Malaria Journal

BioMed Central

Research

Open Access

Is the Mbita trap a reliable tool for evaluating the density of anopheline vectors in the highlands of Madagascar?

Rémi Laganier[1], Fara M Randimby[1], Voahirana Rajaonarivelo[1,2] and Vincent Robert*[1,2]

Address: [1]Unité d'Entomologie Médicale, Groupe de Recherche sur le Paludisme, Institut Pasteur de Madagascar, B.P. 1274, Antananarivo 101, Madagascar and [2]UR Paludisme afro-tropical, Institut de Recherche pour le Développement, B.P. 434, Antananarivo 101, Madagascar

Email: Rémi Laganier - remi.laganier@wanadoo.fr; Fara M Randimby - randimby@yahoo.fr; Voahirana Rajaonarivelo - nirajao@yahoo.fr; Vincent Robert* - robert@pasteur.mg

* Corresponding author

Published: 19 November 2003

Malaria Journal 2003, **2**:42

Received: 19 October 2003
Accepted: 19 November 2003

This article is available from: http://www.malariajournal.com/content/2/1/42

Abstract

Background: One method of collecting mosquitoes is to use human beings as bait. This is called human landing collection and is a reference method for evaluating mosquito density per person. The Mbita trap, described by Mathenge *et al* in the literature, consists of an entry-no return device whereby humans are used as bait but cannot be bitten. We compared the Mbita trap and human landing collection in field conditions to estimate mosquito density and malaria transmission.

Methods: Our study was carried out in the highlands of Madagascar in three traditional villages, for 28 nights distributed over six months, with a final comparison between 448 men-nights for human landing and 84 men-nights for Mbita trap, resulting in 6,881 and 85 collected mosquitoes, respectively.

Results: The number of mosquitoes collected was 15.4 per human-night and 1.0 per trap-night, i.e. an efficiency of 0.066 for Mbita trap vs. human landing. The number of anophelines was 10.30 per human-night and 0.55 per trap-night, i.e. an efficiency of 0.053. This efficiency was 0.10 for indoor *Anopheles funestus*, 0.24 for outdoor *An. funestus*, and 0.03 for *Anopheles arabiensis*. Large and unexplained variations in efficiency were observed between villages and months.

Conclusion: In the highlands of Madagascar with its unique, highly zoophilic malaria vectors, Mbita trap collection was poor and unreliable compared to human landing collections, which remains the reference method for evaluating mosquito density and malaria transmission. This conclusion, however, should not be extrapolated directly to other areas such as tropical Africa, where malaria vectors are consistently endophilic.

Background

Malaria transmission is ordinarily calculated as the product of the density of anopheline vectors per human and the infectivity of this anthropophilic fraction of mosquitoes. Up to now all-night stationary direct bait collections (also called human landing collections) with a human acting both as bait and collector has been the reference method for evaluating mosquito density per human. Most of the time, mosquitoes that land on human skin are collected before they have bitten but, clearly, this method exposes men to mosquito bites. Therefore, alternative methods are needed and there have been many attempts to develop new strategies and traps, with varying degrees of success [1].

Mathenge and collaborators [2] have published a complete description of a new trap design. The 'Mbita trap':

- is baited by one human protected from mosquito bites;

- allows the human to sleep *ad libitum*;

- consists of a modified conical bednet made of white cotton cloth (not netting) that concentrates in its upper part the heat and various odours produced by the human bait; the apex is made of netting and forms a funnel with a small round hole (5 cm in diameter) at its base that permits the entrance of mosquitoes but impedes their escape; a netting panel is fixed halfway up the net to separate the upper mosquito chamber from the lower human chamber;

- is inexpensive to produce, does not require any maintenance, and is simple to use.

Mathenge et al [2] provide evidence of its efficacy in trapping laboratory-reared *Anopheles gambiae* released in a screen-walled greenhouse in the Mbita Point ICIPE field station, near Victoria Lake, Kenya. When compared side-by-side with similar samples of mosquitoes, the Mbita trap caught 43.2 ± 10% of the number caught by human landing collections. Clearly, if such success were verified in the field with wild mosquitoes, this trap would become an attractive alternative for mosquito surveillance.

The aim of this study was to evaluate the success of Mbita traps in sampling mosquitoes in the field conditions of Malagasy highlands with special references to two indicators, the anopheline vector species and the anopheline density per human. In other words, we made a comparison of methods between the Mbita trap and human landing collection.

Methods

Description of the study area

The study was carried out in three traditional villages on the western fringes of the central highlands of Madagascar, Antananarivo province, Tsiroanomandidy prefecture. These villages were:

- Andranonahaotra (ANH), 1,002 inhabitants, 400 zebus, coordinates 19°00'34"S 46°25'21"E, altitude 920 m, Ankadinondry-Sakay commune (Fig 1),

- Soanierana (SOA), 1,274 inhabitants, 160 zebus, 19°08'42"S, 46°25'26"E, 900 m, Mahasolo commune,

- Analamiraga (AMG), 900 inhabitants, 390 zebus, 19°14'35"S, 46°16'22"E, 885 m, Maroharona commune (Fig 2).

These three villages follow a general line NE-SW and are separated by 14 km for ANH-SOA and 17 km for SOA-AMG.

The area has one rainy season from November to April. Mean annual rainfall is 1,600 mm. The mean temperature between December and May is 23,9°C ranging from 21,9°C to 24,9°C. Rice fields generate a number of breeding sites for various mosquitoes including anophelines. Rice production is the main activity of villagers. In this region, most people (>99%) do not use bednets and zebus are kept within the village at night.

In the twentieth century, the central highlands of Madagascar have experienced large malaria outbreaks. A national programme for preventing malaria epidemics, with CAID ("Campagne d'Aspersion Intra-Domiciliaire" of insecticide) performing DDT spraying of house walls at 2 g/m^2. The houses in the study area are normally covered by this treatment, but the last insecticide treatment was carried out pre-1998 in AMG, in 2000 in SOA and 2001 in ANH, i.e. >60, 36 and 24 months respectively before the beginning of this study.

The study protocol was approved by the Ministry of Health of Madagascar.

Human landing collections

Adult male volunteers were placed in a room ordinarily used as a bedroom or out-of-doors in places protected from the rain. According to WHO recommendations [3], mosquitoes were collected with glass tubes closed by cotton plugs as they landed on the exposed lower legs of adult humans (Fig. 3). Malaria prophylaxis was offered. In each village, collections were performed monthly for two consecutive nights from 18.00 to 06.00. Each night, four houses were used and, for each house, two men were sited indoors and two outdoors, working in six-hours shifts. The total number of men per night was 32 divided in two teams of 16.

Mbita trap collections

Mbita traps were provided by Dr. Mathenge at cost price (10 US dollars each). They were used as described [2] and baited with a man resting in bed and in the trap for 12 hours from 18.00 to 06.00 (Fig. 4). In each village, in parallel to human landing collections, three traps were used per night, with one outdoors, and two indoors in separate bedrooms without people other than this under the trap in order to avoid local competition between the trap and other more accessible people for mosquitoes. Bedrooms

Figure 1
View of the village of Andranonahaotra. Habitat and villagers are typical of highlands of Madagascar.

chosen for Mbita trap collections were used one single night each month (i.e. 4 different bedrooms per village and per month). At 06.00, when the human bait left the trap, an experienced technician collected the mosquitoes with an aspirator.

Mosquito procedures and data analysis
Mosquito species were assessed using morphological characteristics. For *An. gambiae s.l.*, a sample of 50 females per village and per month was tested by PCR [4] (this sample was obtained by human landing, pyrethrum spray, and artificial pit shelter collections). As only *Anophelesarabiensis* was observed in a sample of over 1,100, hereafter, any *An. gambiae s.l.* were assigned to *An. arabiensis*. Ovaries of anopheline vectors were examined for parity using the Detinova technique [5]. The origin of the blood meal of anophelines found fed in traps was assessed by ELISA [6]).

The number of mosquitoes caught by each method was recorded. By definition, one human-night referred to the unit of human landing collections i.e. the activity of mosquitoes on one human during the whole night. A trap-night referred similarly to the activity of one trap during the whole night. The efficiency of the Mbita trap (Δ) is defined as the number of mosquitoes collected per trap-night divided by the number of mosquitoes collected per human-night in similar conditions of location (indoors and/or outdoors) and time (nights of observation). A positive correlation was also searched for *An. funestus* samples between Mbita trap and human landing collections using the Pearson's coefficient correlation.

Figure 2
View of the village of Analamiranga. Zebus are kept in the village during the night in these highlands of Madagascar.

The results are from December 2002 to May 2003 in AMG (i.e. 12 nights with 192 men-nights for human landing collections and 36 traps-nights for Mbita trap collections) and December 2002 to March 2003 in SOA and ANH (i.e. 8 nights with 128 men-nights and 24 traps-nights in each of these 2 villages).

Results

The whole data set consists of 6,899 mosquitoes for human landing and 85 for Mbita trap collections. Mosquitoes landing on humans belonged to 26 mosquito species (10 Anophelinae and 16 Culicinae) and those collected with Mbita traps to eight species (three Anophelinae and five Culicinae) (see Additional File 1 for the complete data used to performed this analysis).

Mosquito species with less than five specimens in human landing collections were excluded from the analysis (i.e. a total of 18 mosquitoes with 2 Anophelinae and 16 Culicinae, all human landing, that represented 0,26% of the whole data set) and results presented hereafter concern 6,881 and 85 mosquitoes, respectively, belonging to 17 species (Table 1). The ratio of the total numbers of Anophelinae/Culicinae was 2.02 for the human landing catch and 1.18 with Mbita trap collections ($p = 0.015$ by exact Fisher's test). On average one man-night collected 15.36 (10.27 Anophelinae and 5.09 Culicinae) and one trap-night collected 1.01 mosquitoes (0.55 Anophelinae and 0.46 Culicinae).

Overall, the efficiency of Mbita traps vs. human landing collections (Δ) is 0.066. This Δ is not influenced by the

Figure 3
Indoor landing collection of mosquitoes. The man who acts as baits actively collects mosquitoes that land on his legs.

indoor/outdoor location (Δ = 0.050 indoors and 0.098 outdoors, $p > 0.99$ by exact Fisher's test). For *Anopheles funestus*, Δ is 0.103 indoors and 0.237 outdoors ($p = 0.074$), for *An. arabiensis*, Δ is 0.070 indoors and 0.000 outdoors ($p = 0.28$), whereas for *An. funestus*, variations of Δ were analysed per village and month (original data used to perform this analysis are in Tabl. 2). Δ was 0.036 in AMG, 0.963 in SOA and 1.212 in ANH ($\chi^2 = 165.7$, df = 2, $p < 10^{-4}$) with values that varied inversely with the density of this species in human landing collections. Δ was also highly variable between months and ranged from 0 to 6.8 (maximum in February, outdoors, SOA) without clear tendencies that would provide clues to explain this variation.

Beside this analysis of efficiency, a correlation was searched for *An. funestus* samples between Mbita trap and human landing collections. No statistically-significant positive correlation either for the indoor or the outdoor samples was evidenced (indoor, Pearson's coefficient correlation $r = -0.21$, $n = 14$, $p = 0.47$; outdoor, $r = 0.20$, $n = 14$, $p = 0,50$). Another similar analysis using log-transformed values (+1) did not modify the conclusions.

The two *An. gambiae s.l.* collected in Mbita traps were from indoor trap at AMG on January. Both were nulliparous and unfed and were identified as *An. arabiensis*. Among the 43 *An. funestus* collected in the Mbita trap, one was collected fully fed in an indoor trap and had taken its blood meal from zebu. Eighteen *An. funestus* were examined for ovaries, 12 were parous and six were nulliparous,

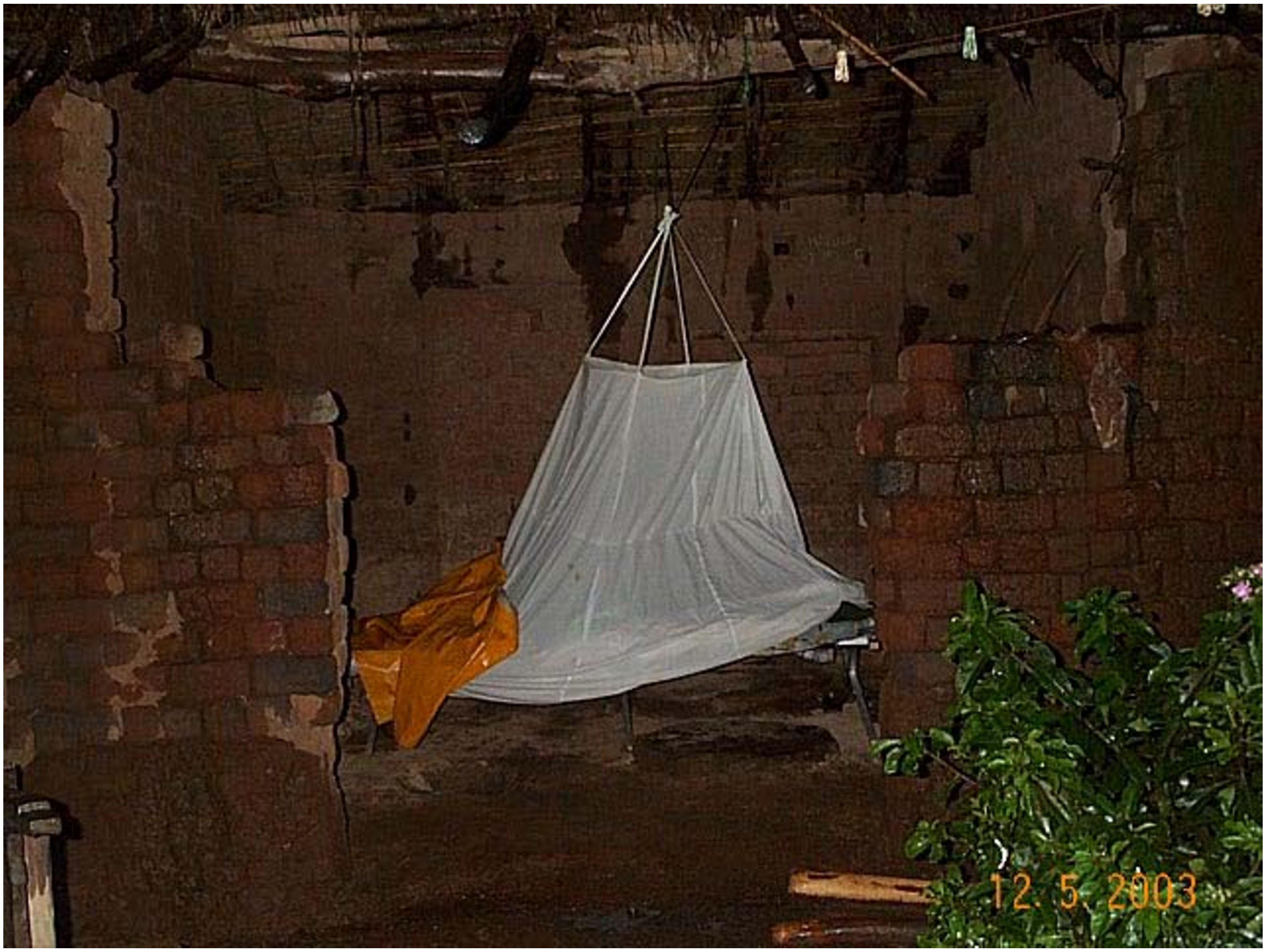

Figure 4
View of an outdoor Mbita trap. The man who acts as bait is sleeping and protected from mosquito bites. By design, mosquitoes enter in the trap via a funnel-shaped entry-no-return port at the bottom of the trap.

i.e. with an excess of nullipars relative to those sampled by human landing collections (85% of parous among 1,512 mosquitoes, collected either indoors or outdoors without difference in the parity rate) (p = 0.04 by exact Fisher's test).

Discussion

The efficiency of the Mbita trap compared to human landing collections is very poor for all species of mosquitoes (with the possible exception of *An. funestus* which will be discussed below). This low efficiency observed in the highlands of Madagascar with wild mosquitoes is in complete contradiction with previous published results [2] obtained in semi-field conditions using laboratory reared *An. gambiae* and in field conditions of rural Kenya [7]. We hypothesise the main reason for these discrepancies resides in the well known zoophilic/exophilic trophic preferences of Malagasy mosquitoes [8]. During the study, the antropophilic rate for *An. arabiensis* was 0.00 for those collected indoors by pyrethrum spray collections (only 12 fed females tested) and 0.016 for those outdoors resting in pit shelters (318 tested, unpublished data). This zoophilic/exophagic behaviour may be antagonist to the entry in the trap that is thought as a positive response to convective heat currents and various odours produced by the human bait in the trap.

The efficiency of the Mbita trap seamed less poor for *An. funestus*. In some cases, a trap efficiency was observed which was higher than that of human landing collections.

Table 1: Number and density of mosquitoes collected indoor and outdoor by human landing collections and Mbita trap collections.

	INDOOR				OUTDOOR				TOTAL			
	Number of mosquitoes		Density of mosquitoes		Number of mosquitoes		Density of mosquitoes		Number of mosquitoes		Density of mosquitoes	
	Man 224 m-n	Mbita 56 m-n	per man 224 m-n	per Mbita 28 m-n	Man 448 m-n	Mbita 84 m-n	per man	per Mbita	Man	Mbita	per man	per Mbita
An. coustani	242	0	1.080	0	907	0	4.049	0	1149	0	2.565	0
An. squamosus	197	0	0.879	0	651	1	2.906	0.036	848	1	1.893	0.012
An. arabiensis	116	2	0.518	0.036	304	0	1.357	0	420	2	0.938	0.024
An. mascarensis	33	0	0.147	0	464	0	2.071	0	497	0	1.109	0
An. funestus	469	12	2.080	0.214	1032	31	4.665	1.107	1501	43	3.350	0.512
An. rufipes	7	0	0.031	0	14	0	0.063	0	21	0	0.047	0
An. maculipalpis	25	0	0.112	0	131	0	0.585	0	156	0	0.348	0
An. pharoensis	3	0	0.013	0.000	7	0	0.031	0	10	0	0.022	0
Cx. univitatus	15	0	0.067	0	67	2	0.299	0.071	82	2	0.183	0.024
Cx. antenatus	244	1	1.089	0.018	1002	9	4.473	0.321	1246	10	2.781	0.119
Cx. quinquefasciatus	125	5	0.558	0.089	191	20	0.853	0.714	316	25	0.705	0.298
Cx. decens	14	0	0.063	0	36	0	0.161	0	50	0	0.112	0
Cx. giganteus	40	0	0.179	0	152	0	0.679	0	192	0	0.429	0
Cx. poicilipes	3	0	0.013	0	28	1	0.125	0.036	31	1	0.069	0.012
Ae. tiptoni	3	0	0.013	0	11	0	0.049	0	14	0	0.031	0
Ae. fowleri	3	0	0.013	0	12	0	0.054	0	15	0	0.033	0
Ma. uniformis	69	0	0.308	0	264	1	1.179	0.036	333	1	0.743	0.012
TOTAL *Anopheles*	1092	14	4.861	0.250	3510	32	15.728	1.143	4602	46	10.272	0.548
TOTAL Culicinae	516	6	2.304	0.107	1763	33	7.871	1.179	2279	39	5.087	0.464
GRAND TOTAL	1608	20	7.165	0.357	5273	65	23.598	2.321	6881	85	15.359	1.012

Man = mosquitoes collected during the night by human landing catches Mbita = mosquitoes collected during the night by Mbita trap m-n = nomber of "men-nights"

Table 2: Monthly variations of density of *An. funestus* per man and per night by human landing collections and Mbita trap collections

		INDOOR				OUTDOOR				TOTAL			
		Number of *An. funestus*		Density of *An. funestus*		Number of *An. funestus*		Density of *An. funestus*		Number of *An. funestus*		Density of *An. funestus*	
Months 16 m-n	Villages 4 m-n	Man 16 m-n	Mbita 2 m-n	per man 32 m-n	per Mbita 6 m-n	Man	Mbita	per man	per Mbita	Man	Mbita	per man	per Mbita
Decembre	AMG	9	0	0.56	0	45	0	2.81	0	54	0	1.69	0.00
	SOA	0	3	0.00	0.75	3	0	0.19	0	3	3	0.09	0.75
	ANH	3	0	0.19	0	11	3	0.69	1.50	14	3	0.44	0.50
January	AMG	50	0	3.13	0	127	0	7.94	0	177	0	5.53	0
	SOA	4	0	0.25	0	18	1	1.13	0.50	22	1	0.69	0.17
	ANH	6	4	0.38	1	13	3	0.81	1.50	19	7	0.59	1.17
February	AMG	19	0	1.19	0	202	0	12.63	0	221	0	6.91	0
	SOA	7	0	0.44	0	14	12	0.88	6.00	21	12	0.66	2.00
	ANH	16	5	1.00	1.25	13	0	0.81	0	29	5	0.91	0.83
March	AMG	63	0	3.94	0	153	1	9.56	0.50	216	1	6.75	0.17
	SOA	23	0	1.44	0	36	3	2.25	1.50	59	3	1.84	0.50
	ANH	1	0	0.06	0	4	0	0.25	0	5	0	0.16	0

Table 2: Monthly variations of density of *An. funestus* per man and per night by human landing collections and Mbita trap collections

April	AMG	239	0	14.94	0	334	8	20.88	4.00	573	8	17.91	1.33
May	AMG	29	0	1.81	0	59	0	3.69	0	88	0	2.75	0
Total of numbers and Mean of densities	AMG	409	0	4.17	0.00	920	9	9.58	0.75	1329	9	6.92	0.25
	SOA	34	3	0.67	0.19	71	16	1.11	2.00	105	19	0.82	0.85
	ANH	26	9	0.41	0.56	41	6	0.64	0.75	67	15	0.52	0.63
Grand Total of Numbers and Mean of Densities	469	12	1.75	0.25	1032	31	3.78	1.17	1501	43	2.76	0.58	

Man = mosquitoes collected during the night by human landing catches Mbita = mosquitoes colleced during the night by Mbita trap

But no correlation was highlighted between Mbita trap and human landing collections.

Unfortunately, there was no explanation for the large variations in trap performance and the unreliability with the reference method constituted by the human landing collection. Why is the efficiency of the trap higher in villages with low density in human landing collections? Why is the efficiency higher outdoors? One fact is that anthropophilic behaviour is not positively linked to this efficiency: the rate for indoor *An. funestus* was 0.33 in AMG, 0.61 in SOA and 0.19 in ANH (unpublished data obtained from about 400 mosquitoes collected by pyrethrum spray collections) i.e. a higher efficiency was observed in the village with a lower anthropophilic rate. The anthropophilic rate for exophilic *An. funestus* was 0.10 in the three villages (from about 200 mosquitoes resting in pit shelters), i.e. the higher efficiency was observed outdoors with the lower anthropophilic rate. These data are in contradiction with the hypothesis on zoophily stated in the previous paragraph. Is there a density dependent factor that acts on the efficiency of Mbita trap, as suggested by observations in the three villages? Does an unbaited Mbita trap would collect mosquitoes? All these questions remain open.

Conclusions

The efficiency of the Mbita trap appears to be poor and/or unreliable compared to classic human landing collections in the highlands of Madagascar. Our findings do not corroborate those obtained in the previous experiments in semi-field conditions in a greenhouse using laboratory-reared *An. gambiae* [2] or in field conditions of rural Kenya [7]. A possible explanation for this discrepancy is the marked zoophilic preferences of Malagasy mosquitoes (including *An. arabiensis* and *An. funestus*) which preclude their entry into a human baited trap. Human landing collections remain the gold standard method for evaluating mosquito density and, thus, malaria transmission in the highlands of Madagascar. However, this conclusion cannot be extrapolated to areas, such as most of tropical Africa, where malaria vectors are consistently endophilic and anthropophilic.

Authors' contributions

RL supervised the study, provided the data sheet, and drafted the manuscript. FR and VoRa participated in the data collection and actively contributed to the interpretation of the findings. ViRo conceived the study, analysed the results, and drafted the manuscript. All authors read and approved the manuscript.

Additional material

Additional File 1

Complete data set of mosquitoes collected by human landing collections and Mbita trap collections. This excel file proposes two sheets. One sheet presents the total number and density of mosquitoes per species and endophilic/exophilic behaviour. The other sheet presents the total number and density of mosquitoes per species and villages.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1475-2875-2-42-S1.xls]

Acknowledgements

We extend our thanks to the medical entomology staff of the Institut Pasteur de Madagascar for their participation in the field trials. We are also grateful to villagers who kindly gave us access to their homes, thereby making this study possible. We would also like to thank Evan Mathenge and Carlo Ayala for providing the Mbita traps and the photos, respectively and acknowledge Bart Knols, Arthur Talman and two anonymous reviewers for improving the manuscript. Funds were obtained from IRD and Institut Pasteur through the ACIP "Population parasitaire des moustiques".

References

1. Service MW: **Mosquito ecology: field sampling methods.** *Barking: Elsevier* 21993.

2. Mathenge EM, Killeen GF, Oulo DO, Irungu LW, Ndegwa PN and Knols BGJ: **Development of an exposure-free bednet trap for sampling Afrotropical malaria vectors.** *Med Vet Entomol* 2002, **16:**67-74.
3. WHO: **Manual on practical entomology in malaria. Part II. Methods and techniques.** *Geneva: World Health Organization* 1975.
4. Scott JA, Brogon WG and Collins FH: **Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction.** *Am J Trop Med Hyg* 1993, **49:**520-529.
5. Detinova TS: **Age grading methods in Diptera of medical importance with special reference to some vectors of malaria.** *Geneva: World Health Organization* 1962. [*Monogr Ser* **47**]
6. Beier MS, Schwartz IK, Beier JC, Perkins PV, Onyango F, Koros SJK, Campbell GH, Andrysiak PM and Brandling-Bennett AD: **Identification of malaria species by ELISA in sporozoite and oocyst infected Anopheles from western Kenya.** *Am J Trop Med Hyg* 1988, **39:**323-327.
7. Mathenge EM, O Omweri GO, Irungu LW, Ndegwa PN, Walczak E, Smith TA, Killen GF and Knols BGJ: **Comparative field evaluation of the Mbita trap, CDC light trap and the human landing catch for sampling of malaria vectors in western Kenya.** *Am J Trop Med Hyg* in press.
8. Chauvet G, Coz J, Gruchet H, Grjebine A and Lumaret R: **Contribution à l'étude biologique des vecteurs du paludisme à Madagascar. Résultats de 5 années d'étude.** *Med Trop* 1964, **24:**27-44.