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A Simple Method for Sampling Indoor-Resting Malaria Mosquitoes *Anopheles gambiae* and *Anopheles funestus* (Diptera: Culicidae) in Africa

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ABSTRACT Sampling indoor resting African malaria vectors is traditionally done by hand catches with oral or mechanical aspirators and pyrethrum spray catches (PSCs). In this study, we designed and briefly evaluated an inexpensive but practical alternative by using a cloth resting box or wicker resting basket and a ceiling net. Evaluations were performed in greenhouse and field situations in rural Kenya by comparing capture rates of *Anopheles gambiae* s.l. and *Anopheles funestus* (Giles) in these traps to hand collections and PSCs. A resting box and a ceiling net when used together collected more mosquitoes than a single collector using a hand-held aspirator but only one-third the number collected by PSCs. At sites where PSCs are impractical, a resting box and ceiling net can be effectively used as an alternative to hand catches in malaria surveillance.

KEY WORDS resting box, ceiling net, Kenya, *Anopheles*, adult mosquito collection

Entomological surveys associated with malaria prevention and control efforts require the sampling of adult mosquitoes (WHO 1992, Service 1993, Service and Townson 2002). In Africa, this largely involves techniques that collect females inside human dwellings. Such techniques include exit traps, bed-net traps, hand aspirator and net collections, drop net collections, and human landing catches (Service 1993). Collections involving human bait are unethical if they expose human subjects to bites from infected mosquitoes. Alternatives that do not expose human subjects include carbon dioxide and light traps as well as animal-baited traps. The effectiveness of these methods varies, but their cost and practicality often preclude their adoption in communities in poor, disease-endemic countries.

The indoor resting behavior of important malaria vectors such as *Anopheles gambiae* and *An. funestus* are well documented (Haddow 1942; Smith 1955, 1962; Fay et al. 1997). Because of this behavior, sampling vector populations resting indoors can be a valuable part of surveillance and control programs (WHO 1992, Lindblade et al. 2000). Resting mosquitoes provide greater variety in terms of sex, bloodmeal status, age, and gonotrophic condition than host-seeking mosquitoes (Service 1993).

Hand collections, made with a mouth or mechanical aspirator, and knockdown pyrethrum spray catches (PSCs) are the two methods commonly used for collecting mosquitoes resting indoors (WHO 1992, Service 1993, Service and Townson 2002). However, the variable skill and initiative of collectors can greatly influence the samples collected within dwellings. Pyrethrum spray catches are more expensive and cause human exposure to potentially hazardous insecticides. Because the chemicals used in spray catches have varying degrees of repellency and persistency, dwellings are normally sampled no more than twice a week (Service 1993).

Because some of the world's most important vector species rest inside human and animal dwellings, the development of better techniques for sampling indoor-resting mosquitoes has great value in entomological surveys. New methods tested to date have had limited success. One study tested the use of plywood resting boxes as a method to sample indoor mosquitoes, but it found the boxes to be effective only with high densities of mosquitoes in low humidities (Yasuno et al. 1977). Resting boxes made of cardboard and black muslin cloth attracted between one-third and two-thirds of the *Aedes aegypti* (L.) collected indoors with a modified vacuum cleaner aspirator (Edman et al. 1997, Kittayapong et al. 1997). An insecticide-impregnated fabric (IIF) trap has been developed and used for sampling indoor-resting mosquitoes; however, in most rural African settings the materials needed for constructing the trap are not available, and the specimens collected are dead (Das et al. 1997). A

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Kenyan study used a 1.5- by 1.8-m (5- by 6-foot) reed ceiling mat from which hand catches of indoor-resting anophelines were made. This method was not compared with other methods for sampling indoor-resting mosquitoes to assess its comparative efficacy (Sexton et al. 1990).

The objective of our study was to develop and assess the relative utility of a sampling method incorporating artificial resting sites as simple and inexpensive alternatives to hand catches and PSCs. Three specific objectives guided our efforts: 1) to identify, using hand catches, the preferred indoor resting sites of common Afrotropical malaria vectors *An. gambiae* s.l. and *An. funestus* (Giles); 2) to evaluate the efficacy of traditional indoor hand collections made by an experienced collector; and 3) to test the efficacy of simple resting boxes for sampling populations resting indoors in semifield conditions (i.e., a screen-walled greenhouse). These evaluations were carried out to determine which materials are attractive to resting mosquitoes, the preferred placement of resting harborages inside houses, and the efficiency of the new sampling methods.

The method that emerged, a resting box in combination with a resting net, was then tested against 15-min hand catch collections conducted by an experienced collector. Promising results in greenhouse and field situations led us to explore the development of another resting box option, wicker baskets. The efficacy of the baskets was only evaluated with *An. gambiae* s.s. mosquitoes under greenhouse conditions.

Materials and Methods

Site Descriptions. All studies were conducted in Suba District, Nyanza Province of Western Kenya. Greenhouse trials were conducted at the Thomas Odhiambo Campus (Mbita Point) of the International Centre of Insect Physiology and Ecology (ICIPE). Fields trials were conducted in the village of Luanda Nyamasare, located ≈12 km away. The area has two rainy seasons. The long rainy season occurs from March to June, and the shorter rainy season occurs from October to December. Malaria is considered holoendemic in the region and is typically most prevalent near the end of each rainy season (Mutero et al. 1998). A more detailed description of the area has been given previously (Geissler et al. 2000, Knols et al. 2002, Mathenge et al. 2002, Okech et al. 2003).

Indoor Resting Habitats of *Anopheles*. From 27 March to 18 April 2004, at varying times of the day (0825–1645 hours), 32 human dwellings in Luanda Nyamasare were visited by an expert mosquito collector. All resting mosquitoes were collected with an oral, plastic-tube aspirator (hand catches). This method is widely used for determining preferred indoor-resting sites (WHO 1992, Service 1993, Service and Townson 2002).

The collector thoroughly searched each dwelling and transferred collected mosquitoes to paper cups with net covers. As much time as was needed to collect all observable mosquitoes was taken; the time spent in each house was recorded. Searching involved looking under and behind furniture, behind curtains, and around cooking utensils and other household items. Live specimens were anesthetized with ethyl acetate to aid identification. Twenty-one of the sampled dwellings were constructed of wood and mud with corrugated iron roofs. Seven were made of wood and mud with thatched roofs, three where made completely of corrugated iron and wood, and one of grass reeds and wood. Nine of the most productive dwellings were revisited 5–10 d after the first collection to increase the sample size (a total of 41 collections). Of these nine, one house was made completely of corrugated iron and wood, three were made of mud and wood with thatched roofs, and five were made of mud and wood with corrugated iron roofs. None of the 32 dwellings sampled had ceiling boards. All of the dwellings were rectangular with the exception of a single circular house (made of grass reeds and wood). Dwellings were always divided into two smaller rooms by a hanging cloth, reed mats, or mud wall.

The approximate height where each mosquito was collected was recorded as high, medium, or low. "High" was designated ≈161–240 cm from the ground (above the collector's shoulders). "Medium" was ≈81–160 cm from the ground (between the collector's waist and head). Mosquitoes caught below the collector's waist were designated as "low" (≈0–80 cm from the ground). Surfaces and objects from which mosquitoes were collected were recorded and divided into five categories: 1) wall/floor/door; 2) plastic items, e.g., bottles and tubs; 3) cloth items, e.g., clothes and bed-nets; 4) permanent wooden items unlikely to be moved, e.g., beds and cabinets; and 5) temporary items, i.e., likely to be disturbed or moved, e.g., lanterns or bicycles. The total number collected in area deemed "hidden" (not in plain view of the collector) was noted. For example, a mosquito collected on a plastic item located in a hidden area would have been recorded as "plastic" and "hidden." These categories are similar to those used in studies conducted in India (Pal et al. 1960, Wattal and Kalra 1960).

Evaluation of Hand Collection Methods. Twenty-one dwellings of similar size were sampled by the collector from 1 to 16 April 2004. Thirteen were made of wood and mud with corrugated iron roofs, seven were made of wood and mud with thatched roofs, and one was made of corrugated iron and wood. All of the dwellings were rectangular. Mosquitoes were collected by hand aspirator until no more mosquitoes could be found. The time needed to hand collect all mosquitoes was noted. To provide varying densities of resting mosquitoes, collection times were deliberately varied (0825–1630 h).

Immediately after searching a dwelling, a PSC was performed. The PSC involved removing all large pieces of furniture, covering the floor with white bed sheets. Insecticide was first sprayed from outside of the house onto the eaves, windows, and door before entering the dwelling and spraying the entire inside of the house. All doors and windows remained closed for ≈10 min to allow for mosquito knockdown. Collectors

Fig. 1. Cloth resting box (left) and the wicker basket resting box (right).

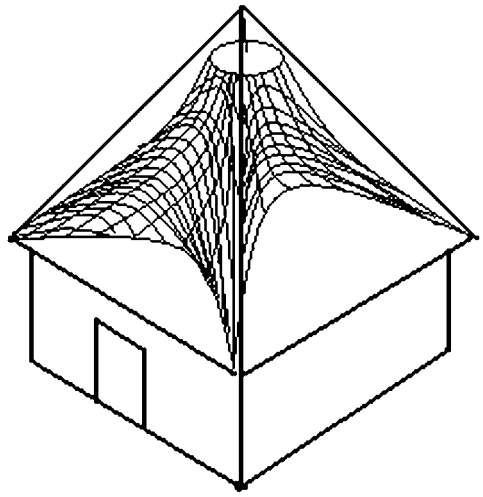

Fig. 2. Resting bed-net hung in hut.

then reentered the dwelling and used forceps to collect mosquitoes from the sheets and place them in plastic vials. A commercial-grade pyrethroid insecticide (Doom Fast Kill, Mortein, Australia, active ingredients d-phenothrin and imiprothrin) was used due to availability and safety. The combined number of mosquitoes collected from the PSC and hand collection was calculated to provide a total catch for each dwelling. The percentage collected by hand was calculated from this total.

Greenhouse Evaluation of a Cloth Resting Box. Greenhouse trials were conducted from 3 April to 15 June 2004 at the Mbita Point station. A 30- by 30- by 30-cm cloth resting box, similar to the design described by Crans (1989), was made of cotton cloth sewn to fit a 2-cm-thick galvanized wire frame. Bright blue cloth was sewn to cover the outside of the box and black cloth covered the inside (Fig. 1). The colors were chosen to create a dark space inside the box. A flap made of mosquito netting (mesh size 196) with a sleeve was sewn to the top of the box. This flap was closed over the open side to facilitate capture when the number of mosquitoes in the box was large.

The box was hung ≈50 cm above the floor with a string from the ceiling of an experimental hut made of plywood (3.2 by 2.7 by 1.7 m) located inside a modified screen-walled 11.4- by 7.1- by 4.2-m greenhouse (Cambridge Glass House Co. Ltd., Huntingdon, Cambridgeshire, United Kingdom). The height chosen for the box was slightly above a person sleeping on a grass mat and conserved floor space in huts with many occupants. The box was hung in the far right corner, opposite the door, and away from the direct light that entered from the door. The open side faced the center of the hut. The experimental hut and greenhouse are described in detail previously (Mathenge et al. 2002).

Each night, female *An. gambiae* s.s. from the Mbita strain colony at one of three different densities (low, 50; medium, 100; and high, 200) were released into the greenhouse at ≈2100 hours. All test mosquitoes were allowed to feed on a 10% sucrose solution for at least 24 h after emergence. Half of the females were blood-fed on a human arm for 10 minutes daily for three nights before release. This was to ensure a mix of unfed, blood-fed, gravid, and half-gravid females tested.

The resting box was checked hourly from 0700 to 1200 hours and again at 1500 hours. All mosquitoes caught in the box were removed with an oral aspirator and counted each time the box was checked. After the final collection of the day, two collectors searched out and removed the remaining females in the greenhouse.

Comparison of Resting Box–Net Method to Hand Collections. Trials were performed from 13 April to 30 June 2004 in the same village in which the previous field studies were run. Preliminary trials suggested that a double size blue bed-net (Supanet, Nairobi, Kenya) could facilitate the capture of mosquitoes that were resting high in the house. The net was hung from the highest point inside a dwelling and then spread out and tied to the top of the eaves, assuming the same shape as the inside roof (Fig. 2). To aid in collection, the resting net could be lowered to within reach of the collector by slowly releasing the string used to hang the net. Mosquitoes agitated by the movement of the net generally flew up into the center of the net where they were easily captured by oral aspirator.

Three houses of similar size and construction were chosen to compare the efficacy of three methods of indoor-resting collection in a Latin Square design. All dwellings had walls of mud and wood. Two dwellings had thatched roofs, and one dwelling had a corrugated iron roof. The three collection methods evaluated were 1) a cloth resting box (described above) with a resting net, 2) a plain cardboard box of similar size to the cloth resting box (42 by 21.5 by 25 cm) with a resting net, and 3) hand collection for 15 min by using a plastic tube mouth aspirator. The cardboard box was included in the Latin Square because it was the simplest box design available in the study area.

The time (15 min) chosen for hand collection was based on previous results (i.e., from the survey of

indoor-resting habitats) and on recommendations by WHO (1992). Immediately after mosquito collections at each dwelling, a PSC was performed (as described above). Dwellings were sampled every 3 d to allow ample time for the insecticide spray to dissipate. The number of test days was based on preliminary observations. Collections were made from 0800 to 1000 hours, when the huts were relatively cool and the occupants were generally outside. Collected mosquitoes were identified as *An. gambiae* s.l., *An. funestus*, culicines, or unidentified anophelines. The Latin Square was run for a total of 27 experimental days in three complete house rotations. The time required to complete the sampling of the resting box and resting net was recorded.

Evaluation of a Resting Basket. From 15 May to 13 July 2004, trials were run in the greenhouse by using a brown wicker basket (30 cm in height; 28-cm mouth diameter) with black cotton cloth insert (Fig. 1). The basket was hung ≈50 cm from the ground inside the experimental hut in a modified, screen-walled greenhouse as in the previous trials. Each night, one of three different densities of female *An. gambiae* s.s. mosquitoes (low, 50; medium, 100; and high, 200) from the Mbita strain colony were released into the greenhouse at ≈2100 hours. Methods for the resting basket were the same as described for the cloth box. Ten trials were run at low and high densities, and 32 trials were run at medium density.

Data Analysis. A Kruskal–Wallis test was performed to analyze the percentage of mosquitoes caught at varying heights in the dwellings (SAS Institute 2001). The total number of mosquitoes collected during the Latin Square experiment by using each method (hand collection, cloth resting box and resting net, and cardboard box and resting net) was calculated. For each experiment, the total number of mosquitoes caught by an individual method (*TN*) was added to the numbers caught by PSC performed after the same method giving an actual overall total (*OT*). The percentage of the overall total number (*POT*) of mosquitoes collected using each method was calculated with the following equation:

$$TN \div OT = POT \qquad [1]$$

The percentages captured by each of the methods in the Latin Square on each test day were transformed using the following equation:

$$\arcsin\sqrt{x} \qquad [2]$$

where x is the percentage of mosquitoes captured by a method on each test day. The results were then analyzed using the Tukey multiple comparison procedure (SAS Institute 2001).

The percentages of mosquitoes recaptured by both the resting basket and resting box were compared using a type III sum of squares test (SAS Institute 2001).

Table 1. Percentage of total number of mosquitoes (437) collected from each location

Location	% total
Wall/floor/door	34
Furniture (more permanent)	30
Cloth/bed-net	24
Temporary items	8
Plastic items	4
Hidden (protected)[a]	26

[a] The category "hidden" was noted separately and describes those mosquitoes collected on a location category that was out of plain view of the collector (i.e., the collector needed to move or look under furniture to find the resting mosquito).

Results and Discussion

Indoor Resting Habitats of *Anopheles*. Mosquitoes in the dwellings were collected from a variety of different substrates. Approximately one-quarter of the collected mosquitoes were from areas hidden from view (Table 1). There was no significant difference ($\chi^2 = 0.97$, df = 2, $P = 0.61$) in the number of mosquitoes collected at the low ($n = 176$), medium ($n = 111$), and high ($n = 119$) elevations in the dwellings surveyed. Results showed no particular substrate preference on which mosquitoes rested. Therefore, the materials for the box design (cloth with wire frame) were chosen because for their economy and safety for the occupants.

For mosquito species that rest indoors, it is generally thought that males and females prefer to rest in dark, low areas (Bidlingmayer 1994). One would expect, therefore, to find *Anopheles* resting at low heights because they would be further away from the main indoor light source, the eaves, yet close to localities often occupied by humans (e.g., beds and chairs). Although we found a large number of mosquitoes resting at heights of 0–80 cm, this number was not significantly different from the number resting above 80 cm.

About one-quarter of the mosquitoes (26%) were found in hidden areas, not in plain view of the collector. The technician used as much time as needed to complete the search of each dwelling during the initial part of the study. We assumed that the majority of the mosquitoes not found by the technician but collected in the PSC performed after the search were resting above the reach or out of sight of the collector. We decided that our collection method needed to account for mosquitoes resting out of the reach of hand collections, usually on the exposed underside of roofs (Schofield and White 1983, Lindsay et al. 1995).

Accuracy of Hand Collection Methods. Of the 531 mosquitoes captured in 21 dwellings by both hand collection and PSC performed afterwards, the hand collection method captured 32.2% (171 of 531) of the total collection. The collector took a mean of 10.8 ± 5.0 min to collect all mosquitoes taken by hand catches in each dwelling. The training of the technician is a critical component of the success of this method because the mosquitoes are extremely difficult to locate

Table 2. Percentage of the overall number of mosquitoes found in each dwelling over the 27 test dates for the three methods tested

Date	Hand collection	Cloth box/ resting net	Cardboard box/ resting net
13 April	6.7	56.0	2.2
16 April	9.6	12.0	85.7
19 April	48.1	34.6	18.9
22 April	14.7	14.2	9.3
25 April	14.5	25.0	20.5
28 April	28.5	28.7	11.9
1 May	9.0	63.2	5.2
4 May	0.0	25.0	14.2
7 May	8.3	0.0	31.6
10 May	4.6	35.7	0.0
13 May	0.0	0.0	23.6
16 May	37.0	10.7	21.5
19 May	13.3	38.4	3.2
22 May	17.2	17.3	42.3
25 May	34.7	20.0	45.6
28 May	3.8	20.0	15.0
31 May	14.8	23.2	48.0
3 June	28.8	21.4	20.8
6 June	14.4	66.6	18.7
9 June	5.1	19.4	30.7
12 June	11.1	0.0	17.0
15 June	33.3	60.7	12.5
18 June	4.1	32.5	37.5
21 June	33.3	10.0	37.5
24 June	22.8	10.5	9.0
27 June	6.6	29.7	46.1
30 June	30.0	14.2	16.6
Mean % for each method ± SE	15.9 ± 12.9a	27.1 ± 18.5b	23.3 ± 18.6a,b

Methods followed by the same letter are not significantly different ($P \leq 0.05$). Note: $F = 3.37$; df = 2, 50.

and capture. The impact of training is borne out by the results presented here.

Evaluation of Cloth Resting Box. The total mean percentage of *An. gambiae* s.s. recaptured during the 30 greenhouse trials of cloth resting boxes was 36.1 ± 9.9%. Because this percentage was similar to that of our hand collections (32.2%), a direct field comparison of the two methods was planned. However, we felt that a cloth resting box placed near the floor of a natural dwelling would not efficiently sample mosquitoes that prefer to rest on the underside of the roof. Thus, a resting net was developed and tested as a compliment to the resting box.

Comparison of Resting Box–Net Method to Hand Collections. Results of the Latin Square trial indicated that a cloth resting box in combination with a resting net collected a significantly higher percentage of the total number of mosquitoes resting in a dwelling compared with the hand collection method. The cloth resting box with a resting net (*POT* = 27.6%, 282 of 1,020) caught 1.7 times more mosquitoes than the hand collection method (*POT* = 14.2%, 166 of 1166). The cardboard resting box with a resting net (*POT* = 24.1%, 256 of 1,060) caught 1.5 times more mosquitoes than the hand collection method. Mosquito catches from the cloth resting box with a resting net were significantly greater ($F = 3.37$; df = 2, 50; $P = 0.05$) than collections made by hand (Table 2). There was no significant difference between the cloth resting box with a resting net ($P = 0.97$) and cardboard resting box with a resting net nor between the cardboard resting box with a resting net and the hand collection method ($P = 0.93$) (Table 2). The time taken to complete sampling using a resting box and ceiling net (8.0 ± 3.9 min, $n = 31$) was less than the 15 min suggested by WHO (1992) for hand collection). The mosquitoes collected by all three methods (boxes, ceiling nets, and hand aspiration) were predominately *An. gambiae* s.l. (Table 3).

Evaluation of an Indoor-Resting Basket. To provide a practical alternative to the resting box, a resting basket was developed and tested under greenhouse conditions. The resting basket recaptured 33.8% ± 8.9 ($n = 52$) of released experimental mosquitoes. No

Table 3. Percentage of the total mosquitoes collected by each trap tested the Latin Square

Type of trap	*An. gambiae* s.l.	*An. funestus*	Culicine	Unidentified anopheline
Cloth box, $n = 51$	90.2	7.8	2.0	Nil
Cardboard box, $n = 20$	85.0	10.0	Nil	5.0
Resting net,[a] $n = 408$	93.2	4.4	2.2	0.2
Hand aspiration, $n = 144$	92.4	6.2	0.7	0.7

Note: n is the number of trials run for each method. No mosquitoes were collected with a mark of "nil."
[a] Collections from the two resting nets tested were compiled together.

significant difference was found in the total percentage of recaptured mosquitoes between the resting basket and resting box methods ($F = 0.51$; df = 1, 76; $P = 0.47$). Because there were no significant differences in these recapture rates, the resting basket seem to be an acceptable alternative. Materials necessary to construct baskets are cheaper and more readily available to malaria control workers in many African villages.

The cloth resting box or basket in combination with a ceiling net has a number of advantages over the traditional hand collection method. Mosquitoes are easier to see in the black cloth box and in the bright blue net and the collector only needed to search a small area rather than the entire house. The time needed to complete a house search is only two-thirds that commonly recommended for hand collections (WHO 1992).

Resting boxes and nets also have advantages over the PSC technique. Although PSC captures most, if not all, mosquitoes resting in a dwelling, the cost of sustained use is often beyond the means of poor countries. Pyrethrum spray catches also expose the investigators and the members of the household to potentially harmful chemicals (Service 1993). Our study showed that cloth resting boxes or baskets used along with ceiling nets can be a practical alternative surveillance method for local malaria control programs to sample indoor resting mosquitoes. Even simple cardboard boxes, as tested in this study, may provide a suitable alternative to the hand collection method.

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