Parassitologia 35: 5-9, 1993

A NEW ODOUR-BAITED TRAP TO COLLECT HOST-SEEKING MOSQUITOES

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Abstract. A new odour-baited entry trap which releases an air stream containing chemical cues collected from a bait, has been used successfully to collect West African mosquito species, most of them important vectors of malaria, such as *Anopheles gambiae* s.l. and *An. funestus*. 85% of the yield consisted of live, unfed and partially fed specimens. The trap has some practical and theoretical advantages over similar sampling techniques.

Key words: mosquito sampling, odour-baited trap, host-seeking, malaria vectors, West Africa.

Human baited collections are crucial for the direct evaluation of the degree of contact between human beings and mosquito vectors of disease. At present three main catch methods are available for this purpose: landing, bed-net, and drop-net catches (Service, 1976). The first is widely adopted as the most reliable estimate whenever the man-biting rate is needed in epidemiological surveys, but, apart from being too dependent on the skills and motivation of the catchers, it is ethically unsatisfactory, time consuming and very labour demanding.

Bed-net catches can be standardized more easily, but, as long as the bait is protected from being bitten, most of the mosquitoes are lost before the morning, and periodical collections must be made during the night. Moreover, for several species, the numbers and proportions caught may be different from those in biting catches. Evidently, this depends on the behaviour of each species, how they respond to the net, and the degree of obstruction to the entry of mosquitoes. Furthermore, the bed-net may constitute an attractive stimulus in itself, as the collection of resting individuals from unbaited nets, and of bovid-fed specimens from human-baited bed-nets suggest (Colless, 1959; Akiyama, 1973). A drawback drop-nets have in common with bed-nets is that the catch must be interrupted regularly to collect trapped mosquitoes.

Despite the usefulness of human-baited collections in ecoepidemiological studies, they have been little changed over the years to improve their standardization. Traps are one obvious way to standardize catches, but the animal-baited traps currently available can be used only with small or medium-sized vertebrates (Service, 1976). Light traps located near a host may also provide adequate samples of mosquitoes, but the role of the light is still poorly understood, and the response of mosquitoes to it varies according to its intensity and wavelength, and also according to the species concerned (Wilton and Fay, 1972). So, even if samples are not biassed with respect to parameters such as parous and sporozoite rates, as compared to human baited catches (Lines *et al.*, 1991), it is not known whether or not the sample obtained is the actual 'host-seeking' fraction of the population.

From the 1970s onwards, the study of the behaviour of tsetse flies has led to the development of remarkable improvements in sampling and control techniques (see Colvin and Gibson, 1992). The addition of ox odours to visual targets generally increases the catch several times, with a high dose-response effect. The isolation of some active components of host odour has advanced both the understanding of tsetse behaviour and the feasibility of new control techniques.

With the tsetse model in mind, an odour-baited trap was developed to study the host-seeking behaviour of mosquitoes in the field. Its design and trial are presented here. The main feature of the trap is that it isolates the odours produced by the host from other cues such as visual features of the bait or its convected or radiant heat. This is a first step in the dissection and analysis of the odour-mediated behaviour of host-seeking mosquitoes. Possibly this might lead to the development of new sampling tools of epidemiological interest, by analogy with what has been achieved for tsetse flies.

General description

The underlying idea of the method is to exploit the anemotactic behaviour of mosquitoes 'searching' for a host (Dow and Morris, 1972; Bowen, 1991; Edman, 1991), by guiding them into an odour-baited entry trap (hereafter OBET) from which air-borne chemical cues, used by mosquitoes to locate a host, are released. The same general principle has already been exploited in wind tunnel field studies for the assessment of the maximum flight speed of mosquitoes (Gillies and Wilkes, 1981) and sandflies (Killick-Kendrick *et al.*, 1986).

The apparatus has three main components: a source of chemical cues, an air current generator, and the trap.

The chemical cues can be varied; in the prototype model we used a tent to collect the volatiles and gases produced by a host, but we also used fractions of these whole host emissions, as well as pure compounds such as carbon dioxide. The air-stream transfers the chemical cues from the tent to the trap, and creates a current of air flowing from the OBET, which enables mosquitoes to find their way upwind into the trap. The air stream emanates from the entrance of the OBET only, and the trap collects mosquitoes without necessarily killing them.

Technical description

The OBET consists of a steel wire frame 40 cm high × 40 cm wide × 60 cm long, covered on each side with white mosquito netting (Fig. 1). In the centre of the rear side and about 10 cm inside the frame, two 10-cm diameter metal rings support a 50-cm long plastic tube which links the trap and the source of odours *via* inflated 'lay-flat' polythene tubing; one end of the 50-cm tube is "closed" with netting to prevent mosquitoes from flying up it. A white cloth sleeve fitting over it, facilitates the removal of mosquitoes.

In the centre of the front (entry) side and about 10 cm inside the frame are two 20-cm diameter rings to support a foam rubber cylinder (with 1-cm thick walls) that constitutes the trap entrance. Another 16-cm diameter ring is placed 15 cm inside the front to support a disc of mosquito netting. These five rings all have their centres on the same axis. The entrance cylinder is inserted into the trap up to about 14 cm, so that a gap of only about 1 cm is left between the internal edges of the entry cylinder and the mosquito netting disc. The whole trap except for the front opening and the rear ring is covered with polythene sheet to ensure that odours are released through the front hole only. To retrieve captured mosquitoes, the plastic rear tube is removed and an arm inserted through the cloth sleeve inside the rings to catch mosquitoes with an electric aspirator. The entrance cylinder is plugged with cloth during this collection phase.

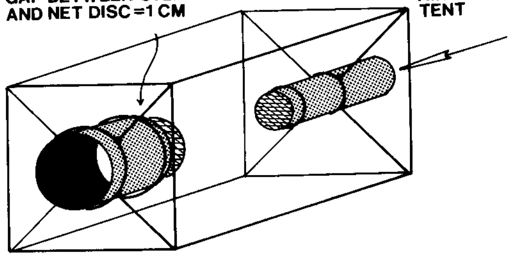

Fig. 1. A stylized drawing of the OBET showing the plastic tube to which the lay-flat tubing is connected (narrow cylinder) and the foam rubber tube which is the entrance for mosquitoes (wider cylinder). Cross-hatched areas show discs of mosquito netting. Steel wire rings, netting around the cage and cotton sleeve around the plastic tube are not shown.

The host-holding tent is made of heavy-duty polythene sheeting folded so as to have a cuboid shape and is suspended from each upper corner by strings on poles. Dimensions of the tent can be adapted to the particular bait used; for human hosts and goats we used a tent 2 m long × 1.5 m wide × 1.5 m high, and for calves a tent 2.5 m long × 1.5 m wide × 1.7 m high. Two rectangular windows (100 × 20 cm) covered with mosquito netting are cut on each lateral side to allow an input of "clean" air. On the centre of one end, a 10-cm diameter hole is cut and fitted with a 12-V DC fan. The trap is then connected to this by the 'lay-flat' tube. This could be of any desired length; in our field trials we used tubing up to 12 m long. The air stream is sufficient to inflate it to its full diameter.

The fan is powered by a 12-V rechargeable battery and the air flow and speed adjusted by regulating the distance of a plastic screen from the inlet side of the fan. A finer regulation can be achieved with an electronic regulator. We used a mean air speed of 0.5 m/s as measured with a hot-wire anemometer at the centre of the trap entrance.

RESULTS AND DISCUSSION

The OBET was tested in two sudanese savanna villages in Burkina Faso during the beginning of the dry season (October-December 1992). About 1500 mosquitoes were caught in 76 trap nights. Species collected included *Anopheles pharoensis*, *An. rufipes*, *An. ziemanni*, *An. wellcomei*, *An. squamosus*, *Culex poicilipes*, *Cx. quinquefasciatus*, and other unidentified *Culex* species, but by far the most abundant species caught with human baits were *An. gambiae* s.l., *An. funestus* and *Mansonia* spp. (Table 1). More than 85% were unfed or partially fed; only 5% were fully fed and the rest were gravids. To avoid high mortalities in the trap, the mosquitoes were collected early in the morning (5-6 a.m.) which gave a ca. 100% survival rate without any sugar solution being provided.

Table 1. Mean number of specimens collected in the OBET catches with different types of odour bait. Number of replicates: human bait 52, animal bait 6, carbon dioxide 18.

Species	Human	Animal	Carbon dioxide
An. gambiae s.l.	18.2	1.2	0.8
An. funestus	6.4	0.7	0.4
Anopheles spp.	0.3	0.7	0.3
Mansonia spp.	1.9	3.3	0.1
Other culicines	0.6	0.5	0.2

During the month of October, a small-scale experiment was carried out to look at the response of mosquitoes to different baits inside the tent. For three nights a human being, a calf and three goats were put into separate tents, changed each night to avoid position bias. The percentage species composition of these collections is shown in Table 2. Because of the low numbers caught, we have pooled the data of the highly anthropophilic species *An. gambiae* s.l. and *An. funestus*, and compared them to all the other species collected. In spite of the low overall yield, the catches on human and animal baits are highly significantly different.

Table 2. Species composition of the OBET catches with different odour bait types. The numbers in the animal bait column include catches made with a calf in a tent and three goats in another one. Species relative frequencies for calf *vs.* goats are not significantly different, but the calf caught about 5 times more mosquitoes than the goats. G statistic with William's correction = 12.97; d.f. = 1; $p < 0.005$.

Species	Human	Animal
Total Catch (n)	24	38
Percent:		
An. gambiae s.l. + *An. funestus*	75	29
Other species	25	71
	100	100

The OBET was used successfully outdoors, placed on top of a 150-cm high stand (Fig. 2) and also as an entry trap fitted to the windows of local mud huts (Fig. 3). Trials with no bait in the tent and no chemical cue released from the trap caught no mosquitoes (except for one unfed *An. funestus* individual) in 26 replicates. This supports the hypothesis that the mosquitoes caught were responding to the bait and possibly 'searching' for a blood meal. The change in the species composition of the catch with different baits, also suggests there is a certain degree of specificity in the response of mosquitoes to the stimuli provided with the air flow.

As with other automatic collection equipment such as light traps, the OBET eliminates some of the intrinsic variability of non-mechanical sampling techniques. Compared to human baited collections it does not require trained personnel to perform the catches, and it is independent of the ability of the catcher to capture landing mosquitoes. However, the correlation, if any, between OBET catches and human-baited collections is not known yet, and further studies are needed on this aspect.

Unlike other kinds of baited trap, which depend on the natural diffusion of the bait's odours, and sometimes on a device to suck mosquitoes approaching the bait into a collecting bag (Service, 1976), the OBET exploits an air-stream current that is directional and lets the mosquitoes "choose" to fly up it and into the trap. Moreover, there are no attractants other than the chemical cues of the bait, so that the bias problems sometimes encountered with

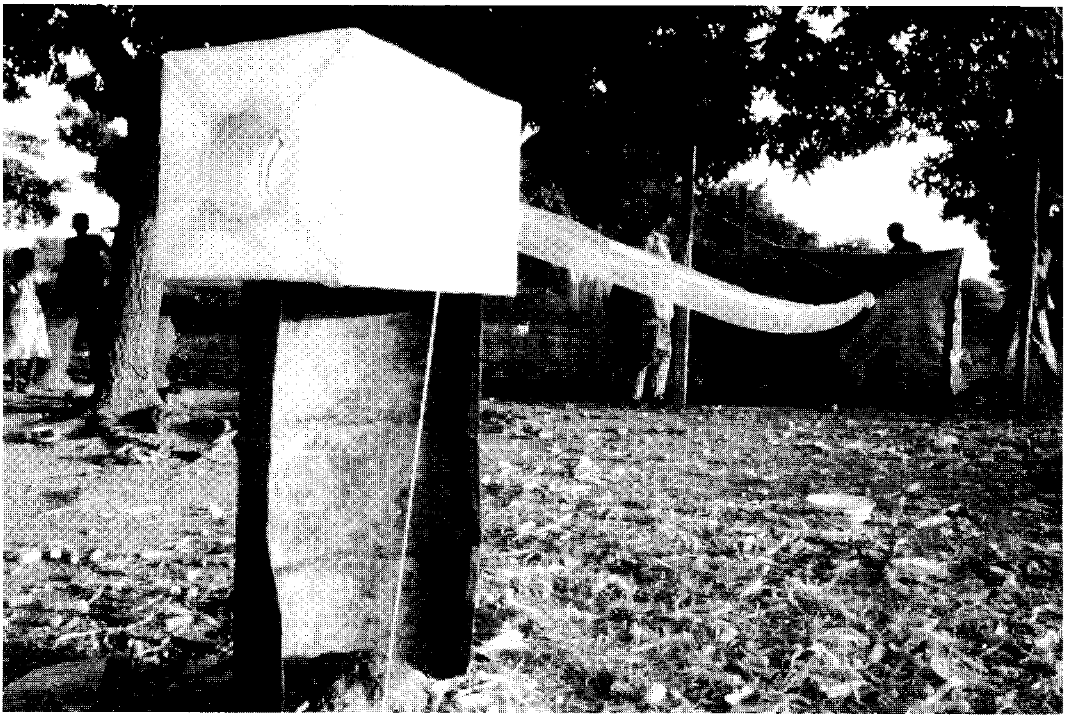

Fig. 2. The odour baited entry trap operated outdoors on top of a 150-cm high stand, showing the inflated "lay-flat" polythene tubing connecting the host-holding tent (in the background) to the trap.

Fig. 3. Measuring the mean air speed at the entrance of an odour baited entry trap fitted to a window of a house. On the left, the host-holding tent with the inflated polythene tubing directed inside the house for connection to the trap.

other techniques (e.g. light or sound) are unlikely to complicate interpretation of the catch.

The trap can be used either indoors or outdoors, and any host can be used as the bait without altering the trap itself. A possible source of bias when sampling outdoor biting species may arise from the design of the entrance opening which might inhibit exophagic species from entering. The influence of the air speed on the behaviour of the mosquitoes approaching the trap and the role of odour concentration needs also to be investigated. However, Gillies and Wilkes (1981) have shown that *Anopheles ziemanni* and *Mansonia* spp. were most successful in reaching the electrocuting grid inside a field wind tunnel at 0.5-0.8 m/s air speed, as measured at the grid level.

At present this collection technique requires electric power and a source of chemical cues (either a living host inside a tent or a flow of carbon dioxide). This may make it impractical in some situations. When synthetic and/or extracted attractant substances become available, it should be possible to develop a simpler and more easy-to-handle version of the trap, though an electric fan will probably still be necessary. We also suggest that the trap should be useful for comparative analysis of attractiveness of different blends of odours.

Acknowledgements

We would like to thank Dr W. Takken and B.J. Knols for profitable discussions on mosquito behaviour, and Drs D.R. Hall and A. Cork for their practical suggestions. Field trials were carried out at the Centre National de Lutte contre le Paludisme of Ouagadougou thanks to the Ministry of Health of Burkina Faso and the Direzione Generale per la Cooperazione allo Sviluppo of the Italian Ministry of Foreign Affairs. The authors are most grateful to the personnel and the entomological staff of the Centre. The work is part of the project 'Behavioural studies on malaria vectors' funded by the European Community under contracts No. TS3-CT92-0101 and TS3-CT91-0032.

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