** s.l., ***A. funestus*** and ***A. pharoensis-were*** compared with respect to their prespraying NBC/PSC ratio and median biting hour, and their response to propoxur. Here also the adjusted residual MBR showed a positive correlation with the prespraying NBC/PSC ratio, but the correlation with the prespraying median biting hour was negative.

From the above it is likely that the prespraying NBC/PSC ratio is a good indicator of the degree of exophily. As shown by the combined figures for 1971 (Fig. 18), this ratio increases as the wet season progresses. This suggests that exophily increases in the course of the wet season, probably in relation to the increasein vegetation (63, 80).

Effect of propoxur on the indoor-resting density, the exit-trap collections, and the outdoor collections

In the sprayed villages, the numbers of vectors collected by PSC were very small: in about 1½ years after the first spraying, i.e., during 2 wet seasons and the intervening dry season, 2197 hut collections yielded 57 *A. gambiae* s.l. females and 8 A. *funestus* females. The huts used as capture stations were sprayed with propoxur like any other hut. Of the 57 *A. gambiae* s.l., 21 were caught in the wet season of 1972, 33 in the wet season of 1973, 3 in the intervening dry season. The captures were concentrated towards the end af the intervals between successive spraying rounds. They were also concentrated in certain villages, and, within villages, in certain huts. The comparatively small variation between villages with respect to PSC after spraying showed no correlation with the large variation in response to propoxur, as assessed from the NBC (see p. 80), and is probably to be explained by random variation between huts with respect to the adequacy of spraying.

The numbers collected by ETC in the sprayed villages were also very small: 1150 trap-nights (in huts which were sprayed like any other hut) yielded *8 A. gambiae* s.l. females and a single *A. funestus* female.

Village clusters No. 3 and 4 (area B) were also assessed by outdoor collections from artificial shelters. In the wet seasons of 1971 (baseline), 1972 and 1973 (intervention), 160, 176 and 160 collections respectively yielded 172, 15 and *62 A.gambiae* s.l. females, and 24, 13 and 2 *A. funestus* females. Nearly all were collected in cluster No. 3. In the unsprayed comparison cluster No. 2, there were 80,88 and 80 collections in 1971, 1972 and 1973 respectively; they yielded only 12, 2 and 18 *A. gambiae* s.l. females and no *A.funestus.*

Sporozoite rates and inoculation rates

During the whole intervention period, only 20 sporozoite-positive vectors, all *A. gambiae* s.l., were collected in the sprayed follow-up villages, 2 in pit-shelters in Sugungum and 18 by NBC. Each of the 6 sprayed villages assessed by NBC contributed to the 18 positives found, and the numbers contributed by different villages were very similar, varying only between 2 and 5. The sporozoite rates of *A. gambiae* s.l. collected by NBC in the wet seasons of 1971, 1972 and 1973 in the treated and un-

Table 9

Sporozoite rate of *A. gambiae* s.l collected by NBC, before, during and after the application of control measures

Village clusters	Treatment (in 1972-1973)	Sporozoite rate *(%)* of *A. gambiae s.l.* in the wet season, by NBC				
		1971 (21 Jun.- 7 Nov.1	1972 (22 May- 22 Oct.)	1973 (18 Jun.- 4 Nov.)	1974 (29 Jul.- 15 Dec.)	1975 (14 Jul.- 30 Nov.)
1,2	None	1.7 (24/1403)	3.2 (19/594)	1.4 (25/1780)	0.5[a] (1/188)[a]	3.0[a] (10/335)[a]
3, 4	Propoxur	1.4 (3512580)	0.4 (1/252)	0.7 (7/1005)	–	–
6, 8	Propoxur and low-freq.MDA	2.6 (52/1966)	2.8 (3/107)	0.4 (2/506)	–	–
5, 7	Propoxur and high-freq.MDA	3.8 (65/1713)	2.4 (2/83)	1.5 (3/196)	0.4 (9/1896)	1.3 (16/1233)

a In 1974 and 1975, only 2 NBC per wet season in only one village cluster.

treated village clusters (Table 9) do not demonstrate any large effect of propoxur, nor any marked increase in the effect by the addition of mass drug administration; however, it should be noted that these results are based on very small numbers of sporozoite positives. The estimated inoculation rate, on the other hand, was decreased greatly by the treatments, because of the great reduction in the man-biting rate. The cumulative entomological inoculation rates for the wet seasons of 1971, 1972 and 1973 (see Table 4) show a reduction from 132 to 0 and 10 in Sugungum and from 18 to 5 and 2 in Rafin Marke; there was little year-to-year change in the untreated comparison villages.

The capture of 18 positive A. gambiae s.l. by NBC in the sprayed villages suggests a large population of positive vectors because the sampling fraction is very small: 48 man-nights of NBC per fortnight out of 50 000 x 14 = 700 000 man-nights per fortnight in the whole of the sprayed area, or a fraction of 7 out of 100 000. The 18 positives captured may therefore correspond to a total of 18 x 100 000/7, i.e., about 250 000 sporozoite-positives.

Vector behaviour

No new information was collected during the intervention phase regarding the frequency of blood meals or the degree of anthropophily. It has already been mentioned that the indoor MBR decreased more than

the outdoor MBR (see p. 80 and Table 7), and this probably reflects selection of the more exophagic fraction of the population (see p. 70).

With respect to resting, there was certainly a marked decrease in the proportion of the population that was found resting indoors. This must be explained to a large extent by a marked increase in indoor mortality. Whether there was also a selection for exophilic behaviour will be discussed below (p. 92).

Age composition and longevity

The effect of propoxur on the age composition as determined by the Detinova method (see Table 6) could be seen by comparison with the 197 1 data and with the unsprayed villages. In 1972 and 1973, ***A. gambiae*** s.l. was, on the average, younger in the sprayed than in the unsprayed villages. The difference was significant but not very large. Only 22 of the ***A. gambiae*** s.l. captured in the sprayed villages were classified by the Polovodova method; 8/22, or 36%, had oviposited twice or more; in the unsprayed villages, the corresponding proportion was 75/173, or 43%, i.e., very similar ($\chi^2 = 0.16$; $p>0.60$) (43).

The interpretation of changes in age composition in terms of longevity is considered in the next section.

Vectorial capacity

Among the components used to estimate the vectorial capacity (see pp. 74-75) the application of propoxur affects ***ma, p,*** and possibly, ***a.*** The effect on ***ma*** was measured directly (see pp. 77-80); the effect on ***p*** has to be deduced from the change in age composition (see the preceding section). A given reduction in average age can, however, be interpreted in more than one way and the method adopted has a large effect on the computed vectorial capacity (119). A commonly used method of interpretation assumes implicitly that there is a uniform reduction in longevity, and a new ***p*** is calculated on that basis. If P is the proportion taking their second or later meal and the interval between blood meals is 2 days, then $p = \sqrt{P}$. We get, from Table 6 in 1972, in the unsprayed villages: $p = \sqrt{0.80} = 0.89$; in the sprayed villages: $p = \sqrt{0.66} = 0.81$. This by itself would reduce the vectorial capacity, for n = 10, by a factor of $\{(0.89)^{10}/(-\ln 0.89)) \div \{(0.81)^{10}/(-\ln 0.81)\}$, or about 5. This factor has been called the longevity factor of insecticidal impact by Garrett-Jones & Grab (67). If, however, longevity is not reduced uniformly, i.e., if some individual vectors have a higher than average probability of avoiding exposure after every meal, the impact on vectorial capacity is much smaller. If the vector population is composed of 2 fractions, one

completely endophilic (and exposed) and the other completely exophilic (and not exposed), then among the vectors collected by NBC after application of the insecticide the fraction belonging to the unexposed population is approximately P_2/P_1, where P_2, P_1 = the proportion of "old" vectors (e.g., taking their second or later meal) in the presence or absence of the insecticide, respectively.[a] In the same example as above, 0.66/0.80 = 0.83, or 83%, of the vectors collected after spraying would be unexposed; the exposed would make only a negligible contribution to vectorial capacity; and the longevity factor would be: 1/0.83 = 1.2, i.e., practically negligible in comparison with the effect on density.

Nonuniform exposure, due to nonuniform resting behaviour, is ***a priori*** more likely than the reverse, It fits also better with some other aspects of the data (119). In particular, under the hypothesis of nonuniform exposure, in the extreme form outlined, the mosquitos resting outdoors after feeding would always be the same, and the proportion resting indoors after feeding, estimated before spraying, should be equal to the proportion exposed among those taking their first meal, estimated after spraying. The first proportion was estimated as 0.47 (see p. 71); the second proportion is estimated by $(1 - P_2/P_1)/(1 - P_2)$, i.e., from Table 6, 0.51 in 1972 and 0.36 in 1973, which is not too different from the first proportion.

A direct proof of nonuniform resting behaviour was provided by the finding, in the Garki district, of an association between resting behaviour and the frequency of certain chromosomal inversions within each of the 2 species of the ***A. gambiae*** complex found in the various localities (see p. 98 and ref. 32).

The vectorial capacity during the intervention period was calculated twice, i.e., once according to the usual but implicit assumption of uniform exposure and once according to the probably more realistic assumption of nonuniform exposure; the latter was simplified even further by assuming that all vectors collected by NBC in the sprayed villages had a normal expectation of life, i.e., by neglecting the small "longevity effect". This means that the vectorial capacity was obtained by multiplying ***ma,*** estimated by NBC, by the same factors as before spraying (see pp. 74,275). Table 32 in Chapter 10 shows the seasonal average vectorial capacities, calculated in this way, for the period 1971 to 1973, i.e., the baseline and intervention phases, in the 4 villages used to test the transmission mode1 previously fitted to baseline data; 2 of the villages

[a] After spraying, very few of the exposed become "old" (e.g., take a second blood meal). Practically all "old" vectors collected after spraying (fraction P_2 of the total) belong to the unexposed fraction: that fraction has the same proportion of "old" individuals as before, i.e., for each "old" individual there are $(1 - P_1)/P_1$ young ones. Therefore, in the sample collected after spraying, the proportion belonging to the unexposed population is $(P_2 + \{(1-P_1)/P_1\}P_2)$ which simplifies to P_2/P_1.

were untreated, while 2 (Sugungum and Ungwar Bako) were treated with propoxur alone. During the 3 seasons of the intervention period-i.e., the wet season of 1972, the 1972-1973 dry season, and the wet season of 1973-the vectorial capacity was 0.66, 0.044, and 2.83 in Sugungum, i.e., 30, 2, and 129 times the critical vectorial capacity (see Chapter 10). In Ungwar Bako, the corresponding vectorial capacities were 0.068, 0, and 0.24, or 3, 0 and 11 times the critical value.

When these vectorial capacities, calculated under the assumption of nonuniform exposure, were used as mode1 input, they produced a reasonably realistic parasitological output (see Chapter 10); when the alternative vectorial capacities, calculated under the assumption of uniform exposure, were used instead, the expected impact of propoxur on malaria was systematically and markedly larger than the one actually observed, i.e., the parasitological output was clearly less realistic than under the assumption of nonuniform exposure (119).

After-effect of Propoxur, after Discontinuation of Spraying

In 1974-1975, after the end of the intervention phase, observations continued in village clusters No. 2, 5 and 7, i.e., in the village clusters included in the seroimmunological study. In clusters No. 5 and 7 (area Al), previously treated, the entomological observations were limited to the wet season, at the same frequency as previously. In cluster No. 2, untreated throughout, observations were made in the wet season at reduced frequency, but the transition from dry to wet season was observed in some detail in 1975 (see pp. 59-60). The results of these observations are described in the present section.

Vector density

The effect and after-effects of propoxur in 2 villages sprayed in 1972 and 1973 (Rafin Marke and Nasakar), as compared to the untreated village of Ajura (Table lO), were as follows: (1) the man-biting rate (MBR), reduced in 1972 and 1973, has already returned to "normal" in 1974; (2) the ratio between indoor and outdoor MBR, reduced in 1972-1973, is still reduced in 1974 and 1975, relatively more SO in Rafin Marke than in Nasakar; (3) the indoor resting density (IRD), reduced to very low levels in 1972-1973, increases in 1974-1975, but returns to "normal" only in Rafin Marke, and only in 1975; (4) the ratio between the biting rate and indoor-resting densities, considerably increased in 1972-1973, decreases

Table 10

Man-biting rates (MBR) and indoor-resting densities (IRD) of *A. gambiae s.l.* in 3 villages in the wet seasons of 1971 through 1975, i.e., before, during and after spraying with propoxur[a]

Village and treatment	MBR and IRD	1971 (21 Jun.-7 Nov.)		1972 (19 Jun.-5 Nov.)		1973 (18 Jun-4 Nov.)		1974 (29 Jul.-15 Dec.)		1975 (14 Jul.-30 Nov.)	
Ajura (2-553), compact part, untreated throughout[b]	MBR In	12.4	(40)	4.48	(40)	11.0	(40)	13.6	(8)	21.6	(8)
	Out	8.60	(40)	5.00	(40)	11.1	(40)	10.1	(8)	20.4	(8)
	In/Out	1.44	(63)	0.90		0.98		1.35		1.06	
	IRD	18.1		4.53	(68)	7.94	(69)	6.57	(14)	12.7	(13)
	MBR (In)/IRD	0.68		0.99		1.39		2.07		1.70	
Rafin Marke (5-154), sprayed with propoxur in 1972 and 1973	MBR In	8.00	(36)	0.35	(40)	1.05	(40)	4.61	(36)	5.38	(40)
	Out	4.25	(36)	0.90	(40)	2.13	(40)	8.03	(36)	10.1	(40)
	In/Out	1.88		0.39		0.49		0.57		0.53	
	IRD	2.95	(39)	0.02	(48)	0.02	(47)	0.44	(41)	3.76	(46)
	MBR (In)/IRD	2.71		17.5		52.5		10.5		1.43	
Nasakar (7-218), sprayed with propoxur in 1972 and 1973	MBR In	13.8	(36)	0.08	(40)	0.25	(40)	9.73	(40)	3.20	(44)
	Out	25.9	(36)	0.65	(40)	1.60	(40)	27.3		[illegible]	(40)
	In/Out	0.53		0.12		0.16		0.36	(40)	10.26	
	IRD	39.1	(52)	0.04	(46)	0.08	(48)	2.34	(47)	1.51	(45)
	MBR (In)/IRD	0.35		2.00		3.13		4.16		2.12	

a In parentheses: the number of collections (1 collection = 1 man-night or 1 PSC in 1 hut).

b In Ajura in 1974-1975 there were only 2 night-biting collections in each wetseason; only the corresponding pyrethrum spray collections are used in the table; the inclusion of the 4 PSCs available results in only minor changes.

in 1974-1975, but returns to "normal" only in Rafin Marke and only in 1975.

The relationship of the night-bite catch **(NBC),** along with the PSC and ETC, to the wet-season rainfall in the 2 sprayed villages (Fig. 19) shows that in 1974 and 1975, when the rains came earlier, there was no corresponding shift in the NBC to earlier dates. This delay of the NBC in relation to the rainfall in 1974-1975 is probably related to spraying with propoxur in 1972-1973 because, in 1971-1973 in the untrèated comparison villages, the NBC curve did follow shifts in the rainfall curve. Fig. 19 also shows that in the first half of the wet season of 1974 and 1975 in the villages sprayed in 1972 and 1973, the PSC and ETC were low in relation to the NBC, in comparison with the second half of the same wet seasons or with the wet season of 1971.

Sporozoite rates and inoculation rates

In the villages treated with propoxur (plus high-frequency MDA) the sporozoite rates of the A. gambiae s.l. collected by NBC were still low in the 2 years after the intervention period (see Table 9). In 1974 the rate was 0.4%, i.e., even lower than in the intervention period; in 1975, the rate was 1.3%, i.e., about the same as in 1973, the second year of intervention. It should be noted that in the wet season of 1974 4 rounds of chloroquine were administered to those aged less than 10 years (see Chapter 3). The untreated village is of limited value for comparison, because too few samples of the NBC were taken there. There was no significant difference of sporozoite rate between the indoor and outdoor NBCs. The sporozoite rate of the ***A. gambiae*** s.l. collected by PSC in village clusters No. 5 and 7 was 3.2% (4/126) in 1974-1975, i.e., significantly higher than in the NBC in the same period. There was no significant difference before spraying or in the untreated comparison village. Two positive ***A. funestus*** were collected by NBC in 1974 in village cluster No. ***7.***

The cumulative entomological inoculation rate, over the entire wet season (see Table 4) scarcely increased in Rafin Marke in the 2 years after the intervention period; but in Nasakar, where it had been relatively much more reduced during the intervention, it increased clearly in 1974 and 1975, although not approaching the very high pretreatment level.

Vector behaviour

The precipitin test was performed on fed ***A. gambiae*** collected by PSC in 1975. The human blood index was high: 0.93 (357/382). There was no difference between the baseline and post-intervention phases, nor be-

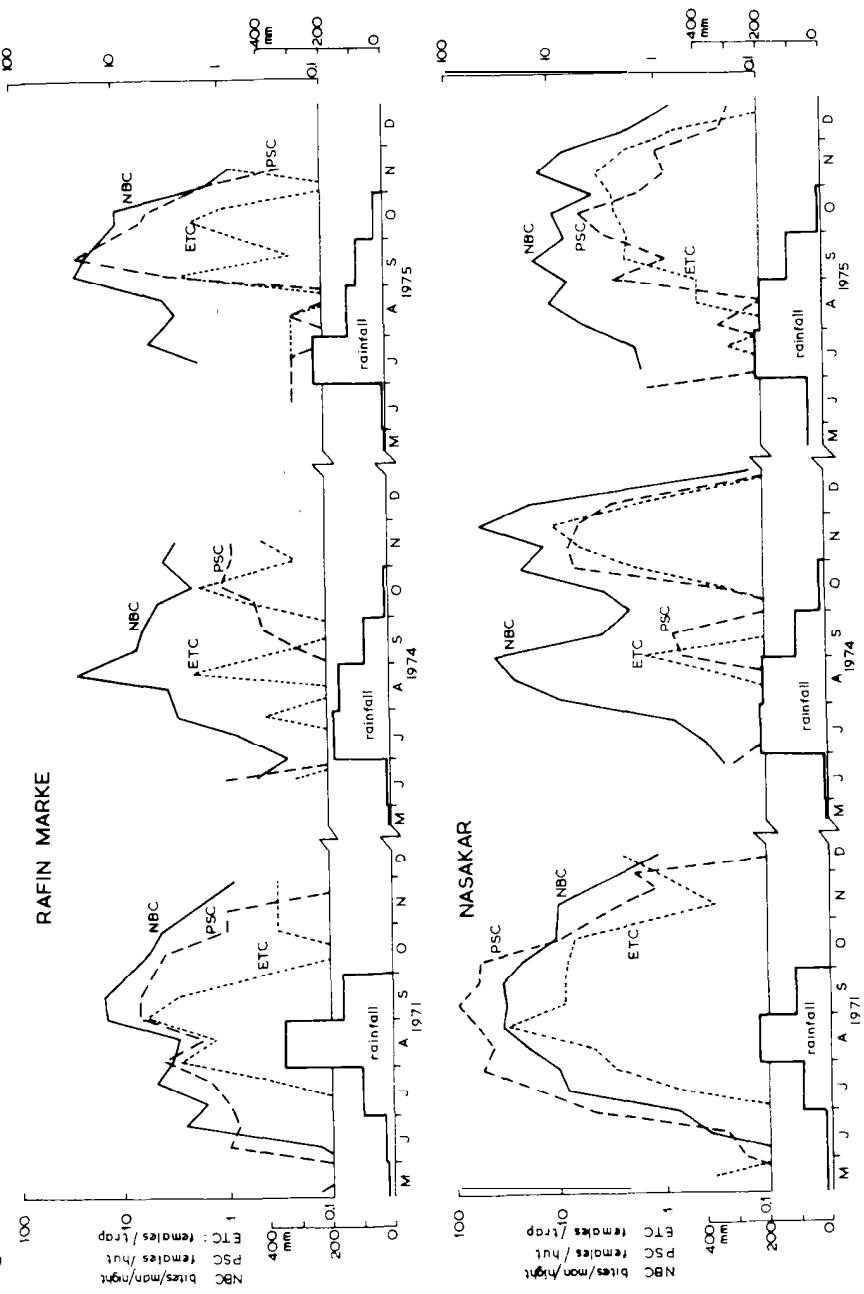


Fig. 19. Rainfall and density of *A. gambiae* s.l. in 2 villages in 1971 before spraying, and in 1974 and 1975 after discontinuation of spraying

tween previously treated and control villages, nor between compact and scattered villages.

The increase in the degree of exophagy, manifested by the decrease of the ratio between indoor and outdoor MBR, which had been observed during the intervention period, persisted in the post-intervention periods of 1974 and 1975 (see Table 10). This effect tended to fade away in the late wet season, when the bioassay indicated little or no insecticidal effect of the sprayed walls (see below). In October-December of 1974 and 1975, in the previously sprayed villages the ratio MBR(IN)/MBR(OUT) was 0.49 and 0.37, versus 0.53 in 1971.

In 1974, in the sprayed villages, there was still a reduction in the proportion of the vector population found resting indoors, as shown by the persistence of a high ratio MBR/IRD (see Table 10). In 1975, this effect had subsided in Rafin Marke, but not yet in Nasakar. This effect of propoxur has to be assessed in comparison with the spontaneous variation of the ratio MBR/IRD in Ajura, shown in the same table. The effect was mainly present in the first half of the wet season (see Fig. 19), when a persistent insecticidal effect of propoxur was demonstrable by bioassay (see below).

Age composition and longevity

In 1974 and 1975, since the age composition of the ***A.gambiae*** s.l. population was apparently back to normal in the sprayed villages (see Table 6), the longevity of these vectors had probably returned to normal also.

Bioassay and chemical tests

Air and wall bioassay tests were performed in previously sprayed huts and in control huts. The sprayed huts had not been renovated since the last spraying; the control huts had never been sprayed. The bioassay used ***A. gambiae*** s.l. females caught in villages that had never been sprayed, or their F, offspring (Table 11). In the wet seasons of 1974 and 1975, there was still a marked insecticidal effect, both by contact and airborne, which subsided in the latter. part of the wet season, when the relative humidity of the air decreases; there was no relationship between mortality and temperature. In the wet season of 1976, the insecticidal effect was somewhat smaller than in 1974 and 1975, but still easily demonstrable.

Chemical analyses of wall-mud specimens for propoxur were performed by the WHO Anopheles Control Research Unit at Kaduna. Only in 1 sample (10 x 10 x 2.5 cm deep), collected on 4 August 1975, was a trace of propoxur (0.028 - 0.093 g/m^2) detected.

Table 11

Bioassay tests (air and wall) conducted with *A. gambiae* s.l.[a] in huts sprayed with propoxur in 1972 and 1973

Date	Days after last spraying (6th round)	Test	Nasakar				Control[b]			
			N	% mortality imm.[c]	% mortality 24 h	RH (%)	N	% mortality imm.[c]	% mortality 24 h	RH (%)
5Sep. 1974	335	air	122	100		60-89	83	8.4	10.8	
2Oct. 1974	362	air	95	100	–	82-83	31	3.0	16.0	–
2Oct. 1974	362	wall	120	100	–	82	31	10.0	16.0	
31 oct. 1974	391	wall	109	20.0	52.0	32-36	56	0.0	3.5	45
11 Dec. 1974	432	wall	89	10.0	21 .o	18	47	0.0	0.0	18
31 Jul. 1975	664	air	96	75.0	100	63-79	70	2.9	12.9	67-80
7 Aug. 1975	671	wall	120	100	--	59	60	0.0	11.7	52
24 Sep. 1975	719	air	90	88.9	98.9	6676	60	0	1.7	
24 Sep. 1975	719	wall	120	100	–	76	60	0	8.3	70
30 oct. 1975	755	wall	118	1.7	'il.9	18	64	4.7	10.9	20
24 Aug. 1976	1053	air	119	49.6	52.9	56-71	66	1.5	7.6	52-64
24 Aug. 1976	1 053	wall	115	84.3	93.0	66	56	3.6	8.9	5965
30 Sep. 1976	1 090	air	77	39.0	53.2	58-76	48	4.2	4.2	52-64
30 Sep. 1976	1090	wall	116	75.9	87.9	53	60	0.0	13.3	68

a Fernales caught in the freshly fed state in villages that had never been sprayed, or their F_1 offspring (some of the controls on 24 Aug. 1976, plus all those used on 30 Sep. 1976), also freshly fed, except for the wall bioassay of 30 Sep. 1976, when 70% were unfed.

b On 5 Sep. 1974:Garki; on 2 Oct. 1974:Ajura; later:Achauya.

c Immediate.

Compact versus Scattered Settlements

In order to interpret any possible parasitological differences between the compact and scattered settlements (see Chapter 5), it was interesting to compare them in terms of entomological findings. NBC, ETC and ORC were performed only in compact settlements, but in 3 of the village clusters the PSC was split between a compact and a scattered settlement (see Chapter 2, Table 1 and Fig. 1). Within each of the 3 clusters, the indoor-resting density (IRD) of A. gambiae s.l. was found to be greater in the scattered part: 39 versus 18 females per hut in cluster No. 2, 21 versus 10 in cluster No. 8, and 60 versus 39 in cluster No. 7, all in the wet season of 1971, i.e., before intervention. The difference was very consistent: the scattered settlement had the higher IRD in 23 out of 30 comparisons ($p<0.01$). The difference between the clusters was, however, larger than the difference between compact and scattered settlements in the same cluster. There was no difference between the 2 types of settlement with respect to ***A. funestus,*** but no comparison was made in the 2 clusters with high densities of ***A. funestus,*** i.e., clusters No. 1 and 3.

The comparison between the 2 types of settlement with respect to the human blood index (HBI) in the PSC was made only in 1975. There was no significant difference: the HBI(PSC) was 1.00 (40/40) and 0.87 (48/55) in the compact and scattered parts of cluster No. 2 (never treated), 0.95 (160/168) and 0.95 (186/196) in the compact and scattered parts of cluster No. 7 (area A1).

There were, on the average, slightly more sleepers per PSC catching station in the scattered settlements (2.3 per hut) than in the compact settlements (1.8) of the same 3 village clusters. This is unlikely to explain any significant part of the difference in IRD (see p. 54 and Table 3).

We do not know whether the proportion of blood meals taken on man that are followed by rest indoors is the same in the scattered settlements as in the compact ones (see pp. 70-71), but it is unlikely to be larger, because "indoors" represents a much smaller fraction of the environment in the scattered settlements. Therefore, the findings regarding the indoor-resting density and human blood index can probably be interpreted as reflecting a higher man-biting rate in the scattered settlements. The fed/gravid ratio was the same in the scattered as in the compact settlements or slightly lower.

In 1972-1973, cluster No. 2 was left unsprayed; the scattered part continued to have a higher indoor-resting density of ***A.*** gambiae s.l. than the compact part: 7 versus 4.4 in the wet season of 1972, 27 versus 8 in the wet season of 1973. In clusters No. 7 and 8, which were sprayed, very few mosquitos were caught by PSC, in the scattered as well as in the compact settlements (see p. 84).

Anopheles gambiae and *A. arabiensis*

For a variety of technical and operational reasons, it has not been possible to include the systematic identification of the 2 sibling species of the ***A. gambiae*** complex in the main entomological sampling scheme. The available information cornes mainly from concurrent work by a WHO consultant (31) and from preliminary surveys in 1969-1970 (145). Further observations by Shidrawi in 1971-1973 were not available at the time of writing. Whereas the preceding sections have treated ***A. gambiae*** s.l. as a single species, the present section will describe the information available regarding the abundance and characteristics of the two species. The possible consequences of treating ***A. gambiae*** s.l. as a single species with respect to the understanding of the epidemiology of malaria are considered in the Discussion section.

In the study area, ***A. arabiensis*** (species B) of the ***gambiae*** complex was nearly always and everywhere the dominant species. ***A. gambiae s.s.*** (species A) was, however, found in nearly every single village from which at least 20 mosquitos were identified, and is probably present everywhere. There was some variation between villages with respect to the relative abundance of ***A. gambiae*** S.S., although no geographical pattern was detected. The abundance of both species increased markedly in the wet season; this increase was more pronounced for ***A. gambiae s.s.,*** which reached its maximum abundance towards the middle of the main breeding season. The proportion of ***A.gambiae s.s.*** in the total of ***A. gambiae*** s.l. varied according to method of collection and from year to year (Table 12). In the PSC it ranged between 6% in 1969 and 46% in 1972. The comparison between captures on donkeys (97/1053, or 9.2%, being ***A. gambiae*** S.S.) and on men (548/1769, or 31 .O%, being ***A. gambiae*** S.S. for all NBC and net-traps, in the absence of propoxur) strongly suggests that this species is more anthropophilic than ***A. arabiensis.*** The comparison between PSC, NBC, net-traps and ETC, in the absence of propoxur, does not suggest any clear-tut difference between ***A. gambiae*** and ***A. arabiensis.***

Propoxur was applied in 1972 and 1973, and only a ***few A. gambiaes.1.*** were captured by PSC in the sprayed villages; in the small samples examined, the proportion ***of A. gambiae*** did not differ significantly from what it was in the unsprayed villages. In 1971, i.e., before spraying, there was also no significant difference in the PSC: 373/1585 or 23.5% of ***A. gambiae*** S.S. in the villages to be sprayed versus 69/253 or 27.3% in the villages to be left unsprayed. In 1974, i.e., in the first year after spraying, the proportion of species A among ***A. gambiae*** s.l. was smaller in the previously sprayed villages than in the controls, both in the PSC and in the net-traps. The effect of propoxur on the relative abundance of the

Table 12

Proportion of *A. gambiae* s.s. as percentage of *A. gambiae s.l. (A. gambiae* s.s. and *A. arabiensis),* by year, method of collection and treatment[a]

Sampling method	Propoxur[b]	1969 !Sep.-Dec.)		1970 (Jul.-Oct.)		1971 (Aug.1)		1972 (Jul.-Sep.)		1973 (Aug.-Sep.)		1974 (Jul.-Sep.)	
PSC		5.9	(41/690)	11.8	(24412060)	24.0	(442/1836)	46.1	(967/2096)	27.4	(120/438)	24.4	(290/1187)
	+	–		–	–	–	–	47.6	(10/21)	20.0	(2/10)	8.9	(5/56)
Net-trap outdoors, humanbait		–	–	–	–	25.3	(108/427)	39.7	(93/234)	21.3	(12/527)	40.0	(182/455)
	+	–	–			–		–	–	–	–	*29.9*	(106/355)
NBC, outdoors		–	–			10.0	(3/30)	–		–	–	52.1	(50/96)
	+	–	–	–	--	–	–	–		–	–	–	–
Capture on donkeys outdoors		–		–	–	*0.0*	(0/30)	13.8	(16/116)	3.5	(17/482)	15.1	(644/425)
	+	–	–	–			–			–	–	–	–
ETC		–	–	–		–	–	–		–	–	42.2	(106/251)
	+	–	–	–	–	–	–	–	–	–	–	–	–

Sampling method	Propoxur[b]	1969 (Sep.-Dec.)		1970 (Jul.-Oct.)		1971 (Aug.)		1972 (Jul.-Sep.)		1973 (Aug.-Sep.)		1974 (Jul.-Sep.)	
ORC		–	–	–		0.0	(0/36)	—	—	—	—	5.1	(11/217)
	+	–	–	–	–			–	–	–	–	–	–
Adult females reared from larvae or pupae		--	–	–	–	–	–	–	–	–	–	–	–
	+	–	–			–	–	34.4	(32/93)	—	—	—	–

[a] Mainlyfrom Coluzzi et al. (31) for 1971-1974; from Shidrawi (145) for 1969-1970.
b Applied in 1972-1973, but still effective in 1974 .

sibling species was thus not very striking; it was, in addition, rather unsystematic when individual villages were considered (31). It should, however, be noted that the sampling scheme was not specifically designed to measure this effect and that the samples identified were small.

If the variation between villages with respect to the effectiveness of propoxur could not be explained by variation in the gambiae/arabiensis ratio, it was, however, related to the intraspecific frequency of certain chromosomal inversions, which are themselves related to differential resting behaviour (32).

Table 13

Percentage of blood-meals found positive for man by the precipitin test in *A. gambiae* s.s. and *A. arabiensis* collected by PSC in Jirima village and in the adjacent nomadic Fulani camp, both unsprayed[a]

Species	Village	Nomadic Fulani camp
A. gambiae s.s.	*100.0* (129/129)	*78.6* (66/84)
A. arabiensis	*82.9* (165/199)	30.4 (102/335)
Total	89.6 (294/328)	40.1 (168/419)

a From Coluzzi et al. (31).

Table 14

Sporozoite rates *(%)* of *A. gambiae* s.s. and *A. arabiensis* collected by PSC in unsprayed villages[a]

Village	*A. gambiae s.s.*	*A. arabiensis*	*P*
Jirima village	4.0 (8/199)	0.0 (0/329)	<0.001
Jirima nomadic Fulani camp	4.3 (3/69)	0.0 (0/182)	0.02
Ajura, Kwaru, Baribari, Gwadawa	9.4 (50/530)	4.0 (11/274)	<0.01
Total	7.6 (61/798)	1.4 (11/785)	<0.001

a From Coluzzi et al. (37).

In the unsprayed villages, Coluzzi also compared A. gambiae S.S. and A. arabiensis with respect to the source of blood meals and the sporozoite rates (Table 13). In the compact village of Jirima (unsprayed), where few large animals were present, both species had fed mainly on man, but the proportion fed on man was significantly larger in A. gambise S.S. In the adjacent temporary Fulani camp, where cattle outnumber men by about 10 to 1, 79% of A. gambiae had fed on men, versus only ***30%*** of ***A. arabiensis.*** These results are in obvious agreement with those given above regarding captures on men and donkeys. In examinations of the salivary glands (Table 14), ***A. gambiae*** was found to have a consistently and significantly higher sporozoite rate than ***A. arabiensis.***

Very few hybrid adult females were found: 2 out of 2750, or 0.07%, in 1969-1970 (145); 4 out of 9699, or 0.04%, in 1971-1974 (31). Hybridization must be very rare, or hybrids must have reduced viability, or both.

In summary, both ***A. garnbiae*** and ***A. arabiensis*** are present throughout the Garki district; ***A. arabiensis*** is the dominant species; the relative abundance of ***A.gambiae*** increases during the main breeding (wet) season; ***A. gambiae*** is the more anthropophilic and has a higher sporozoite rate. No obvious difference was detected between the species in exophagy and exophily, and no clear-tut difference was demonstrated regarding the reduction of their populations by propoxur.

Discussion

The estimation of the vectorial capacity

Given the presence of a particular malaria parasite in a particular human population, the distribution of the parasite in that population (endemic level,- epidemics, etc.), and its consequences (morbidity, mortality, etc.) are determined by the "rate of contact" between persons (see also Chapter 10). This "rate of contact" is best expressed by the vectorial capacity, as defined by Garrett-Jones (66), i.e., $ma^2 p^n/(-\ln p)$; the vectorial capacity is a daily rate of potentially infective contact between persons through the vectors (here "potentially infective" refers to the survival of the vector through the incubation period or "extrinsic cycle"). The main contributions of entomology to the study of the epidemiology and control of malaria can therefore be classified under the following headings: (1) measuring the vectorial capacity and its natural variation; (2) "explaining" the vectorial capacity in terms of the natural environment and its unspecific modification by man; (3) measuring the reduction in vectorial capacity resulting from defined specific vector control measures (or eventually, measures aiming at a reduction of man-vector

contact, without reduction in the number of vectors). The entomological component of the Garki project was designed in terms of the first and third of these categories. In addition to the above, entomological observations contribute a direct estimation of the inoculation rate.

However important the concept of vectorial capacity, the definition involves certain simplifying assumptions which may be questioned: (1) survival of the vector is not affected by age; (2) survival of the vector is not affected by infection; (3) vector and vertebrate populations mix at random; (4) the vector population is homogeneous with respect to susceptibility to infection, host choice, incubation period, and survival. Furthermore, even if the definition of the vectorial capacity is accepted, its estimation, in this project as well as in others, is open to many questions. The estimation of each of the component factors (ma, a, *p,* and n, see p. 74) is subject to bias and random error, e.g., if the vector is partly zoophilic, and if animal blood meals are less likely to be followed by rest indoors, then a is systematically overestimated by PSC; indeed the estimation of ma by any other method except the NBC is very likely to be even more biased. Still, it would be of great practical importance to be able to relate quantitatively the entomological situation to its malariological consequences, and to attempt this we need to estimate the vectorial capacity (or some alternative "contact rate").

The following approach was therefore adopted: (1) sampling methods were standardized as rigorously as feasible, and a high sampling frequency was adopted; this should fix the biases and reduce the random errors, and thus allow comparison between different times or places; whether success was achieved can, to a certain extent, be tested by comparing independent estimates of the same variable, such as vector density, or by comparing systematic subsamples of the data (e.g., the sums of odd-numbered and even-numbered collection cycles in a given place; a preliminary exploration of this with respect to density showed a strong concordance, suggesting that sampling frequency was adequate); (2) the estimates obtained for the entomological variables are tested for their ability to "explain" any parasitological differences observed (see Chapter 5), and in particular, the estimated vectorial capacity is used as input into a transmission model, which calculates the resulting parasitological situation; comparison with the parasitological situation actually observed will test simultaneously the model's structure and the numerical estimates used (see Chapter 10).

A particular difficulty regarding the estimation of the vectorial capacity in Garki was that, for various technical and operational reasons, the 2 species of the *A. gambiae* complex could not be distinguished in the estimation of the vectorial capacity. This problem is discussed below (see pp. 104-105).

Transmission in Garki and its natural variation

The main vectors of human malaria in Garki are A. gambiae, A. arabiensis and *A. funestus. A.pharoensis* is probably a vector also; its contribution to transmission was very small, however.

The level of transmission is certainly very high, whether one judges by the man-biting rate, by the inoculation rate, or by the vectorial capacity. There is a well-known large seasonal variation. There was also a relatively large variation between villages and between successive years. This has to be taken into account in evaluating the effect of control measures. The observed variation in the volume of rainfall could explain the variation in the density of ***A. funestus*** from year to year but not the concurrent variation in the density of ***A. gambiae*** s.l., nor the differences between villages. Rainfall was not analysed for patterns, but it could still be done; no detailed information was collected regarding the relief and nature of the soil.

The effect of propoxur

Propoxur produced only mediocre control, in the sense that: (1) the vectorial capacity remained well above its critical level (the minimum level required to maintain endemic ***P. falciparum*** malaria); and (2) the inoculation rate remained at a readily measurable level. After spraying, transmission was due almost entirely to ***A. gambiae*** s.l., to the exclusion of ***A. jùnestus.*** The epidemiological consequences of this relatively modest impact of residual spraying are considered in Chapters 5 and 10. Possible reasons for the mediocre result could, ***a priori,*** be the following in any combination: (1) the very high baseline vectorial capacity and inoculation rate; (2) insufficient coverage, either in space (too many huts missed) or in time (delay of first round of spraying or excessive interval between rounds); (3) ineffectiveness of the insecticide; (4) immigration of vectors ***(A. gambiae*** s.l.) from unsprayed villages; (5) exophilic behaviour of a significant part of the man-biting ***A. gambiae*** s.l. population. These various possibilities will be considered in turn. The cytogenetic differences which may underlie certain of these explanations, in particular with relation to exophily, are considered in the next section.

The baseline vectorial capacity and inoculation rate were certainly very high, and remained relatively high even despite a large reduction. Coverage in space (proportion of huts sprayed) was very high, by direct independent assessment. Coverage in time (timing of spraying rounds) and effectiveness of the insecticide were also quite good, judging from the PSC, ETC and bioassay tests. Immigration of vectors ***(A. gambiae*** s.l.) from unsprayed villages was tentatively accepted to explain the results of the small-scale preliminary trial of propoxur (see p. 76) but the

same features-greater reduction in IRD than in NBC (IN), and greater reduction in NBC (IN) than in NBC (OUT)-were observed in the much larger main trial. Furthermore, the variation between villages in the relative effectiveness of propoxur in controlling ***A. gambiae*** s.l. was independent of the distance from unsprayed villages, and the distribution of sporozoite-positive ***A. gambiae*** s.l. was also independent of distance from unsprayed villages. Furthermore, the differential effect of propoxur on the ***A.gambiae*** s.l. populations of different villages is stable from one year to the next and may have a genetic basis (see p. 98; and refs. 32, 115), which would be incompatible with large-scale migration of ***A. gambiae*** s.l. between villages. For all these reasons, immigration of ***A. gambiae*** s.l. from unsprayed areas is unlikely to have been an important factor (it could, however, be very important in cases where control of the ***local*** vector population is more effective).

This leaves as a determining factor, in addition to the high baseline level of transmission, the possibility of exophilic behaviour of a signifitant fraction of the ***A.gambiae*** s.l. population. This is suggested by various observations: (1) the high prespraying ratio of the MBR to the IRD (see p. 70); this ratio has been interpreted in a similar way by Hamon et al. (79) in Bobo-Dioulasso; (2) the correlation between the differential effect of propoxur in different villages and their prespraying MBR/IRD ratio (see p. 80 and ref. (115); (3) the fact that most of the difference between the effect of propoxur on ***A. gambiae*** s.l. in Garki and the (much greater) effect of fenitrothion on ***A.gambiae*** s.l. in Kisumu, Kenya (64) can be "explained" by the difference between the two places in the prespraying MBR/IRD ratio of their ***A.gambiae*** s.l. (115). The relevant cytogenetic findings are discussed in the next section. The exophilic and endophilic individuals are probably relatively consistent in their behaviour. This is suggested, indirectly, by the agreement between the estimated prespraying proportion of ***A. gambiae*** s.l. blood meals followed by rest indoors and the estimated postspraying proportion of vectors exposed after their first meal, under the hypothesis of consistent behaviour (see p. 87 and ref. 119). But consistent behaviour of individual vectors was also demonstrated directly by cytogenetic findings (see next section). The consistency of the exophilic behaviour of individual vectors affects the impact of an insecticide on transmission and has therefore to be taken into account in the evaluation of insecticidal impact (see 119).

The consequences of these findings and interpretations with respect to the planning of malaria control will be discussed in Chapter 11. It is already obvious that the following are important (see 114). (1) Estimation of the baseline vectorial capacity; the development of better methods would be useful. (2) Prediction of insecticidal impact; in Garki (and in

Kisumu) the impact was related to the prespraying ratio between the man-biting density and the indoor-resting density; in Garki, it was also related to intraspecific cytogenetic variation. It is not certain that a method of prediction, which would be both practical and of general value, can be developed, but research in this field, e.g., on field indicators of resting behaviour, would be useful. (3) Evaluation of insecticidal impact, in a field tria1 or a control programme; this is discussed in a later section (see pp, 105-106).

A. gambiae, A. arabiensis and intraspecific cytogenetic variation

The only members of the ***A. gambiae*** complex identified in Garki were A. ***gambiae*** S.S. (species **A)** and ***A. arabiensis*** (species B), the latter being more abundant. This corresponds to what was known regarding the geographical distribution of the members of the complex.

According to a recent review of the ***A. gambiae*** complex (163), the main differences between the 2 species, with respect to transmission are the following: (1) ***A.gambiae*** S.S. is strongly and uniformly anthropophilic and endophilic; ***A. arabiensis*** is less anthropophilic and endophilic, but with considerable geographical variation (related to cytogenetic variation); (2) where the 2 species are sympatric, ***A. arabiensis*** is probably the longer-lived, but the evidence is indirect, limited and sometimes discordant; (3) both species have probably the same intrinsic susceptibility to infection with ***P.falciparum,*** but here also the evidence is limited and sometimes contradictory; (4) residual insecticides readily control populations of ***A. gambiae*** S.S., but their effect on populations of ***A. arabiensis*** varies with the degree of endophily; residual insecticides tend to Select ***A. arabiensis*** out of a mixed population of the 2 species, and to Select exophilic cytotypes out of a natural ***A. arabiensis*** population.

In Garki, ***A. arabiensis*** was indeed less anthropophilic than ***A. gambiae*** S.S. There was, however, no clear-tut difference regarding endophily and no clear-tut selection of ***A. arabiensis*** by the application of propoxur, nor could the variation between villages in the ***gambiael arabiensis*** ratio explain the differences in effectiveness of propoxur (115). The latter was, however, related to the frequency, within each of the 2 species, of certain chromosomal inversions (32). In Kisumu, Kenya, ***A. gambiae s.s.*** was the dominant species (M.W. Service, persona1 communication), but to explain the magnitude of the difference between the effect of fenitrothion in Kisumu and that of propoxur in Garki on the respective ***A.gambiae*** s.l. populations it is necessary to postulate that ***A. arabiensis*** was more endophilic in Kisumu than in

Garki. The intraspecific cytogenetic findings from Kisumu were not available at the time of writing.

The association between resting behaviour and certain chromosomal inversions (32) is a direct proof that resting behaviour (e.g., exophily) is a relatively stable characteristic of the individual. This is important for the interpretation of changes observed after spraying (see 119). The observed differences between the species in human blood indices are insufficient to explain the corresponding differences in sporozoite rates; the discrepancy could be explained by greater longevity of A. gambiae S.S., but other explanations cannot be ruled out (e.g., greater susceptibility of ***A.gambiae,*** greater overestimation of the man-biting habit of ***A. arabiensis)*** **.**

The continuation of entomological observations after the intervention period provided an opportunity of looking for evidence of behavioural selection. No firm conclusion can, however, be drawn. The persisting increase in the ratio between the outdoor and indoor NBC of ***A.gambiae*** s.l. probably cannot be explained by a change in the relative abundance of the 2 species (i.e., by interspecific behavioural selection), and could suggest intraspecific behavioural selection, but we cannot rule out the simpler explanation that is due to the actual persistence of propoxur, proven by the bioassay tests.

Since the 2 species of the ***A. gambiae*** complex that were present could not be distinguished in the estimation of the vectorial capacity in Garki, it is important to try to evaluate the resulting error. The question was explored by simulation as follows. It was assumed that the 2 species did not differ with respect to π, nor in susceptibility to infection. The 2 species could differ with respect to ***a*** andp, and also with respect to the proportion of blood meals, taken on either man or animal, that are followed by rest indoors, which would affect the estimation of ***a*** from the PSC. Simulations were made under the following assumptions: (l)A. ***gambiae*** S.S. takes all its blood meals on man and all of them are followed by rest indoors: ***A. arabiensis*** takes only 40% of its blood meals on man, and half of the blood meals taken on man and one quarter of those taken on animals are followed by rest indoors. (2) The expectations of life, in days, of ***A. gambiae*** and ***A. arabiensis*** respectively are 5 and 5 (i.e., $p = \exp(-1/5) = 0.819$), or 4 ***(i.e.,p*** = 0.779) and 6 ***(i.e.,p = 0.846),*** or 6 and 4. (3) The 2 species can be present in any proportion. (4) ***n*** is 10, and the number of blood meals per vector per day is 0.5. (5) Parameters are estimated as in this project, i.e., ***ma*** is estimated by NBC, ***a*** by half the human blood index in the ***PSC,p*** by the square root of the proportion of the NBC that are taking their second or later meals. (6) The vectorial capacity is estimated either with or without identification of the two species, in both NBC and PSC, and the two estimates are compared with

each other and with the "true" vectorial capacity, known by construction.

The simulations give the following results. (1) If the 2 species have the same longevity, or if the anthropophilic species (A. gambiae S.S.) is also the short-lived species, then both estimates are overestimates, but they do not differ from each other by more than 2%. (2) If the anthropophilic species (A. gambiae S.S.) is the longer-lived one, then distinguishing the species leads to overestimation of the vectorial capacity, while failing to distinguish them leads to underestimation, except when the anthropophilic species tends to disappear; the largest difference between the 2 estimates occurs when A. gambiae and ***A. arabiensis*** form 37% and 63 % of the NBC, respectively: then failing to distinguish the species leads to an underestimation by 23%, while distinguishing them leads to an overestimation by 7%. (3) Whatever the longevities, the worst estimate is obtained when in fact only the zoophilic species is present: this leads to an overestimation by 43%, because of the bias in estimating ***a,*** but in this case, the identification of the species makes no difference.

It is unlikely that the species differ in reality as much as is assumed in these simulations, in particular with respect to longevity. It is thus unlikely that the failure to distinguish between ***A. gambiae*** S.S. and ***A. arabiemis*** in the estimation of the vectorial capacity was a major source of error as compared to the other possible sources.

Evaluation of insecticidal impact

The following points are of interest. (1) Among collection methods, PSC, ETC and NBC were all indispensable; in the hands of the project staff CDC miniature light-traps were not a satisfactory substitute for the NBC (146), but a reliable trapping substitute for the NBC is obviously desirable; it would not necessarily have to be unbiased. (2) Spontaneous variation between villages and between years was rather large and it is important to take it into account: e.g., ignoring it would lead to the false conclusion that propoxur was less effective in 1973 than in 1972 (see p. 80). This stresses once more the necessity for both adequate baseline data and adequate controls. (3) The insecticidal effect of propoxur (as assessed by bioassay) persisted for at least 3 years after the intervention period, and some changes in the vector populations (e.g., an increase in the ratio of the outdoor NBC to the indoor NBC) persisted for at least 2 years. For operational reasons, different insecticides are sometimes evaluated in succession in the same area, with an interval of 1 or 2 years; this may be insufficient. (4) TO translate the change in density and age composition, observed after spraying, in terms of transmission, one has to make an assumption regarding uniformity of exposure, and the usual

(but implicit) assumption of uniform exposure maximizes the calculated reduction in transmission. Nonuniform exposure is, however, more plausible ***a priori*** and also more apt to explain the changes observed after spraying; nonuniform exposure is also suggested directly by cytogenetic findings (32). (5) In evaluating an insecticide, the area required to minimize immigration of vectors is a vexed point; there is, obviously, no general solution. Some observations presented above suggest that in the study zone the migration of vectors between villages was relatively unimportant (see p. 102), and that in that zone the entomological evaluation of an insecticide does not require a very large area.

Summary and Conclusions

The level of transmission in the Sudan savanna is very high indeed: the man-biting rates of ***A. gambiae*** s.l. and ***A. funestus*** reached seasonal peaks of 174 and 94 bites/man/night (averages of 8 man-nights); the vectorial capacity (contact rate between persons through the vectors) reached a seasonal peak of about 40, i.e., about 2000 times the critical value required to maintain endemic malaria; the cumulative entomological inoculation rate reached a maximum of 145 sporozoite-positive bites in 1 year (out of which 132 were in the wet season).

In the Sudan savanna there are large seasonal, yearly and local variations in the level of transmission. In most villages, the vectorial capacity drops below its critical level for about half of the year (this does not necessarily prevent transmission, given the large reservoir of parasites), while in some it remains well above the critical level throughout the year. The variations from year to year are relatively important; over a period of 3 years, these variations followed the variations in total rainfall in the case of ***A.*** funestus but not in the case of ***A. gambiae*** s.l. Villages, even when not obviously different on inspection and located only a few kilometres apart, differ in vector density, anopheline fauna ***(A. funestus*** has a very uneven distribution) and probably in vector behaviour and karyotypes. Among 8 villages, the cumulative inoculation rate ranged from 18 to 145 sporozoite-positive bites in one year. The local and yearly variations stress the need for adequate baseline and comparison (control) data for a correct evaluation of the impact of control measures.

Propoxur produced only a mediocre result in terms of transmission in the Sudan savanna: the vectorial capacity remained well above the critical level required to maintain ***P. falciparum,*** and the inoculation rate remained quite readily measurable. This mediocre result was observed notwithstanding the high standard of the operations, the high coverage achieved and the good bioassay tests. The result was also little affected

by the size of the treated area, as shown by the comparison between the village-scale preliminary tria1 and the main intervention area covering 165 villages. The mediocre result is due essentially to the high level of the baseline transmission and to the relative exophily of ***A. gambiae*** s.l. This relative exophily is reflected in a high prespraying ratio between the man-biting and indoor-resting densities. Substituting another insecticide for propoxur would probably not have altered the result very much. In particular, the much greater impact of fenitrothion on ***A. gambiae*** s.l. in Kisumu, Kenya, than that of propoxur in Garki was largely predictable on the basis of the lower prespraying ratio between the man-biting and indoor-resting densities, and is therefore largely explicable in terms of vector behaviour .

The effect of residual spraying in reducing ***A.gambiae*** s.l. populations, and hence transmission, varies significantly between villages. The variation is related not to variations in coverage of the spraying operations, nor to distance from unsprayed villages, but probably to variation in the amount of exophily, reflected in the prespraying ratio between man-biting and indoor-resting densities. As stated in the previous paragraph, this prespraying ratio is to a certain extent a predictor of the effectiveness of residual spraying. The amount of exophily is a relatively stable characteristic of villages and is also associated with genetic differences within individual species of the ***A. gambiae*** complex (see next paragraph). The vector population attached to a village appears thus as relatively isolated genetically most of the time. This would also fit with the observation that the effect of spraying was little affected by the size of the sprayed area. As a consequence of the relative autonomy of the local vector populations, a large area is not required for the entomological evaluation of vector control measures in this particular environment. Possible variations in the amount of exophily should be taken into account in evaluating insecticides.

A. gambiae s.l. in the study area is composed of ***A. gambiae*** S.S. and ***A. arabiemis.*** The dominant species is usually ***A. arabiemis*** but the relative abundance of the 2 species varies between times and places in ways which are not explained. ***A. gambiae*** S.S. is the more anthropophilic and has higher sporozoite rates; no clear-tut difference was demonstrated regarding exophily or effectiveness of propoxur. The vectorial capacity was estimated as if ***A. gambiae*** s.l. were a single species; appropriate simulations show that this is unlikely to have introduced a large error in the estimate. The cytogenetic investigations of Coluzzi suggest that resting behaviour and exposure to propoxur are related less to the relative abundance of the 2 species than to the intraspecific frequency of certain chromosomal inversions, some of which may be associated with a relatively stable behaviour pattern of the individual.

A commonly used method of translating the effect of an insecticide on the density and age composition of vector populations into an effect on transmission assumes implicitly a uniform reduction in longevity, i.e., a uniform exposure to the insecticide consequent on a uniform resting behaviour. This tacit assumption is the most optimistic possible regarding insecticidal impact on transmission, but it is not plausible ***a priori*** nor is it compatible with the finding of an association between resting behaviour and genetic variation. Moreover, the assumption of nonuniform exposure leads to more realistic expectations regarding the effect of the insecticide treatments on the prevalence of ***P. falciparum*** (see Chapter 10). It is preferable, in the African savanna at least and probably elsewhere, to evaluate insecticidal impact on transmission under the assumption of nonuniform exposure, or even to assume that the vectors caught biting man after spraying have a normal expectation of life. A nonuniform resting behaviour creates the possibility of behavioural selection.

Some effects of spraying with propoxur on vector populations, such as a decrease in the ratio between indoor and outdoor biting densities, or an increase in the ratio between the biting density and the indoor-resting or exit-trap dendity, remain detectable 2 years after the last application. The sprayed walls demonstrated an insecticidal effect by bioassay in the first half or two-thirds of the breeding season for at least 3 years after the last applicatioh. Furthermore, in the post-intervention phase, the sporozoite rate of the man-biting population was low notwithstanding a probably normal longevity and an elevated gametocyte rate (see Chapter 5), and this sporozoite rate was lower than in the indoor-resting population, which was not the case before intervention. Some of these changes could be due to behavioural selection, but the actual persistence of propoxur on the sprayed surfaces may be sufficient to explain them.

Pyrethrum spray collection and exit-trap collections are insufficient for the entomological evaluation of residual spraying, and should be supplemented by collections on human baits (or a substitute collection method, if an adequate one can be found).