Density independent feeding success of malaria vectors (Diptera: Culicidae) in Tanzania

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Abstract

The possibility of density dependent feeding success in the African malaria vectors *Anopheles gambiae* Giles (*sensu lato*) and *A. funestus* Giles was investigated by examining the gonotrophic status of mosquitoes from 423 collections made in a Tanzanian village. Up to 500 mosquitoes were caught in any single 35 minute indoor resting collection. Estimates of the total numbers of mosquitoes resting were made by removal sampling. These indicated that the efficiency of resting collections decreased with increasing mosquito population. Of 1904 mosquito blood meals tested by ELISA, 95% were of human origin. There was no decrease in the proportion of mosquitoes fully fed at high population densities and the only demonstrable avoiding action by hosts in response to high densities of mosquitoes was the increased use of bednets during the wet season. The late biting cycle of these mosquitoes precludes many other density-dependent host defence mechanisms because the hosts are asleep at the time of attack. African malaria vectors have evolved with man and perhaps their evasion of density-dependent host responses has reinforced their preference for human blood.

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Introduction

Vertebrate hosts often show increasing avoidance be-
haviour when they are attacked by an increasing number of
blood sucking insects (Edman & Kale 1970; Klowden & Lea,
1979, Walker & Edman, 1986). Therefore as the density of
insects increases, a smaller proportion obtains the blood
meal required for egg development. Avoidance behaviour
by hosts has thus been postulated as a possible density-de-
pendent population regulation mechanism affecting several
haematophagous insect species (Edman *et al.*, 1972, 1974;
Edman & Scott, 1987; Randolf *et al.*, 1992). Avoidance can
occur either as an immediate host behavioural change in
response to attack, or as longer term behavioural modifi-
cations on the part of whole host populations.

The study of avoidance and its effects has generally
been confined to the interactions of insects and non-human
vertebrates, even though human populations may also show
density dependent avoidance. In addition to active defensive
behaviour, such as the use of a fly swat, one avoidance
mechanism available to individual humans is the use of
mosquito bednets. Birley & Charlwood (1987) thought that
these were used in direct response to the population density
of mosquitoes in villages on the coast of Papua New Guinea.
The proportion of people owning them was much higher in
villages with large populations of mosquitoes than in those
with much smaller ones. Despite this dependence of the
overall availability of nets on average mosquito densities,
mosquito nets were always used when they were available
so the nets did not introduce density dependent feeding
success to the mosquito populations within a village.

As the population of *Anopheles gambiae* Giles *sensu lato*
(Diptera: Culicidae) increased in experimental huts in The
Gambia so did the proportion that had not taken a full
meal (Lindsay *et al.*, 1992). This was attributed to density
dependent feeding success by these mosquitoes. Unfortu-
nately the proportion of pregravid insects (recent
emergences that would not anyway have taken a full
feed) was not estimated in this study. Thus it is not clear
whether feeding success in man-biting mosquitoes depends
on density.

The malaria vectors of sub-Saharan Africa spend much
of their time resting inside people's houses. Hence one
simple way of assessing their feeding success is to estimate
the proportions of fully fed and partially fed females from
resting collections, made in the early morning. The total
numbers of mosquitoes in the house can be estimated from
removal sampling. This was done over a two year period
in southern Tanzania. The results are discussed in relation
to the behavioural strategies adopted by these mosquitoes
to evade possible avoidance mechanisms of the host.

Methods

Study site

The study took place in the village of Namawala (8°09'S
36°24'E) close to the town of Ifakara in southern Tanzania

as part of the Kilombero Malaria Project[1] (KMP) (Kilombero
Malaria Project, 1992; Smith *et al.*, 1993; Teuscher, 1993).
Namawala, a village built on three north-south ridges, lies
1-2 km south of the northern escarpment of the Udzungwa
mountains at the edge of the Kilombero valley, a flat plain
some 20 km wide.

The village was established by bush clearance after
independence as a result of government policy of establish-
ing communal villages. Within the main body of the village
there are approximately 190 houses. These are generally
mud walled thatched huts with small windows and little or
no ventilation. Many houses have a relatively short life span
(2-6 years). During the present study less than 1% of the
villagers used bednets or other anti-mosquito devices.
People sleep on the floor, or on string beds with woven
straw mats which when they are large enough are very
comfortable, despite the fact that they can act as ideal
resting sites for bedbugs.

The majority of people in the village are subsistence
farmers, the main crops of the region being rice and maize,
cultivated in the plain. Clay is the predominate soil type
close to the river, although most of the farming is done on
the typical black cotton soil. During the growing season,
many people spend a substantial proportion of their time in
so called shamba houses close to their fields. A sizeable
minority of people are also engaged in extracting timber
from the Udzungwa mountains. Two kilometers from the
main population centre there is a hamlet of 15 houses
(Kikulukutu) situated in the valley itself.

When censused in 1989, the village had a population of
1138 people. The age structure of the population is typical
for this part of the world, and standard malariometric indices
(such as malaria incidence, prevalence, spleen rates and
antibody titres) indicate intense malaria transmission (Smith
et al., 1993).

The area has two rainy seasons. The main rains start in
March and extend through to May and the short rains occur
in December and January. A cool, dry season follows the
long rains in June and July (Tanner *et al.*, 1991). As this dry
season extends into November temperatures rise, ground
water levels drop and there is extensive burning of arable
land and bush. Agricultural activity is resumed during the
short rains (when rice is planted) and, although surface water
remains scarce, because of a raised water table vegetation
remains verdant throughout the second hot dry season
(February to March) before the long rains.

There are relatively few domestic animals in this part of
Tanzania. In a survey of 111 households there were 283
adults, 230 children, 524 chickens, 9 ducks, 30 cats and 36
dogs. The absence of cattle is due partly to the endemic
trypanosomiasis associated with the game which appears
during different seasons and which may act as alternative
hosts for mosquitoes.

Population densities of the malaria vectors *Anopheles
gambiae* Giles (*sensu lato*) and *A. funestus* Giles are highly
seasonal and light trap collections show thousandfold
changes from one season to another (Smith *et al.*, 1993).

Resting collections

Between 1990 and 1992 resting collections of
mosquitoes were carried out by individual collectors. Each
caught resting mosquitoes, with the aid of a torch and an
aspirator, for 35 minutes per room, between 7:30 and

11:00 h. The number of mosquitoes collected in each five minutes was noted.

Estimation of total mosquito numbers

Total mosquito numbers in the bedrooms were estimated by fitting an exponential decay curve to the number of mosquitoes collected in each five minute interval (Southwood, 1978) and integrating the fitted curves to estimate the total number of mosquitoes which would have been caught if the collection had been continued indefinitely.

Let the number removed in the ith time interval be n_i, which we assume to be proportional to the total number still remaining. In order to put the decay curves for different collections on the same scale, we express each value of n_i, as a proportion of n, the total number collected in that 35 minute period. Hence:

$$n_i = n\,\beta_0 \exp(\beta_1 i) \qquad (1)$$

Where β_0 (which is proportional to the initial rate of recovery of individual mosquitoes) and β_1 (the decline over time in this rate) are parameters to be estimated which might depend on the population size. These relationships were investigated by fitting different Poisson regression models by maximum likelihood using a log-transformed version of equation (1).

The proportion of the total mosquitoes in the house removed in the first j intervals was then estimated as $1 - \exp(\beta_1 j)$ and the total number resting in the house as:

$$N = n/(1 - \exp(7\beta_1)) \qquad (2)$$

Classification of mosquitoes and assessment of feeding success

After transfer to the field laboratory, mosquitoes from an unselected subset of the collections were killed, identified and their abdominal stage determined according to the following classification based on that of Sella (1920):

1. Unfed; non-gravid mosquitoes with no blood whatsoever in the abdomen (ovarioles up to and including second stage)
2. Part-fed; mosquitoes with some blood in their abdomen but without the abdomen being distended by the feed (ovarioles at first to early second stage).
3. Fed; mosquitoes with the abdomen fully distended by the blood meal but without indications of egg development (ovarioles at second to early third stage).
4. Semi-gravid; mosquitoes with the abdomen distended by the blood meal and developing eggs (ovarioles at third to fourth stage).
5. Gravid; mosquitoes with the abdomen distended by eggs with at most a trace of blood (ovarioles at fifth stage).

Gravid females were excluded from analyses of feeding success since they had definitely fed at least one day before collection and could therefore be assumed not to have attempted to feed during the previous night. Fed and semi-gravid mosquitoes were considered to have successfully fed, whereas part-fed and unfed females were considered to have been unsuccessful in feeding. On occasion, samples of the unfed, part-fed and fed mosquitoes were dissected in saline and the developmental stage of the follicles and parity status determined. Although most semi-gravid mosquitoes are likely to have fed the night before collection some may have fed the previous night.

The mosquitoes were separated according to species and as to whether they had successfully fed or not on the night before capture. Feeding success (P_s) was defined as the proportion:

$$P_s = (c+d)/(a+b+c+d) \qquad (3)$$

Feed completion (P_c) was estimated as the proportion of fully fed mosquitoes among those who had definitely started a feed during the previous night i.e. $c/(b+c)$.

Relationships between mosquito densities and abdominal stage were analysed using non-parametric (rank) correlations. Logistic regression analysis (McCullagh & Nelder, 1989) was used to adjust for inter-house variation in order to estimate the effect of day to day changes in mosquito densities within a house on P_s and P_c.

Blood meal analysis

The stomach contents of fed anophelines from unselected resting catches were squashed onto filter paper and tested for human, bovine or canine blood determined by a direct ELISA (Beier et al., 1988).

Mosquito net use

The entomological collections analysed here were made during the first part of the project. During this period less than 1% of the villagers used mosquito nets or other forms of anti-mosquito measures. As part of an intervention experiment they were given nets in late 1991. A survey was conducted during the dry season of 1992 to assess net use at times of low mosquito density. Householders were also questioned about net use during the rainy season.

Results

Data from 1183 resting collections, performed by 17 different collectors, were used to estimate the relationship between the numbers of mosquitoes collected and the total resting in the house. Figure 1 gives the main numbers of mosquitoes removed per 5 minutes, broken down according to the totals of mosquitoes removed during the 35 minutes of collection. The figure legend also gives the equation of the best fitting regression model. The fit of the regression model was excellent for all except the lowest density category shown in the figure.

The analysis indicated a highly statistically significant decrease with time in the numbers of mosquitoes removed as the collection proceeded (F=417.9; degrees of freedom (df) 1,8277; $P < 0.001$). However, this decrease was more apparent when the mosquito density was low than at high densities. There was a highly significant association between logarithm of the total numbers of mosquitoes collected and the gradient of this decrease (F=344.8; df 1,8277; $P < 0.001$). The proportion of the mosquitoes in the house which is recovered during the 35 minute collection period therefore depends on the population size. Figure 2a shows the estimated form of this relationship averaged over all collectors. Figure 2b illustrates the relationships estimated by a regression with a different line for each collector.
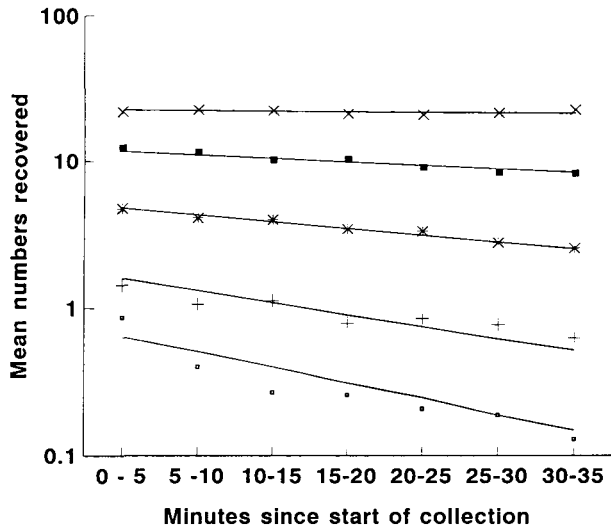
Fig. 1. Numbers of mosquitoes recovered during resting collections by categories of total collected. (·= < 5;   + = 5-9;   * = 10-49; ■ = 50-99; x = > 100).
Lines represent fitted values from the model (based on equation (1) but including additional terms in *n*):

$$n_i/n = 0.32 \ \exp((-0.15+0.055i) \ \ln(n)+0.29i).$$

The species and abdominal status were determined for mosquitoes from collections made in 48 different houses on 109 different dates during the study (corresponding to a total of 423 collections where *A. funestus* was found and 405 with one or more *A. gambiae*). The number of collections and the distributions of numbers of mosquitoes caught are given in table 1. Both species were at their maximum density during the rainy season (March-June) when collections of up to 500 mosquitoes per bedroom were made. Although other species of mosquito were collected in light or landing collections the only other species occasionally caught resting inside houses was *Culex quinquefasciatus* Say.

Regardless of total mosquito density, the proportion of unfed anophelines was always small (table 1). When no allowance was made for differences between houses, the proportion of gravid mosquitoes decreased with increasing mosquito density in both species. In the case of *A. gambiae* this was statistically significant (rank correlation coefficient (r)= −0.117, *P*=0.019) but in *A. funestus* it was not (r=−.001, *P*=0.83).

Table 2 shows the feeding success by species and mosquito density. The rank correlations between the number of mosquitoes caught and the feeding success were negative for both *A. funestus* (r= −0.26, *P* < 0.0001) and *A. gambiae* (r = −0.32, *P* < 0.0001), giving an overall impression that high mosquito densities were indeed associated with poor feeding success. However, most of the variation in feeding success was accounted for by differences between houses. Logistic regression indicated that these inter-house differences were highly statistically significant (Likelihood ratio (LR) tests, *A. funestus*: χ^2=113.9, 27 d.f., *P*=0.0001; *A. gambiae*: χ^2=432.6, 28 d.f., *P* < 0.0001). Such inter-house variations in feeding success can arise as a consequence of mosquito dispersal patterns and are therefore not necessarily related to host avoidance mechanisms. The households with the highest mosquito densities tended to have the lowest feeding success but the rank correlation

was only significant for *A. gambiae* (*A. funestus*: r= −0.19, *P*=0.20; *A. gambiae*: r= −0.41, *P*=0.006).

Avoidance behaviour by hosts in response to increases in mosquito numbers might lead to variation in feeding success between different nights in the same house. However, there was only a small and statistically insignificant relationship between *A. funestus* densities and feeding success on different days within the same household (LR test: χ^2=2.4, 1 d.f., *P*=0.12). In *A. gambiae* when the household effect was allowed for with a logistic model, there was a significant positive association between densities and feeding success (LR test: χ^2=75.9, 1 d.f., *P* < 0.0001). The ratio of P_s for a given collection in a house, to the mean value of P_s for all collections made in that house gives a measure of feeding success which is adjusted for the inter-house variation. Ratios above 1 indicate a higher than expected feeding success. For *A. funestus* there was no obvious relationship between this ratio and the mosquito density (figure 3a). At high *A. gambiae* densities most of the ratios
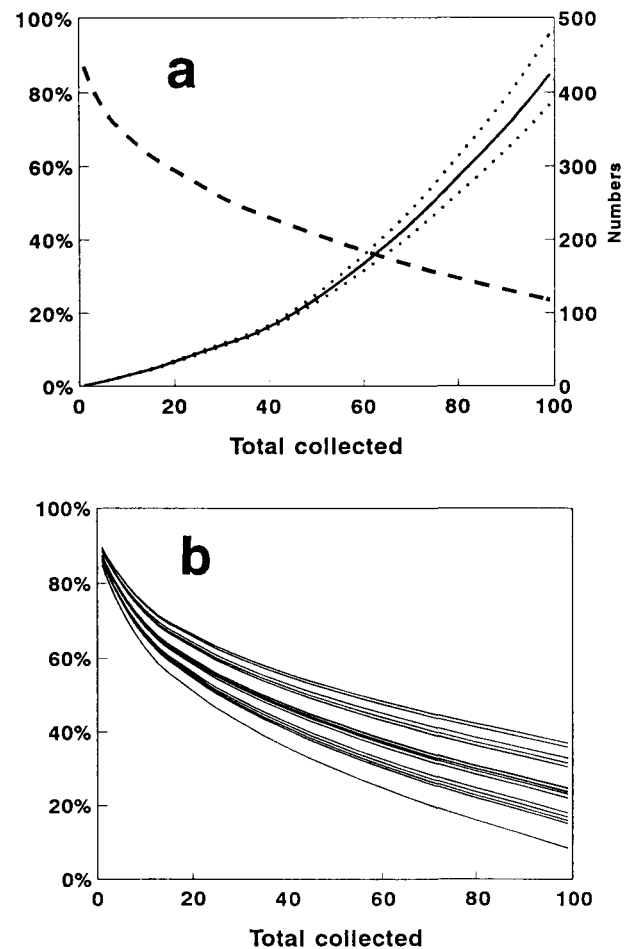

Fig. 2. Estimated efficiency of resting collections according to the total number collected.
a. --- Estimated efficiency (%) from a model aggregating data for all collectors (regression equation as for figure 1.)
— Estimated total mosquitoes resting in the house (...... 95% CI).
b. Estimated efficiency (%) from a model which fitted separate regression terms for each collector.

Table 1. Proportions of mosquitoes by feeding category.

Species	Total no. of mosquitoes caught	No. of catches	Mean proportions of mosquitoes examined				
			Unfed a	Part fed b	Fed c	Semi gravid d	Gravid e
Anopheles	1-4	123	0.041	0.038	0.447	0.090	0.384
funestus	5-9	78	0.064	0.084	0.387	0.180	0.284
	10-49	144	0.036	0.075	0.461	0.164	0.265
	50-99	51	0.032	0.073	0.495	0.141	0.260
	100+	26	0.053	0.052	0.551	0.125	0.219
	Overall	423	0.043	0.064	0.453	0.140	0.300
A.	1-4	82	0.035	0.078	0.390	0.162	0.335
gambiae	5-9	53	0.023	0.070	0.532	0.129	0.246
	10-49	172	0.048	0.111	0.579	0.070	0.192
	50-99	74	0.039	0.147	0.678	0.046	0.090
	100+	24	0.044	0.097	0.727	0.058	0.075
	Overall	405	0.040	0.105	0.562	0.091	0.203

Table 2. Feeding success of mosquitoes by species and density.

No. of mosquitoes caught	P_s			
	Anopheles funestus		A. gambiae	
	mean	S.D.	mean	S.D.
1-4	0.887	0.294	0.841	0.301
5-9	0.774	0.309	0.887	0.193
10-49	0.849	0.185	0.793	0.206
50-99	0.853	0.167	0.791	0.189
100+	0.869	0.066	0.847	0.124
Overall	0.845	0.240	0.817	0.218

sity, A. funestus: $r=-0.32$, $P=0.04$; A. gambiae: $r=-0.27$, $P=0.08$). There was little trend in P_c with mosquito densities within households for A. funestus but A. gambiae was most likely to complete its feeds at higher densities (LR χ^2, 61.8, 1 d.f., $P < 0.001$).

Of 956 blood meals from A. funestus, 905 (94.7%) were of human origin, 15 (1.6% were shown to be canine, and 11 (1.2%) bovine. Corresponding results for the 948 A. gambiae sensu lato tested were 907 (95.7%), 14 (1.5%) and 1 (0.1%), respectively.

Of 312 mosquito net owners surveyed at the end of the dry season of 1992, 139 (45%) were not using the nets, 117 (84%) of these stated that the reason for their non-use was the absence of mosquitoes. All respondents with nets stressed that they used them in the wet season.

were greater than unity (figure 3b). In the latter species, therefore, there is some evidence that within any given house P_s is highest on the nights when densities are high.

The relationships of feed completion (P_c) with mosquito density were similar to those of feeding success (P_s). There were again marked differences between households in P_c for both species. Feeds were more likely to be completed in houses with low average mosquito densities (rank correlations between feed completion and mean mosquito den-

Discussion

The numbers of mosquitoes caught in resting catches are not proportional to the numbers in the houses, but nevertheless can be used to estimate the total densities. The accuracy of the estimate decreases as numbers increase (fig. 2a). Although the efficiency varied between collectors (fig. 2b),
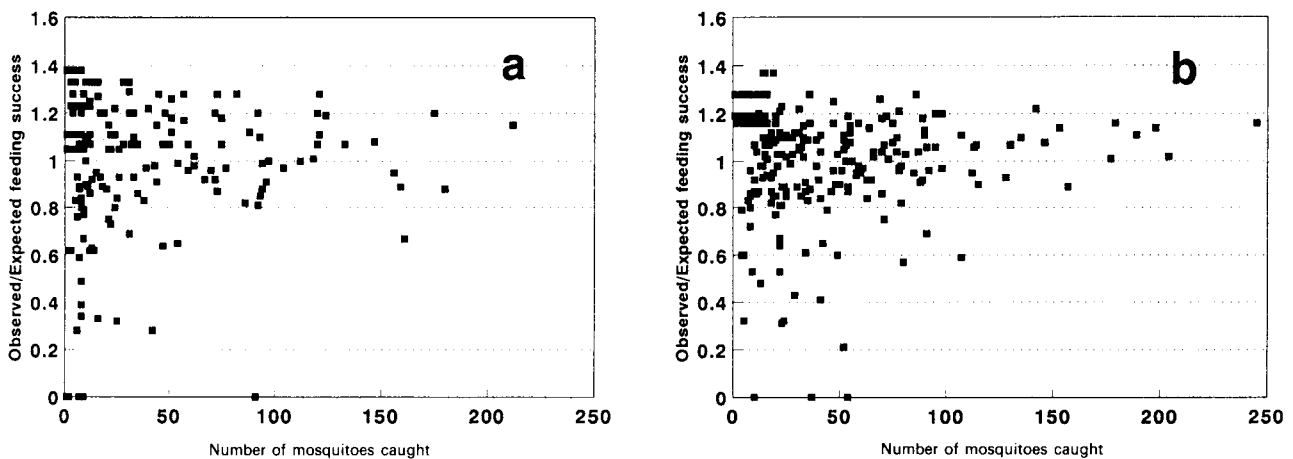

Fig. 3. Feeding success (P_s)/Mean feeding success for the household, by number of mosquitoes caught. (a) Anopheles funestus (b) A. gambiae.

the effect on the estimate of the total number resting in the houses was not large enough to affect our conclusions regarding feeding success at different densities.

Overall, over 80% of both *A. gambiae* and *A. funestus* in the resting catches had succeeded in feeding, but this varied considerably between houses. Virtually all of these mosquitoes had fed on humans. The main determinant of the variation between houses in numbers of *A. funestus* is likely to be the distance from a few fixed breeding sites. Young, pre-gravid, females may not attempt to obtain a full blood meal, even if it is available (Gillies & Wilkes, 1965). Such females may have a more limited dispersal (perhaps related to their need to find a mate). Measured feeding success would then be lower in houses closest to the breeding site. This would account for the negative correlations between average *A. funestus* density and average feeding success and also the negative correlation between the proportion of gravid mosquitoes and mosquito density.

Within any one house, there is little relationship between mosquito density and feeding success for *A. funestus*. In *A. gambiae*, there was some tendency for feeding success actually to increase with increasing mosquito density. *Anopheles gambiae* has transient breeding sites and longer flight range than *A. funestus* (Gillies & de Meillon, 1968), which perhaps make it easier for the newly emerged *A. gambiae* to concentrate in houses with high host densities or other favourable conditions for feeding. A positive correlation between feeding success and density may also arise as a result of underestimation of feeding success at low densities. The completion of feeds showed very similar patterns of variation with mosquito density to those shown by overall feeding success. These were exactly the opposite of what we would expect if density dependent host avoidance mechanisms operated.

When questioned about population cycles of mosquitoes, all the villagers were quite aware of the season of maximum mosquito density. They perceived a drop in numbers during the cool season (which they attributed to the decline in temperature) and perhaps an 'absence' — if they are lucky — in the succeeding hot dry season. This is, however, a relative term since on a number of occasions, as part of other studies, we have caught over 50 blood-fed insects from cursory searches in houses where the owner/occupier had stated that there weren't any inside. When shown the evidence most just said 'Come back in the rainy season!!'

There are also many other blood sucking insects to distract the unfortunate villagers from the anophelines. Bed-bugs were a common and perceived painful pest, populations of which can sometimes reach huge proportions. When questioned, 90% of people in Namawala stated that their houses were infested. At the start of the rainy season, there is also a proliferation of painful biting mosquitoes such as *Mansonia africana* (Theobald) and *M. uniformis* (Theobald) which are active in the early evening before people go to bed and of the audibly obvious, but less painful, *Culex quinquefasciatus*. In the wet season everyone with a bednet used it. Among the non-using owners of bednets in the dry season, 84% gave the absence of mosquitoes as a reason. The increase in use of mosquito nets in the wet season is likely to be a response to the nuisance mosquitoes. Thus mosquito net usage was the only demonstrable density dependent host avoidance mechanism against mosquitoes. It is unfortunate that nets were not used

throughout the year since there was significant transmission of malaria during the dry season (Smith *et al.*, 1993).

Anophelines biting late at night are easier to ignore, even if their bites are painful. The season of peak mosquito densities coincides with the growing and planting season for crops such as rice. Hence most people (including the children) are tired, and are likely to be asleep during the early hours of the morning when both *A. gambiae* and *A. funestus* are biting. Perhaps this helps them to ignore the exceptional biting densities that occur at this season.

In a zoophagic mosquito subject to density dependent selection, there is an advantage in overflowing onto another host as densities increase. Thus selection will tend to limit dependence on a single host species. Such mosquitoes are generally crepuscular biters, often biting animals close to water which is needed for oviposition and then resting in humid vegetation nearby (e.g. *A. triannulatus* from Brazil (Charlwood & Wilkes, 1981; Charlwood & Alecrim, 1989). Many non-African malaria vectors have an intermediate ecology between such species and the highly specialized African vectors. For example *A. farauti* Laveran in Papua New Guinea has catholic feeding habits and can be easily diverted to hosts other than man. It rests outside houses and may bite at any time during the night (Charlwood *et al.*, 1985, 1986a, 1986b).

Although *A. gambiae* and *A. funestus* both belong to species complexes in which most of the other members are predominantly zoophagic, they are among the most synanthropic insects on the planet. They are perhaps the only anophelines which have concurrently evolved in association with humans. Evolution of late night biting has the effect of eliminating density dependent host avoidance mechanisms. This reduces selection pressure that would otherwise favour broader host range, consequently encouraging adaptations which result in more efficient feeding on humans. These mosquitoes therefore spend a very great proportion of their adult life resting inside mud and thatch houses in sub-Saharan Africa and feeding on human blood which they prefer to any other (Gillies & de Meillon, 1968).

Anopheles funestus breeds in permanent water bodies in savannah. Ambulant early man would have spent a considerable time at such places and it was probably in such areas that the first permanent settlements occurred. *Anopheles funestus* is therefore likely to have been the first anopheline to specialize in biting man. It bites later, rests longer in houses and is more anthropophagic than *A. gambiae*, or perhaps any other anopheline. It is dissimilar in behaviour from its widespread relative, the zoophagic species *A. rivulorum* Leeson. Perhaps the key development in the evolution of this host specificity was the elimation of density dependent host avoidance by adopting a late biting cycle.

Anopheles gambiae is similar to its primarily zoophagic sibling species, *A. arabiensis* Patton, implying that the association of *A. gambiae* with man is more recent. Both these species have a late biting cycle and use temporary bodies of water as breeding sites. This suggests that these traits both anteceded the specialization of *A. gambiae* on humans. Since it does not need to remain in the vicinity of large bodies of water, *A. arabiensis* can avoid competition with other mosquitoes by tracing hosts when they are away from water later on in the night. The ancestor of these species would have been preadapted for specialization on an animal that sleeps soundly at night (i.e. man).

Hence the evolution of *A. gambiae* as a specialist human biter, required only minor behavioural modifications because density dependent mosquito avoidance by humans was already evaded because of its pre-existing late biting cycle.

Acknowledgements

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