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A novel window entry/exit trap for the study of endophilic behavior of mosquitoes

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Abstract

To overcome the limitations of currently used window entry/exit traps, we developed an efficient new glue net entry/exit trap (GNT) that is economical, easily transported and assembled, and can be utilized for a variety of studies which aim to investigate the dynamics of mosquito movements between indoor and outdoor habitats. Cage experiments were conducted to determine what percentage of mosquitoes trying to pass through the netting are actually being caught. The GNT caught 97% of female and 98% of male *Anopheles gambiae* s.s., as well as 97.5% of female and 98% of male *Culex pipiens* attempting to cross into a bait chamber adjoining the release cage. During a six day field study, the bedroom windows of 12 homes in Mali were fitted with entry/exit GNTs. Traps without glue were fitted over the inside and outside bedroom windows of an additional 12 homes as a control. A total of 446 *An. gambiae* s.l. were caught attempting to exit dwellings while 773 *An. gambiae* s.l. were caught attempting to enter. The number of males and females attempting to exit dwellings were roughly similar (215 and 231 respectively) while there was a slight difference in the number of males and females trying to enter (382 and 430 respectively). Pyrethrum spray catches (PSC's) conducted inside the dwellings on the last day of the experiment yielded only six females and a single male.

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Keywords

Anopheles gambiae; Culex pipiens; Glue net traps (GNT's); Entry/exit traps; Window traps; Mali

1. Introduction

There are a wide variety of traps available for sampling populations of exophilic mosquito species (for review see Silver, 2008). However, many of the important vector species carrying malaria, filariasis, arboviruses and other pathogens are endophilic and so for epidemiological studies, samples of subpopulations such as blood fed females, those old enough to vector pathogens, and males closely associated with them might be more desirable than sampling the mosquito population as a whole (Bradley et al., 1949; Akiyama, 1973; Dimena et al., 2006). Indoor sampling is usually performed with different types of manual and battery powered aspirators, light traps, hand nets, and pyrethrum spray catches (PSC), while mosquitoes moving either in or out of houses are traditionally collected with different types of entrance and exit traps (WHO, 1975; Silver 2008). The latter are of great importance, as they can provide information on the state of the investigated specimens at crucial physiological stages, before or after interaction with human or animal hosts.

According to Silver (2008), the most commonly used window exit/entrance traps are the Muirhead-Thompson traps, and their various modifications, which are fitted over windows to catch mosquitoes (Muirhead-Thomson, 1947; Muirhead-Thomson, 1948). However, these traps are bulky to transport and the opening on the trap sometimes allows mosquitoes to escape back into the home. Rachou exit traps (Rachou et al., 1965) are also fitted over windows and have a non-restrictive opening which prevents escape of mosquitoes back into the home, but because of this type of opening, frequently needs to be changed over a collection period and requires much manpower. Because the previous traps were too bulky to transport to the field in large numbers, Shidrawi (1965) developed the collapsible window trap. The drawback of these traps is that they are complicated to construct, requiring that wire mesh be welded to a collapsible metal frame.

Our research on mosquito sugar-feeding behavior in rural areas of West Africa necessitated simple means of specimen collection in and around houses. Specifically, we required a simple method for trapping mosquitoes while trying to enter or exit human dwellings. Our goal was to design and test a new window entry/exit trap that can be easily constructed, easily transported, is cost effective and, at the same time, provides a reliable measure of the number of mosquitoes attempting to enter or exit human homes.

2. Materials and methods

This study was conducted in two parts. First, cage experiments were conducted to test the efficiency of the trap design, specifically if the mesh size and glue paint were effective at capturing different species of mosquito without destroying the specimen. This was accomplished by using colored dye to mark the percentage of mosquitoes that escaped capture by the net. Importantly, this also provided a prediction of what percentage of mosquitoes should be caught once the trap was deployed in the field. Next, a six day field

study in Kenieroba village, southern Mali was conducted to evaluate the trap's ease of use and effectiveness at catching mosquitoes entering or exiting local dwellings.

2.1. Trap construction

Our glue net traps (GNTs) were assembled from rigid, dark green plastic garden fencing with 0.2 cm wide net separated by 0.8 cm square holes (True Traders Ltd, Great Yarmouth, UK). Frames were constructed by overlapping two layers of 1.0 cm thick plywood fixed with nails and wood glue (LePage® Wood Glue, Henkel Consumer Adhesives Inc. Avon, OH, USA). The netting was sandwiched between the two wooden frames and secured with screws. Next, the net was painted with glue (Tanglefoot® Glue, Tanglefoot Company, Grand Rapids, MI, USA) to capture insects trying to land or pass (Fig. 1).

The dimensions of the fully constructed GNT's used in the field studies were 70.0 cm length $\times$ 60.0 cm width $\times$ 2.2 cm thick and were designed to fully cover typical local windows. Traps were mounted over the windows by suspension from rope secured by a nail (Fig. 2a, b). By mounting one GNT outside the window and one inside, both entering and exiting mosquitoes could be collected separately. GNTs were mounted simultaneously on inside and outside bedroom windows of 12 homes, and traps without glue were mounted over bedroom windows of an additional 12 control dwellings. Collection of trapped specimens was performed each day for 5 consecutive days; day 6 of the study was reserved for PSC catches.

2.2. Cage experiments

Before widespread deployment in the field, it was critical to test how effective a GNT would be at capturing mosquitoes by determining what percentage would be caught trying to pass the net. For this purpose, a two-chamber system was constructed from strong wire and mosquito netting. The release chamber was rectangular with dimensions of 1.2 m length $\times$ 0.6 m width $\times$ 0.6 m high, with an opening of 0.4 m $\times$ 0.4 m in the center of one of the 0.6 m $\times$ 0.6 m sides. A wooden frame (as described above) was attached with wood glue to the 10 cm wide gauze flaps surrounding this opening, which could be closed at the external side of the wooden frame with a sliding door made from Plexiglas. The feeding chamber was a nearly exact copy of the release chamber but instead was a cube with dimensions of 0.6 m length $\times$ 0.6 m width $\times$ 0.6 m high. It was equipped with two 250 ml beakers with cotton wicks, one containing an attractive colored sugar solution (Attractive Sugar Bait, Westham Innovations Ltd., Tel Aviv, Israel) and the other containing tap water. In the release chamber, only a beaker with water was available to the mosquitoes. The two chambers could be connected with the help of long wing bolts inserted through the frames of the traps. The experiments were conducted with two back-to-back GNT's (as described above) to separate the release chamber from the feeding chamber with attractant. As controls, experiments were also conducted with traps not painted with glue, or an empty frame mounted over the opening between the cages.

Laboratory reared *Anopheles gambiae* s.s. and *Culex pipiens* were used for the cage experiments, which were conducted in Mali and Israel, respectively. In both cases, the specimens were 5 days old and starved 24 h prior to the experiments. The mosquitoes were released at 17:00 h through a sleeve and were allowed to settle down for 30 min before the

sliding door was opened to offer the mosquitoes access to the feeding chamber. The following morning, the GNT's were removed and mosquitoes were counted and checked for colored sugar solution in their gut.

Over nine repetitions, a total of 922 *An. gambiae* s.s. (459 females and 463 males) and 1901 *Cx. pipiens* (961 females and 940 males) were released to test the traps with glue, 1066 *An. gambiae* s.s. (525 females and 541 males) and 1837 *Cx. pipiens* (939 females and 898 males) were released to test the control nets without glue, and 1019 *An. gambiae* s.s. (504 females and 515 males) and 2113 *Cx. pipiens* (1075 females and 1038 males) were released to test the configuration without netting.

2.3. Pyrethrum spray catches (PSCs)

PCSs were conducted according to established protocols (WHO, 1975) to determine the residual density of resting mosquitoes after deployment of the traps. The procedure was conducted by closing the windows and doors and sealing the gaps in the roof with textile, and covering the entire floor (including objects that could not be removed) with white linen sheets (2.0 × 3.0 m). The room was subsequently sprayed with commercial spray cans containing pyrethrum (approx. 1/2 can per room: specification Dichlofos 1.20%, Fenitrothion 0.40%, Tetramethrin 0.15%, Parfum 0.30%, Solvents 22.95%, Propellants 75.00%), first applying to potential escape routes (doors, windows, gaps and eaves) and then the roof or ceiling. The sheets were retrieved after 10 min and the mosquitoes were recovered using forceps. No window traps were used on control homes.

2.4. Field study site

The field experiments were conducted at Kenieroba village (8,33° W & 12.11° N; population census: 2065; 690 houses) located in the northern Sudanian zone, 60–70 km southwest of Bamako on the flood plain of the Niger River. This plain is used for flooded rice agriculture during the rainy season, from May to November, and vegetable crops during the dry season, lasting from December to April. The annual precipitation varies between 750 and 1500 mm, occurring almost exclusively during the rainy season. This period is characterized by intense malaria transmission (Dolo et al., 2003); the prevalence of *P. falciparum* infection increases to 75% of the children less than five years of age, decreasing to 30–50% during the dry season (Dombia 2002; Dolo et al., 2003). The main vectors are *An. gambiae* s.s. (95.5%) and *An. arabiensis* (4.5%), and the mean monthly EIR is 2.8 infectious bites per person with marked seasonal variation (Note that *An. coluzzii* was not considered a separate species at the time of this survey). Long-lasting insecticide treated nets (LLINs) are the main tool for malaria control in this area, with approximately 90% of households possessing at least one bed net.

Twenty four homes distributed throughout the village were chosen for the experiments, twelve received GNTs on the inside and outside bedroom windows and twelve received only PSC treatment on day six as controls. Each home had 1 or 2 residents who were present and using the bedrooms during the study period, but were instructed to keep the bedroom door, and all other doors and windows in the home, closed at all times when not in use. Prior to starting the experiments, homes were checked for gaps between walls and eaves and any

other obvious cracks or spaces where mosquitoes could potentially enter or exit and these were covered with linen sheet.

2.5. Species identification

An. gambiae s.l. mosquitoes caught in the field experiments were counted and stored in 70% ethanol for species identification according to morphology (Gillies and DeMeillon, 1968) and then a random subsample of 75 were identified by PCR (Scott et al., 1993).

2.6. Statistical analysis

General linear model analysis was performed to assess significant differences between daily exit/entry GNTs catch numbers. Analysis was conducted with SPSS software version 20.0 (SPSS Inc., Chicago, IL) and significance was taken at $P < 0.05$.

3. Results

3.1. Cage experiments with *An. gambiae* and *Cx. pipiens*

Results are summarized in Fig. 3a. In the experiment testing the double-sided glue net configuration, a total of 922 *An. gambiae* s.s. (459 females and 463 males) were set free in the release chamber over 9 repetitions. The majority of these mosquitoes ($97 \pm 1.3\%$ of the females and $98 \pm 0.8\%$ of the males) were caught on the net proximal to the release chamber while only a single female was caught on the distal net. A total of 21 mosquitoes over 9 repetitions were not caught by the glue nets. None of the released mosquitoes were stained by the dyed sugar solution, indicating none had fed on the bait presented in the feeding chamber. The control double net without glue allowed 98.0% (1045/1066) of the released mosquitoes ($98 \pm 0.6\%$ of the females and $98.3 \pm 0.8\%$ of the males, $n = 9$ repetitions) to pass the net and feed on the colored bait. The control trap without netting, allowed 96.67% (985/1019) of the mosquitoes ($96.4 \pm 1.6\%$ of the females and $96.7 \pm 1.1\%$ of the males, $n = 9$ repetitions) pass the trap frame and feed on the baits. Fig. 3b shows the percentage of *Cx. pipiens* that were caught by the GNTs and the control traps. Results were similar to those obtained in experiments with *An. gambiae*.

3.2. Field experiments in kenieroba village, Mali

During the 6 days of the field study a total of 1610 *An. gambiae* s.l. (853 females and 796 males) were collected. Random samples of 25 females caught with window entrance traps, 25 caught with exit traps and 25 caught with PSCs and tested by PCR demonstrated that the species composition included 64.00% *An. coluzzii* ($n = 48$), 33.33% *An. gambiae* s.s. ($n = 25$), and 2.67% *An. arabiensis* ($n = 2$).

In total, 231 females and 215 males were captured by the window exit GNTs over a collection period of 5 days. The majority of the females were captured on the first (44.59%) and second days (41.99%), while significantly smaller numbers were captured on the third day (11.26% ; $P < 0.01$), and negligible numbers on the fourth (2.17% ; $P < 0.001$). Likewise, 49.8% and 49.3% of the males were captured on the first and second days, respectively, and only 0.93% of the males were captured on the third day. No mosquitoes were captured on

the window exit GNTs on the fifth day, and a final PSC on the sixth day yielded only 6 females and a single male.

4. Discussion

Effective window traps for large-scale field studies should possess several key characteristics. They should be easy and inexpensive to construct, install and transport to the field but they must also be effective at their primary purpose which is sampling subpopulations of live mosquitoes from both the inside and the outside of homes. Our newly-designed window traps proved to be highly efficient for sampling mosquitoes attempting to penetrate the glue nets. First, cage studies demonstrated the GNT's effectiveness by capturing > 95% of released, laboratory reared *An. gambiae* and *Cx. pipiens* (Fig. 3a, b). Second, the subsequent field test in Mali showed that the majority of mosquitoes inside houses were captured by the exit GNTs. This was demonstrated by the very small number of mosquitoes collected by the PSC procedures on the last day (Table 1b).

According to Silver (2008) and WHO (1975), simply reversing existing window exit traps, such as the Muirhead-Thompson trap, to use as entrance traps does not necessarily provide a reliable sampling of mosquitoes attempting to enter homes; much smaller trap catches have been reported when using exit traps as entrance traps. For example, in a field study in Kenya, van Someren et al. (1958) caught over 14,000 mosquitoes belonging to 30 species from local homes using large window exit traps but when used as entrance traps, caught only 289 mosquitoes belonging to 6 species. Teesdale (1955) also caught considerably fewer mosquitoes using window exit traps as entrance traps. In contrast to traditional window traps such as the Muirhead-Thompson trap, our new GNT design functions effectively as both an exit trap and an entrance trap and can be used simultaneously over a single window. When used as an entrance trap, we caught 773 *An. gambiae* s.l. over the entire study period, which was nearly double the total catch (n = 446) of the exit traps. The traps only caught a significant number of mosquitoes over the first two days before dropping drastically, yet the number of mosquitoes caught trying to enter remained constant over the five day collection period (Table 1a). This may be because mosquitoes do not stay indoors much longer than two days before they must leave to search for sugar outdoors.

Entrance/exit traps are commonly used to measure the effect of insecticide treatments in homes and villages (WHO, 1975; Silver, 2008), specifically on the normal movement, density, and inhibition of feeding of endophilic species. The degree of natural and insecticide induced exophily can be calculated using the ratio of gravid: human blood fed female mosquitoes in exit trap collections (WHO, 1975). If this number is less than 1, the species has natural or insecticide induced exophilic tendencies. Though beyond the scope of this study, our GNT exit traps can provide field workers with a useful tool for calculating this index as the trap does not destroy the body of blood fed females needed for analysis. Almost 95% of the mosquitoes captured on the entrance/exit window GNTs, were trapped by their legs or wings (Fig. 1). This allows for easier storage and for performing additional immunologic, molecular or biochemical investigations. The glue on the nets also prevented foraging insects such as ants from consuming or carrying away the specimens.

Several modifications can be carried out to adapt GNTs for different studies. Although green netting was used in our study, white netting might prove to be more useful as it is more visually trans-parent for many target insects. Larger mesh size can be used for larger species to ensure minimal contamination by the adhesive coating. Several other species collected during this study demonstrate that these traps can also be used without modification to sample a variety of insects, including houseflies, sand flies, beetles and storage pests such as microlepidoptera. GNTs might also be an effective, low-tech, inexpensive method to kill mosquitoes and other pests entering through windows, if glue is used in combination with a pesticide.

We found that once the nets are mounted, a minimum effort is necessary to recoat the nets, and the advantage of the relatively large mesh size is an almost undisturbed air circulation, which made GNTs very popular with the local people. Overall, the setup and collection of GNTs required relatively little manpower. It should also be noted that the total number of mosquitoes caught during the study period was evenly distributed amongst the homes with no single household accounting for more than 13% of the total catch (data not shown).

Herein, we have successfully designed and tested a versatile and simple yet effective tool that can be used to replace existing window traps for the study of endophilic mosquito behavior.

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Fig. 1.
Close-up of two *An. gambiae* s.l. caught in the GNT. Mosquitoes trying to pass the netting get caught by the glue. Note the body almost never touches the glue.

a) Exterior window GNT

b) Interior window GNT

Fig. 2.
Installation of GNTs on the (a) exterior of a bedroom window, and (b) the interior window.

a) Percentage of released laboratory reared *An. gambiae* s.s. caught by GNTs compared to controls (n = 9 repetitions).

b) Percentage of released laboratory reared *Cx. pipiens* caught by GNTs compared to controls (n = 9 repetitions).

Fig. 3.

(a) Percentage of released laboratory reared *An. gambiae* s.s. caught by GNTs compared to controls (n = 9 repetitions). (b) Percentage of released laboratory reared *Cx. pipiens* caught by GNTs compared to controls (n = 9 repetitions).

An. gambiae s.l. collected with window exit GNTs, window entrance GNTs and PSC within 12 experimental and 12 control houses on 6 consecutive days. a) Average catch per experimental dwelling per day and total catch (n). b) Average catch per control dwelling per day and total catch (n).

Table 1

A. Experimental Dwellings							
Trapping Method	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Exit GNT	F	8.58 (103)	8.08 (97)	2.17 (26)	0.42(5)	0	N/A
	M	8.92 (107)	8.83 (106)	0.17 (2)	0	0	N/A
Entrance GNT	F	5.33 (64)	7.08 (85)	8.00 (96)	6.08 (73)	9.33 (112)	N/A
	M	6.17 (74)	7.25 (87)	8.42 (91)	4.83 (58)	5.17 (62)	N/A
PSC	F	N/A	N/A	N/A	N/A	N/A	0.5 (6)
	M	N/A	N/A	N/A	N/A	N/A	0.08 (1)
B. Control Dwellings							
Trapping Method	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
PSC	F	N/A	N/A	N/A	N/A	N/A	15.5 (186)
	M	N/A	N/A	N/A	N/A	N/A	16.5 (198)

* N/A: Not applicable.