A novel experimental hut for the study of entrance and exit behaviour of endophilic malaria vectors

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Abstract

Experimental huts have played a long and illustrious role in the study of mosquito vectors of disease. Here we present a design of a novel, cheap, 12 sided, experimental hut based on the African roundhouse. The panels covering each side can open and act as a door and above each panel there is a shutter that can also open. This allows for a great combination of possible openings for the study of entry and exit behaviour of mosquitoes. Preliminary results describing the exit behaviour of Anopheles funestus when four of the panels were open are described.

1 Introduction

Experimental huts that approximate the design of local houses have long been used to study the behaviour of mosquito vectors of disease [1]. Such huts range from the ’cheap and cheerful’ [2,3] to relatively sophisticated structures [4,5]. Experimental huts enable replicate and controlled collections of mosquitoes to be undertaken. Since sleepers can alternate between huts it is possible to study differences in host attractiveness [6]. Experimental huts are normally designed in such a way that they attract mosquitoes in the same way as local houses but that mosquitoes are not lost to ants and other possible scavengers. Sets of them can be used to determine the effect of experimental interventions, such as the effect of insecticides on mosquito entry rates. While most studies have been concerned with house entry the addition of window traps and a veranda to a hut enables exit behaviour to be studied. Such huts are, however, not only expensive to build but, due to their usual design, cannot be used to determine if there is a preferred direction of exit. This kind of information is of more than academic interest since it may help in the design of novel interventions or traps. Here we describe an inexpensive hut, based on the African roundhouse, or rondaval, that easily allows for such information to be obtained. A combination of possible openings allows for exit rates from up to 12 contiguous sides to be monitored. Some preliminary results with Anopheles funestus Giles from Furvela, a village in southern Mozambique are described.

2 Method

2.1 Description of study site

The study was conducted during September to October 2009 in Furvela (24°43’S, 35°18’E), a village south of Morrumbene District in Inhambane Province.

2.2 Description of huts

The two huts, which cost approximately 250 US$ each to build, are 12-sided, 3.2 m in diameter and 3.15 m high (Fig. 1). Each side consists of a hinged 80 × 160 cm panel made with reed, a local building material much used in this part of Africa. Above each of the panels there is an 80 × 35 cm opening that can be closed with a hinged thatched palm leaf (makuti) shutter and above this there is a 2-4 cm gap through which mosquitoes can enter even when the rest of the house is closed. In other circumstances this gap could be sealed with netting or some other material or could be isolated from the main part of the house by including a netting ceiling between the shutter and the roof. Thus any, or all, of the sides can act as a door for the collectors and exit route for insects. The floor of the hut was made of cement, which extends 50 cm beyond the walls terminating in a 15 × 20 cm water-filled moat designed to exclude ants. The huts were built circa 150 m apart at the edge of the Furvela river valley, the putative breeding site for the mosquitoes in the village. Hut #1 was within 20 m of the authors’ house.
2.3 Experimental design and mosquito sampling

Two experienced mosquito collectors, who had been informed of the purpose of the experiment and agreed to participate in the study, were randomly assigned to sleep in each hut. The collectors entered the huts approximately 10 minutes before sunset and stayed until shortly after dawn. They slept under a mosquito net impregnated with a pyrethroid insecticide (Fendona) on a local bed. Prior to the start of the experiment CDC light-traps were run throughout five nights, to assess whether the mosquitoes were entering the huts during the night. Once entry had been confirmed, exit collections were carried out at three different times of the night: at sunset (17.30-18.30), in the middle of night, (22.00 - 23.00) and before sunrise (04.30-05.30). In each hut, during the collection periods, the door facing each of the four cardinal compass points (NSEW) was kept open to allow mosquitoes to exit. Five minutes before the end of the collection periods the doors were closed. A rectangular mosquito bed net was set up horizontally over each opening in such a way that it sealed the opening (Fig. 1). This prevented host-seeking mosquitoes from outside the hut from attempting to bite the collectors and prevented the escape of mosquitoes attempting to leave. Mosquitoes were collected from the nets using a mouth aspirator. Collectors moved between traps once they were assured that no mosquitoes were left in the previous trap. Depending on the number of insects in the traps sampling took 30 minutes to 1 hr to complete. A torch was used to locate the mosquitoes for the collection in the middle of the night and the start of the last period of collection. Collections were supervised by one of the authors (AK). Times of sunset and sunrise were obtained using a GPS unit (Garmin e-Trex H; Garmin Ltd, Southampton, U.K.). The experiments formed part of the DBL-INS collaborative project, funded by the Bill and Melinda Gates Foundation, designed to turn houses into traps for mosquitoes.

Samples of the unfed mosquitoes collected were dissected according to the schedule described in [7-9]. Briefly: insects were divided into the following categories:

- **Meconium (M):** Virgin mosquitoes with meconium visible in their stomachs.
- **Virgin (V):** With ovarioles at Stage I, spermatheca empty.
- **Plug unfed (Plug):** Sperm in the spermatheca with a mating plug.
- **Nulliparous Stage I (N I’s):** With ovarioles at Stage I with sperm in the spermatheca but without a mating plug.
- **Nulliparous Stage II (N II’s):** With sperm in the spermatheca in which yolk was present in the terminal ovariole.
- **Parous with sacs (Sac):** With a sac with some distension still present - these mosquitoes were considered to have oviposited on the night that they were collected and thus have a two-day feeding cycle.
- **Parous without sacs (No-sac):** The sac from the previous oviposition had contracted and in which, therefore, there had been a delay between oviposition and returning to feed.

2.4 Statistical Analysis

Statistical analysis was performed using Stata v. 9 [10]. Mosquito numbers were log-transformed to reduce the variance and normalize the distribution, later confirmed by the Shapiro-Wilks normality test. Variance was stabilized by applying log [(n+0.5)], which is preferred for performing ANOVA [11]. The pairwise Student t-test was used to analyse the difference in mosquito numbers between huts. Univariate ANOVA was used to analyse the variance of mosquito density, by abdominal condition, between traps (exit direction) and trapping periods. Once a significant difference was detected by ANOVA the Bonferroni unadjusted test, as recommended by Perneger [12], was used to analyse the difference in mosquito density between trap groups and collection periods. Multiple regression analysis was used to determine the possible association between climatic variables and the number of females exiting according to their abdominal condition.
3 Results

A total of 3,152 *Anopheles funestus* were collected in 28 nights of observation. Most (68.3%) of the exiting insects were males. Of the 998 females collected 747 (74.8%) were unfed, 193 (19.3%) had fed and only 58 (5.8%) were gravid. Although only separated by 100 metres the huts differed significantly in the number of insects collected. Pooled unfed and gravid numbers of females (P<0.001) and males (P=0.002) were higher in Hut #2 compared to Hut #1. However, the number of mosquitoes collected in Hut #1 vs. Hut #2 on a nightly basis fluctuated in a similar fashion. More gravid females, in particular, were collected on nights with high relative humidity (Fig. 2).

![Figure 2](image)

**Figure 2.** Humidity, air temperature and number of gravid females (log scale) collected per day.

In both huts most of the exit activity of unfed and fed females occurred during the period between 04:30-05:30 (before sunrise), whilst the greatest exodus of gravid females and males occurred during the first collection period (17:30-18:30). The second collection period (22:00-23:00) was characterized by little or no exit activity (Fig. 3).

Virgin and recently emerged females (with meconium in their stomach) comprised 38.3% of the 467 unfed females dissected from the third period of collection. Recently mated females, with a mating plug in the common oviduct, comprised (14.5%) of the sample whilst the remainder were either previously mated nulliparous insects or parous. In the hut with least mosquitoes (Hut #1) there was no apparent preferred direction of exit (Fig. 4). In Hut #2 at sunset most males were collected from the north-facing exit whilst at dawn they were collected in greatest numbers from the south-facing exit. Exit patterns in females from this hut were similar to the males (Fig. 4).

![Figure 3](image)

**Figure 3.** Relative density (mean±SD) of female *An. funestus*, according to abdominal condition, and males collected exiting Hut #1 and Hut #2. The symbols I, II and III denote the 1st, 2nd and 3rd period of collection. Same letter periods did not differ significantly (P> 0.05).

![Figure 4](image)

**Figure 4.** Direction of exit of *An. funestus* from the experimental huts by time of the night and sex.
3.1 Weather and exit behaviour

Temperature declined during the night from an average of 22.4°C - 28.2°C in the first four hours to 14.6°C - 24.3°C in the last four. No linear association ($r^2=0.038$, $P=0.322$) between the independent variables was found, implying that the calculation of partial correlation coefficients to separate the possible influence of one of the predictors on the other was not required. No significant association was found between environmental conditions and the number of females, other than gravid ones, or the number of males exiting. There was, however, a significant association between outdoor humidity and temperature and the number of gravid females collected ($r^2=0.304$, $P=0.003$). The greatest exodus of gravid females occurred when relative humidity reached 85% and air temperature was 26.5°C (the black area on upper left part of graph of Fig. 5). The possible effect of weather on exodus rates of female mosquitoes was examined by period of the night.

3.2 Period I (17.30-18.30 hrs)

There was a significant association between the number of unfed ($r^2=0.375$; $P=0.012$), gravid females ($r^2=0.31$; $P=0.033$) and outdoor air temperatures. These relationships were best fitted by quadratic polynomial equations: unfed ($y = 37.02 - 2.87$ TEMP + 0.06 TEMP$^2$) and gravid ($y = 57.97 - 4.63$ TEMP + 0.09 TEMP$^2$).

3.3 Period II (22.00-23.00 hrs)

While controlling for the possible effect of co-linearity between independent variables on the number collected, the partial correlation showed a significant association between outdoor relative humidity temperature and the pooled number of females collected ($r^2=0.205$; $P=0.045$ and $r^2=0.272$; $P=0.018$ respectively). Intense egress occurred when relative humidity reached 95% and air temperature 24°C (Fig. 6).

3.4 Period III (04.30-05.30 hrs)

No significant association between these factors and the exodus from the third period was found.

4 Discussion

The huts described are cheap, simple and multipurpose. Although both huts were situated at the edge of the Furvela valley and were only separated by 150 m significantly more mosquitoes were collected in Hut #1 compared to Hut #2. Although the hosts (who did not alternate on different nights) in the huts may have affected the number of hungry females entering them they are unlikely to have influenced the number of male mosquitoes entering them and so we presume that the observed differences were largely due to hut location. Numbers collected from both huts fluctuated in a similar manner but they differed from village houses in Furvela, in that almost 20% of the females leaving had fed compared to the 2% normally collected from village houses (Charlwood & Bragana, in press). Whether the insects had fed on the collectors (who, when not collecting, slept under mosquito bednets) is not known. As pointed out by Gillies and De Meillon [13] in ordinary houses with open doors and windows and much disturbance a considerable exodus of fed and semi-gravid females can take place. It is possible that the experimental protocol was sufficiently disturbing...
to affect the mosquitoes in this way. Unfed females exiting at dawn also included a substantial proportion of mature, mated insects, which, presumably originally entered the huts to feed rather than to rest and left in search of a blood meal elsewhere. Newly emerged insects on the other hand, like males, enter to rest rather than feed [7]. Given the observed patterns of behaviour in the females it is also not certain that the activity patterns of the males were not affected by the experimental protocol. Nevertheless, exit behaviour in the males followed that observed in normal village houses with most activity occurring at sunset [14].

The direction of exit varied according to the direction of the sun but did not follow it in a predictable fashion. Thus we do not know why insects should leave via the door that was at a tangent to the direction of the sun. Experiments with artificial illumination might throw more light on the matter.

5 Acknowledgements

We thank Simião and Emídio for their help during the fieldwork and Nelson Mururre and Antonio Cuamba for their help with sample identification and registration. The experiment was part of the project ‘Turning houses into traps for Mosquitoes’ funded by the Bill and Melinda Gates Foundation.

References


