DRY SEASON REFUGIA OF MALARIA-TRANSMITTING MOSQUITOES IN A DRY SAVANNAH ZONE OF EAST AFRICA

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Abstract. Dry season survival of Anopheles funestus, Anopheles gambiae and Anopheles arabiensis in the Kilombero Valley a dry savannah zone of east Africa, was investigated with over 400 collections from 23 areas, covering 300 sq km of the valley. Anopheles gambiae was found only in association with humans, in forested areas of high annual rainfall, while An. funestus occurred at high densities at the valley edge where large non-moving bodies of water remained. A large population of An. arabiensis was present along the river system throughout the middle of the valley, and mosquitoes probably derived from this population were occasionally caught in villages bordering the valley. No evidence was obtained of aestivation in any mosquito species. Anopheles gambiae was the most long lived, 6.3% compared to 2.0% of the An. arabiensis and 4% of the An. funestus surviving for four or more gonotrophic cycles, the approximate duration of the extrinsic cycle of most malaria parasites. Oocysts of malaria parasites were found in 5.4% of An. funestus and 2.3% of An. arabiensis from villages. Oocyst rates in An. funestus differed significantly between areas but not between houses within areas. Anopheles funestus is the most important dry season malaria vector in the valley, and remains in foci closely associated with groups of houses. All three species survive at high densities but as otherwise hidden refugia populations.

INTRODUCTION

Both Anopheles arabiensis Paton in West Africa and Anopheles gambiae, An. arabiensis and An. funestus Giles are common in the Kilombero Valley, Tanzania. Population densities vary according to season, more so than those described from other areas of Tanzania. Typically at the start of the long or during the short rains, the number of An. arabiensis collected in houses, especially in those bordering the valley, undergoes explosive growth. As the rains continue An. gambiae may become the predominant member of the complex inside houses or in breeding sites. The relative proportion of An. gambiae to An. arabiensis increases with distance from the river and (Lyimo EO, unpublished data). As the rains continue An. funestus becomes the dominant mosquito, particularly in outlying areas where rice is grown. After the rains a cool dry season (July and August) is followed by a hot dry season that can last for several months, especially if the short rains do not arrive. At the end of the rains vector densities decline, the An.funestus population doing so more slowly than either An. gambiae or An. arabiensis. As is the case in other dry savannahs of Africa, the dry season numbers of mosquitoes in villages can drop to levels below detectability using conventional trapping methods.

In this paper we describe the existence of a large population of An. arabiensis from an uninhabited part of the Kilombero Valley and synanthropic, relatively isolated, refugia populations of An. gambiae and An. funestus. The implications for mosquito ecology and malaria control are discussed.

MATERIALS AND METHODS

Description of the study site. The Kilombero river and its tributaries form a nexus of waterways running through the middle of a seasonally inundated plain in southeastern Tanzania (7°44′–9°26′S/35°33′–36°56′E) 250 km long and up to 52 km wide, covering 626,500 hectares at high water, 210–250 m above sea level (Figure 1). The valley is oriented southwest to northeast, between the densely forested escarpment of the Udzungwa Mountains, which rise to 2,576 m above sea level on the northwestern side and the grass covered Mahenge Mountains, which rise to 1,516 m above sea level on the southeastern side. Grasses including Echinocloa pyramidalis and Oryza sp (Graminaceae) dominate the floodplain. A mixed gallery forest with zones of inundation and pockets of swamp forest occurs in the southwestern end of the plain. There is a 45 km long Cyperus papyrus swamp at the southern end of the floodplain. In 1989 an estimated 995 elephant (Loxodonta africana), 30,494 buffalos (Syncerus caffer), 8,414 hippopotamus (Hippopotamus amphibius), 55,769 puku (Kobus vardonii), and 2,920 warthog (Phacochoerus aethiopicus) lived in the Kilombero Valley (Frankfurt Zoological Society aerial survey 1989). Intensive poaching had drastically reduced the numbers of some of these species, especially H. amphibius by the end of the study.

As the water level drops in the dry season much of the vegetation in the valley and around villages dries out and turns brown. Small temporary pools of muddy water develop at the side of the rivers. At the edge of the floodplain, surface water is limited to a few isolated seasonal ponds or lakes (formed when wet season rivers cease to flow), and to manmade ponds close to embankments of the railway which runs along the northwestern edge of the valley (Figure 1). No
rain had fallen in the four months prior to the collections and much of the area was bone dry.

There are roads on both sides of the floodplain with villages and settlements scattered along them (Figure 1). The villages vary in size from tens to hundreds of houses. Three of the villages (Namawala, Area #7; Idete, #8; and Michenga, #11) were included in the Kilombero Malaria Project and have been described in detail elsewhere. Inhabitants of these villages receive an estimated average of more than 300 infective bites per annum. Anti-mosquito measures were not in general use, but from 1992 mosquito nets were widely used in Namawala and Michenga. Most of the villagers are subsistence farmers who spend the growing and harvesting periods, which coincide with maximum mosquito populations, living in temporary farm houses. Many people are migrating into the valley and the landscape of Brachystegia sp. woodland in these areas is disappearing.

Two large villages, Mofu (Area #13) and Merera (Area #14) and a number of, often temporary, fishing camps (Areas 15–20) occur on the Kilombero River several km from the nearest village (Figure 1).

**Mosquito collection.** We have previously demonstrated that 35 minute indoor resting collections provide reasonable estimates of the numbers of insects inside houses particularly at low densities, the great majority of mosquitoes being collected in the first 20 minutes. This time period was therefore chosen for collection because it enabled an increase in sample size without a significant decrease in sensitivity. Between September–October 1992, collections of mosquitoes were undertaken at the Areas designated in Figure 1. Many places could only be reached by foot, bicycle, or canoe. Once a house in any particular Area proved positive for mosquitoes, all houses within a 1- or 2-km radius were searched.

Where they existed, fishermen’s shelters were also searched for resting mosquitoes. At sites along the river mosquitoes were also collected by two men who performed outdoor landing collections between 6 PM and 9 PM.

**Dissection.** Mosquitoes were kept in cool-boxes and provided with water overnight to allow time for blood-meal digestion. The following day they were killed, identified to species group, sexed, and the number of gravid and non-gravid females noted. Gravid females were dissected in saline and midguts examined for oocysts. A logistic model:

$$\log(p/(1 - p)) = \beta_0 + \beta_1N_t + a_i$$

where $p$ is the oocyst rate, $N_t$ is the number of female mosquitoes caught and $a_i$ is a factor associated with the area of collection, was used to determine the relationship between number of insects caught and oocyst rates. Mosquitoes collected from sites along the river were examined on the day of collection. The abdominal condition, mated status, and the ovarian and sac stage of parous insects were determined.

**Species determination.** Anopheles gambiae s.l. were preserved either dry over silica gel or in fixative (isopropyl alcohol or a 3:1 di-ethyl alcohol/acetic acid solution) and later identified to species by DNA probes or by polymerase chain reaction (PCR). At the start of the study a specific DNA probe was only available to identify An. gambiae. Mosquitoes that were identified by the An. gambiae s.l. probe but failed to respond to the An. gambiae s.s. probe were therefore considered to be An. arabiensis.

**Capture-recapture experiment.** A capture-recapture experiment was undertaken in Area #5 in December 1991. Mosquitoes from indoor resting collections were counted, dusted with fluorescent powder and released in the house closest to the breeding site. For the next eight days, 14 of
the 19 houses from the hamlet were sampled for resting mosquitoes. A light-trap was also run in a house in Area #7. Collected mosquitoes were screened under UV light for marks.

**Survival rate estimation.** A major factor determining the vectorial capacity of a mosquito species is the female survival rate. This can be estimated in a number of ways including capture-recapture experiments or dissection of the ovaries. The gonotrophic age of subsamples of An. arabiensis s.l. was obtained by regression of the numbers in each age class with age.

Two models were tested:

1. **Exponential model**
   \[
   \mu(t) = \alpha \quad \text{and} \quad S(t) = N_0 \cdot \exp(\alpha \cdot t)
   \]

2. **Gompertz model**
   \[
   \mu(t) = \alpha \cdot \exp(\beta \cdot t) \quad \text{and} \quad S(t) = N_0 \cdot \exp\left(-\frac{\alpha}{\beta} (\beta \cdot t) - 1\right)
   \]

where \(\mu(t)\) and \(S(t)\) are the mortality and the survival functions respectively and \(\alpha\) and \(\beta\) are constants. The parameters of the models were estimated using maximum likelihood methods, by maximizing the Poisson log likelihood

\[
L = \sum_{i=0}^{r} (N_i \cdot \log(\mu_i(t) - \mu_i(t))
\]

with respect to \(\alpha, \beta\) and \(N_0\) where \(\log(S(t))\) is the goodness of fit of the two models was assessed using Pearson’s \(\chi^2\) test. A fit to the exponential model indicates that survival is independent of age; fit to the Gompertz model indicates that survival decreases with age.

**RESULTS**

**Distribution of species.** A total of 3,812 An. funestus and 3,231 An. gambiae s.l. were found in 409 collections made in 23 areas in September–December 1992–1994 (Table 1). Five hundred and twenty-eight members of the An. complex were identified to species (Table 2), 323 by DNA probe and 205 by PCR. The proportion of positive identifications by PCR (92.3%) was significantly greater than that using DNA probes (74%) (\(\chi^2 = 30.25, P = < 0.001\)). In Areas #1 and #2, close to the forested escarpment of the Udzungwa Mountains, An. gambiae was the predominant species (Table

**TABLE 1**

Site characteristics, number of collections performed and mean numbers (SD) of Anopheles funestus and Anopheles gambiae s.l. collected September–December, 1992–1994. The area of collection is given on Figure 1.

<table>
<thead>
<tr>
<th>Area</th>
<th>Name</th>
<th>Description</th>
<th>An. funestus</th>
<th>An. gambiae s.l.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>n</td>
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<tr>
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<td>Ikile</td>
<td>Village</td>
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<td>9</td>
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<td>Village</td>
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<td>Ruipa</td>
<td>Hamlet</td>
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<td>Relini</td>
<td>Hamlet</td>
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<td>23</td>
<td>Ipera</td>
<td>Hamlet</td>
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**TABLE 2**

Identification of members of the Anopheles gambiae complex by DNA probe or polymerase chain reaction (PCR) from collections made in the dry season, 1992–1994. See Figure 1 for the location of the areas.

<table>
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<th>Area</th>
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<th>Anopheles arabiensis</th>
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<td>37</td>
<td>1</td>
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<tr>
<td>18</td>
<td>Fishing camp</td>
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<td>3</td>
<td>15</td>
<td>DNA</td>
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2). These two areas have a higher annual rainfall than much of the rest of the valley (Figure 1). Only five of the 85 An. gambiae complex identified from other villages bordering the valley were An. gambiae. Of the 4,250 female anophelines collected in other sites along the valley edge, 3,538 (83.5%) were An. funestus. This species was common wherever large non-flowing bodies of water were available. Some of these dried up and the mosquitoes disappeared during the course of the study. Anopheles arabiensis was common at riverine sites, even those uninhabited by humans (Table 1).

It accounted for 249 of the 252 specimens of the An. gambiae complex identified from riverine sites and apart from the two putative An. gambiae specimens from Area #4 and one from Area #19, was the only anopheline mosquito collected away from villages bordering the valley edge.

Mosquito bionomics. There was no evidence of gonotrophic discordance in any of the species at any of the sites. All engorged females, of all species, examined 24 hours after collection were gravid or nearly so. The small numbers of pre-gravid females examined were largely young part-fed insects. Pre-gravid rates in An. funestus and An. arabiensis from villages were similar (Wilcoxon test = \(-0.6985, P = 0.49\), n.s.), while oocyst rates were significantly higher in An. funestus than An. arabiensis. 130 (5.4%) of the 2,397 gravid An. funestus dissected and 17 (3.6%) of the 470 gravid An. arabiensis dissected were infected (Wilcoxon test 3.4035, \(P = 0.007\)) (Table 3). In the logistic model using Area #8 as the baseline area, oocyst rates in An. funestus differed significantly between areas (likelihood ratio test \(X^2 = 21.5, P = 0.02\)) but not between houses within areas (\(X^2 = 0.06, P = 0.81\)). Rates in An. arabiensis were independent both of the area of collection (\(X^2 = 9.86, P = 0.27\)) and of the number caught per house (\(X^2 = 0.39, P = 0.53\)). Only 382 male An. funestus and 153 male An. arabiensis s.l. were collected from these villages. Numbers of males and total females were poorly correlated (Spearman’s correlation; An. funestus \(r = 0.44, P > 0.0, n = 310\); An. arabiensis \(r = 0.23, P = 0.009, n = 136\) as were numbers of males and pre-gravid females (0.26, \(P > 0.001\), for An. funestus and 0.34, \(P = 0.0001\) for An. arabiensis).

The majority (88%, 78 of 89) of engorged An. arabiensis females dissected from shelters at riverine sites or collected from a tent trap were parous or mated nullipars with oocytes at Stage II or III. Of the 121 unfed or part-fed insects examined, 55 (45%) were newly emerged pre-gravid insects with oocytes in which the terminal follicle had not developed beyond Stage I (Table 4). Most (62%) of these young insects had mating plugs, the remainder were virgin. Gravid females were not found in shelters along the river. Fifty-one of 197 pools examined along an uninhabited 15 km stretch of river contained larvae.

Survival rate estimation. An estimated 353 An. funestus females and 155 males were released in the house closest to the breeding site in Area #5 on December 16, 1991; 23% of the females and 8.3% of the males were recaptured in the succeeding 7 days. Fifty-three (65%) of the females and all but one of the males were recaptured in the house of release. Peaks of recapture in females occurred every three days. One marked mosquito was recaptured in the light trap 3.2 km distant five days after release. Daily survival derived from the exponential model for this population was 83.7%.
funestus, An. gambiae, and An. arabiensis appear to occupy distinct hidden habitats during the dry season. The lack of evidence for the existence of aestivating females at low density does not, of course, mean that they do not exist. The problem of distinguishing between absences which are the result of poor sampling and those which are genuine is a significant difficulty.\textsuperscript{22} Nevertheless, previous results indicate that houses with less than 20 resting mosquitoes would have been sampled at approximately 60\% efficiency.\textsuperscript{13} It is therefore likely that we would have found mosquitoes inside houses had they been there. Indeed, whether An. gambiae or An. funestus are capable of aestivating is a moot point. The phenomenon has only been described in An. arabiensis from eastern Sudan, and even there breeding continued throughout the year when suitable water was available.\textsuperscript{3}

Identification of the members of the An. gambiae complex was more reliable by PCR than by DNA probe. The five specimens of An. gambiae from villages where An. arabiensis was the predominant member of the complex were identified by DNA probe. Given the high numbers of insects that could not be identified using this technique, it is possible that they were misidentified. Given the greater dependency of An. gambiae for humid conditions and the conditions in the villages at the time of collection, survival of autochthonous populations of this species at such low densities seems improbable.

Anopheles gambiae was abundant only in the two villages close to the escarpment of the Udzungwa Mountains where annual rainfall is greater than 1,600 mm/year. Such conditions prevail in the upper reaches of the valley, an area that we were unable to examine. No doubt other populations of An. gambiae occur in these areas.

Anopheles arabiensis occurred at low density in houses from the edge of the valley. Low density is generally indicative of an aging population.\textsuperscript{3}\textsuperscript{3} However, many females were recently-emerged pre-gravid insects and rates of infection were correspondingly low. Since water suitable for breeding of this species was non-existent in villages at that time, we suggest that the insects were immigrants from the very large population on the river several km away. As pointed out by Gillies and De Meillon,\textsuperscript{23} "In very dry semi-arid regions, where there are no features to arrest the flight of mosquitoes, it is possible that relatively enormous distances may be covered by individual specimens, although this has not yet been demonstrated." Long distance dispersal of An. arabiensis in the Kilombero Valley would not be surprising given the patchiness and temporary nature of breeding sites, the dispersed hosts, and the open nature of the terrain. The very large An. arabiensis population in the middle of the valley would also argue against a significant proportion of the population elsewhere in the valley surviving the dry season by aestivating. The occurrence of both larvae and adults away from human habitation implies that this was a population dependent upon the game in the valley. Although thought to be reasonably common in the late 1960s\textsuperscript{23} the only other population of An. arabiensis presently recorded in an uninhabited area occurs in the less accessible Kruger National Park in South Africa.\textsuperscript{24} Since the habitat on the river probably represents the archetypal template\textsuperscript{23} for this otherwise important malaria vector, it merits further study.

White\textsuperscript{6} considered that An. arabiensis shows lower inherent longevity than An. gambiae and, in the present study, survival rates in this species were lower than those recorded for either An. funestus or An. gambiae. In the wet season, however, estimated survival rates of An. arabiensis from village houses were much higher.\textsuperscript{18} The majority of insects feed on humans and rest indoors at this time.\textsuperscript{18} The low survival rate in this species on the river may reflect the cost of outdoor resting.\textsuperscript{21}

High densities of An. funestus, the most important dry season vector of malaria in the valley, occurred in houses close to large non-flowing bodies of water. Differences in oocyst rates in An. funestus between the different collection sites probably reflect the size of the local breeding population. Highest mean numbers were obtained from areas where man-made water filled excavations had accompanied the construction of railway embankments. Mosquitoes from these areas had the lowest oocyst rates. Not surprisingly,\textsuperscript{26}\textsuperscript{28} densities were highest in houses nearest water. Indeed, 65\% of the mosquitoes recaptured in the capture-recapture experiment came from the house closest to the breeding site. This was also the house from which most of the insects were collected prior to marking.

Whether there is any significant dispersal of An. funestus between refugia populations, which were generally more than 5 km apart, remains to be determined. However, one female (0.28\% of the total released) was recaptured 3.2 km from the point of release. Dispersal may be triggered when gravid females fail to locate the water source from which they originally emerged or when this dries up. If at least one

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**Figure 2.** Mortality curve of the Anopheles funestus (derived from recapture data), Anopheles arabiensis (derived from dissection data) from Area #16 and Anopheles gambiae from Area #1. Numbers have been adjusted to fit on the same scale.

**Table 5**

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
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<th>Anopheles arabiensis</th>
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46009706
28.800
22.585
21.557
27.126
27.810
35.570
8.886
8.898
8.267
0.041
0.876
0.281
22585
21557
17.591
4.585
0.686
insect per generation moves between different refugia pop-
ulations then genetic homogeneity between them (indepen-
dent of population size) should be maintained.22–25

One of the curious features of the biology of An. funestus
is the apparently slow rate with which it recolonised areas
from which it had previously been eliminated by house
spraying.23 We suggest that if dry season refugia sites are
not re-invaded during the wet season then areas are likely
to remain free of An. funestus longer than would otherwise
be expected. Since dispersal from refugia in the dry season
is apparently restricted, the species should be amenable to
focal control, perhaps by indoor residual insecticide appli-
cation which proved so useful against this species in the past.
Refugia sites are often some distance from roads and, under
a not too rigorous control program might escape detection.
All areas where An. funestus was common were, however,
verdant and could probably be detected from aerial or sat-
eellite imagery.24 How the other twelve or more anophele
species found in the valley9 survive the dry season, however,
remains a mystery.

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