A MOSQUITO BEHAVIOR FRACTIONATOR[1]

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ABSTRACT. A new apparatus, a mosquito behavior fractionator, for testing adult mosquitoes for house entering behavior was developed. The structure, function and operation of the apparatus are described and results of tests of diverse populations of *Ae. aegypti* from East Africa, Kenya are analyzed.

A sample of about 100 mosquitoes of both sexes is released into the central unit of the fractionator. After a minimum of 1 hour of stabilization period, two 200 W incandescent bulbs producing light and heat are switched on. Two solid metal partitions separating the release chamber from the "house" simulating and "open" simulating unit are removed. The tested mosquitoes have a choice to move freely within the three units. After 24 hours of separation, the metal partitions are placed back to their slits, the tested mosquitoes are recovered from the "house" simulating chamber and counted. The mosquitoes from the "open" simulating unit and the release chamber are also recorded.

Results from field experiments with the same populations using a mark-release-recapture method in Shaury Moyo village support the results on behavior achieved by testing in the fractionator. The behavior fractionator could be a valuable tool for testing mosquitoes for domesticity, as well as for genetic studies in mosquito behavior in the laboratory.

INTRODUCTION

A vast body of literature has been accumulated on systematics, distribution, ecology, genetics and vectorial capacity of the yellow fever mosquito, *Aedes aegypti* (L.). Surprisingly little has been done on behavior and inheritance of behavior of this species. It is primarily because of the technical difficulties to measure behavioral traits quantitatively (Mattingly 1967).

By a mark-release-recapture experiment, Trpis and Hausermann (1975) demonstrated differential domesticity in field populations of *Ae. aegypti* in the Rabai area, Kenya, where three different populations of *Ae. aegypti* (domestic, peridomestic and feral) in a relatively small area (ca. 1 mi.^{2}) were detected. Their results indicate that the feral, dark *Ae. aegypti formosus* does not enter houses even if released in the village area. The pale domestic form, *Ae. aegypti aegypti*, breeds independently of natural rainfalls, but in full dependence on man. The peridomestic populations of *Ae. aegypti aegypti* exhibit a wide color variation, and are probably hybrids between the feral and domestic forms. These enter houses only occasionally (15–20%).

To field test mosquito populations for domesticity by the mark-release-recapture method is a very laborious procedure. A reliable laboratory testing method requiring minimum time and assistance is desirable. The purpose of this paper is to present a testing device and procedure for analysis of diverse populations of *Ae. aegypti* for house-entering behavior. We believe that the apparatus will also be a valuable tool in fundamental behavioral genetic studies in other mosquito groups associated with humans at their dwellings.

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DESCRIPTION OF THE BEHAVIOR FRACTIONATOR

A three-cage unit for release and subsequent detection of differences in behavior was developed. The frame was made of wood pieces 30 x 30 mm. The walls were made of hardboard, clear plastic or nylon screen.

1. *Release unit* (Plate I, fig. 1A, 4A; Plate II, fig. 1A, 2A).

The control cage was screened with nylon mesh on the top, back side and partially on the front side. A sleeve was attached to the lower portion of the front side (Plate II, fig. 1j). The release cage was separated from the "house" simulating unit (B cage) by two partitions. The first partition was made of a 2 mm thick aluminum sheet held in a slit (Plate II, fig. 1h); and the second partition was made of 3 mm thick plywood (Plate II, fig. 1g). Six rectangular and 7 circular holes were made in the plywood partition (Plate I, fig. 2a, b; Plate II, fig. 3a, b). Between the release cage and "open" simulating unit (C), only one (metal) partition was made; this partition was also removable (Plate II, fig. 1k).

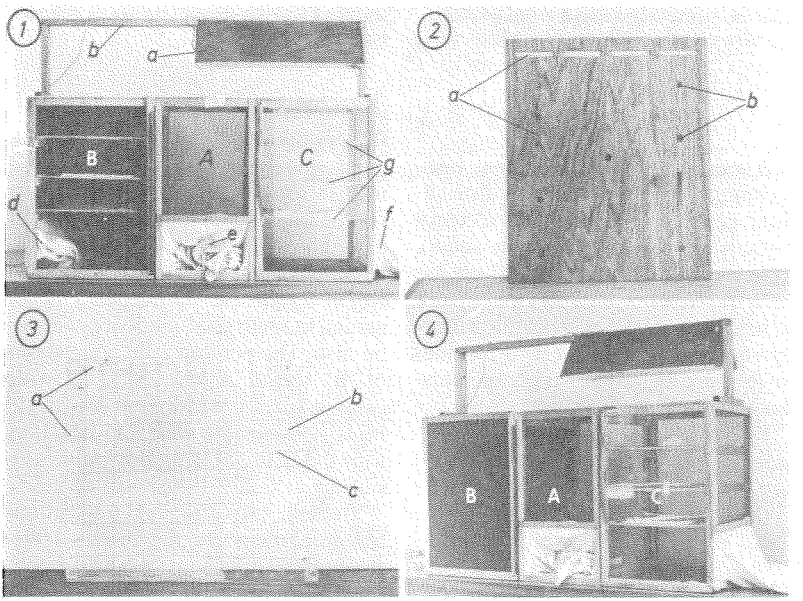

Plate I. The mosquito behavior fractionator.

Fig. 1. Three cage assembly, inside view.
a) Lamp shade for two 200 W incandescent bulbs, b) Holder of the lamp shade, d, e, f) Sleeves for manipulation inside cages, g) Plastic shelves.
A.—Release unit. B.—"House" simulating unit C.—"Open"simulating unit.

Fig. 2. Plywood partition between the (A) and (B) unit.
a) Rectangular holes, b) Circular holes.

Fig. 3. Plastic shelf.
a) Plastic frame, b) Nylon threads.

Fig. 4. The fractionator prepared for testing.
A.—Release unit. B.—"House" simulating unit. C.—"Open" simulating unit.

2. *"House" simulating unit* (Plate I, fig. 1B, 4B; Plate II, fig. 1B).

The "house" simulating unit (B) is closed on four sides with 3 mm thick solid plywood. The front side is closed with 3 mm thick clear plastic inserted vertically in grooves. The partition between this cage and the cage (A) has already been described above. A cloth sleeve is attached to the outer side of the (B) unit (Plate II, fig. 1f). Three removable shelves made of a plastic frame and nylon threads (Plate I, fig. 3; Plate II, fig. 4) are placed in the "house" simulating unit.

3. *"Open" simulating unit.* (Plate I, fig. 1C; Plate II, fig. 1C).

The "open" simulating unit is enclosed on three sides (top, back and left hand side) with nylon screen. The bot-

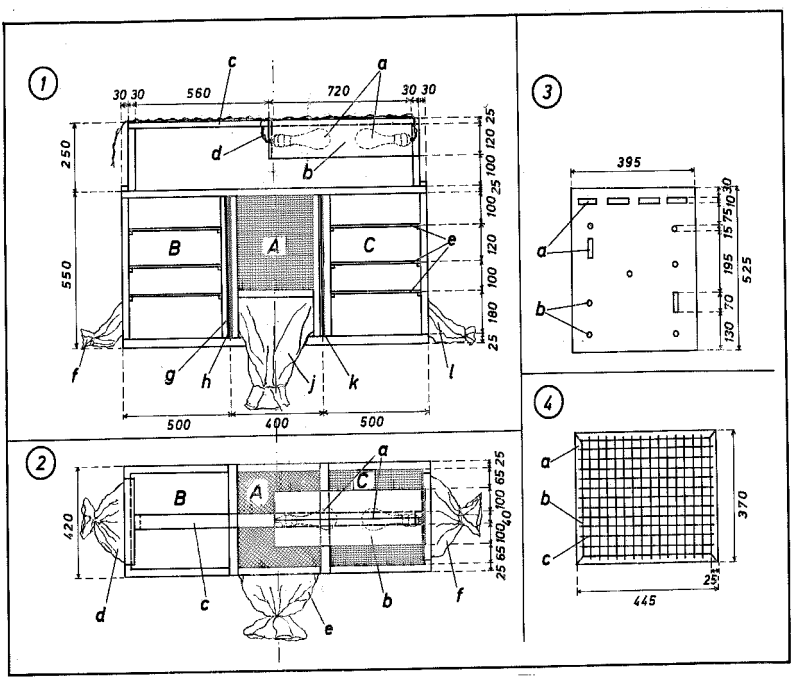

Plate II. Technical drawing of the mosquito behavior fractionator.
(All measurements are expressed in mm.)

Fig. 1. Front view.
A.—Release unit. B.—"House" simulating unit. C.—"Open" simulating unit.
a) 200 W incandescent bulbs, b) Lamp shade, c) Lamp shade holder, d) Electric wire, e) Plastic shelves, f, j, l) Sleeves for work inside the units, g) Plywood partition, h, k) Metal partitions.

Fig. 2. Top View.
A.—Release unit. B.—"House" simulating unit. C.—"Open" simulating unit.
a) 200 W incandescent bulbs, b) Lamp shade, c) Lamp shade holder, d, e, f) Sleeves for manipulating inside the units.

Fig. 3. Plywood partition with holes for entrance of mosquitoes to the chamber (A).
a) Rectangular holes, b) Circular holes.

Fig. 4. Plastic mesh shelf.
a) Plastic frame, b) and c) nylon threads.

tom is made of 5 mm plywood and the wall between the unit (C) and (A) is the removable aluminum partition already described. The front side is covered with clear plastic. Three shelves made of plastic frame and nylon threads identical with those described at the unit (B) are also placed in the unit (C). Several strips of filter paper (50 x 150 mm) were placed on the shelves in an irregular pattern. Similar pattern was developed on the shelves of the "house" simulating unit (B). A sleeve was also attached to the outer side of the unit (C).

4. *Light-heat source*. (Plate I, fig. 1a; Plate II, fig. 1a, 2a).

Two 200 W incandescent bulbs are placed in a plywood shade over the cage unit (C) and half of the release unit (A), (Plate I, fig. 1a).

EXPERIMENTAL PROCEDURE

A population of approximately 100 adult mosquitoes (*50* ♀, *50* ♂) were released into unit (A) and left to stabilize for at least 1 hour. Honey, cellucotton mixture and water in paper cups lined with paper towel were placed on the bottom of the unit (B) and (C). The tests were conducted in an insectary at a temperature between 26–30°C and 70–90% relative humidity. After the stabilization period, the lights were switched on and the two metal partitions (Plate II, fig. 1h, k) removed. The bulbs created both light and heat. The mosquitoes had a choice of either remaining in the release unit or moving to the "house" or "open" simulating unit. The unit (C) is partly shaded with paper strips placed on shelves; thus the intensity of the light and heat decreases toward the bottom of the unit. The "house" simulating unit is relatively dark, temperature substantially lower than in the release or "open" simulating unit and the mosquitoes moving to this unit must have ability to enter dark rooms through small holes. The whole process of separation took 24 hours.

The tests were carried out with laboratory reared mosquitoes derived from eggs, larvae or adult collections from different habitats (domestic, peridomestic, feral). In each test at least two different populations were released. Each population was marked with a different color of fluorescent pigment before release in order to separate them after fractionation. The laboratory reared mosquitoes were between 72–96 hours of age at the time of testing.

After 24 hours the metal partitions were placed back in their slits in order to prevent movement of mosquitoes from unit to unit. The mosquitoes were recovered from the fractionator through the sleeves. The mosquitoes removed from the "house" simulating unit were separated into original populations by means of UV light and the numbers of each population recorded. The mosquitoes from the unit (C) and (A) (which both were lighted) were pooled and subsequently separated the same way as those removed from the unit (B).

RESULTS

In the first series of experiments, three populations of different origin (domestic, peridomestic, feral) were tested in the fractionator for house entering behavior (Table 1). The same three populations were marked with fluorescent pigments, released in Shaury Moyo village, and recaptured in landing-biting catches on man inside houses. The results of recovery from both the fractionator and from houses show a strong similarity indicating that the behavior fractionator is a suitable tool for testing of diverse populations of *Ae. aegypti* for house entering behavior.

Table 1. Comparison of house entering behavior of three selected populations of *Ae. aegypti*, in the behavior fractionator and in field releases.

Strain	Origin	Laboratory test: * Numbers recovered in dark chamber ♀♀	♂♂	Total	% of recovery	Field release: ** Numbers recovered inside houses ♀♀	♂♂	Total	% of recovery
First test:									
MWAMSABU	domestic	26	19	45	60.9	161	153	314	82.6
MNAZI	peridomestic	9	6	15	20.2	32	28	60	15.8
BEJUMWA	feral	6	8	14	18.9	3	3	6	11.6
		41	33	74	100.0	196	184	380	100.0
Second test:									
MWAMSABU	domestic	28	18	46	68.7	99	116	215	80.6
MNAZI	peridomestic	8	6	14	20.9	30	19	29	18.3
BEJUMWA	feral	5	2	7	10.4	11	2	3	1.1
		41	26	67	100.0	130	137	267	100.0

* Release in cages: Both tests 100 per strain, 50 of each sex.
** Release in field: 1. First test 2000 per strain, 1000 of each sex,
2. Second test 900 per strain, 450 of each sex.

Table 2. Recoveries of the domestic and non-domestic populations of *Ae. aegypti* from the dark chamber of the behavior fractionator.

Origin of population (village)	Numbers recovered in dark chamber							
	Domestic populations				Non-domestic populations			
	♀♀	♂♂	Total	% of recovery	♀♀	♂♂	Total	% of recovery
Rabai	39	26	65	80.2	11	5	16	19.8
Simakeni	38	20	58	84.1	9	2	11	15.9
Kipevu	14	15	29	85.3	3	2	5	14.7
Buni	24	12	36	80.0	7	2	9	20.0
Shaury Moyo	28	15	42	81.1	5	5	10	18.9
Total	143	88	281	81.9	35	16	51	18.1

Subsequent experiments using ten diverse populations confirmed the previously achieved results (Table 2). The mosquitoes originated from domestic habitats were always recovered in high percentage (80–84%) in the dark (B), "house" simulating chamber of the fractionator. Recoveries of the non-domestic populations in the "house" simulating unit were substantially lower, ranging between 14.7–20%.

DISCUSSION

The differences in behavior in the domestic and non-domestic *Ae. aegypti* are attributed probably to positive phototaxis of the outdoor breeding populations and negative phototaxis of the domestic mosquitoes, or an ability of the domestic populations to enter dark rooms through holes. The abundance of domestic *Ae. aegypti* in traditional windowless houses and their scarcity in modern buildings of the same village would be in agreement with this hypothesis.

Domesticity as exhibited by *Ae. aegypti* at the Kenya coast is the result of a combination of several behavioral traits such as host preference, choice of oviposition sites, as well as choice of mating and resting sites (Trpis and Hausermann 1975). In combination with an olfactometer test on host preference (Gouck 1972; Trpis, unpublished), the present testing method could be of valuable assistance in determination of the origin of mosquito populations. The behavior of the mosquitoes is of a considerable importance in ascertaining the vectorial capacity. The proposed testing method could also be used in genetic analysis of mosquito behavior in laboratory studies.

References Cited

Gouck, H.K. 1972. Host preference of various strains of *Aedes aegypti* and *A. simpsoni* as determined by an olfactometer. WHO Bull. 47:680–683.

Mattingly, P. F. 1957. Genetical aspects of the *Aedes aegypti* problem. I. Taxonomy and bionomics. Ann. Trop. Med. Parasitol 51:392–408.

Trpis, M. and Hausermann, W. 1975. Demonstration of differential domesticity of *Aedes aegypti* (L.) (Diptera, Culicidae) in Africa by mark-release-recapture. Bull. Entomol. Res. 65:199–208.