Relationship to human biting collections and influence of light and bednet in CDC light-trap catches of West African malaria vectors

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Abstract

The efficiency of miniature CDC light-traps in catching West African malaria vectors was evaluated during two rainy seasons in a village near Ouagadougou, Burkina Faso. Traps were employed both indoors and outdoors using human baits protected by an insecticide-free mosquito-net and different sources of light. Indoors, light from incandescent bulbs increased the catch of *Anopheles gambiae* s.l. (mainly *A. arabiensis* Patton and the Mopti chromosomal form of *A. gambiae* s.s. Giles) and *A. funestus* Giles *c.* 2.5 times as compared to traps whose light bulb was removed. Conversely, the difference was not significant when a UV 'Blacklight-blue' fluorescent tube was compared to the incandescent bulb. Protecting the bait with a mosquito-net increased the catch *c.* 3 times for *A. gambiae* s.l. and *c.* 3.5 times for *A. funestus*. A prototype model of double bednet gave intermediate yields. Outdoors, the addition of incandescent bulbs to unlighted traps did not significantly increase the number of vectors caught, but the addition of the mosquito-net to the unprotected human bait did so by *c.* 1.5–4 times. Thus, the CDC light-trap hung close to a human sleeping under a bednet and fitted with an incandescent bulb, was considered the most practical and efficient in terms of numbers of vectors caught, consequently its indoor efficiency was compared to human landing catches on single collectors and estimated to be 1.08 times and density-independent. Outdoor light-trap catches were either not significantly correlated to biting collections (as for *A. gambiae* s.l.), or density-dependent in their efficiency (as for *A. funestus*); thus, they were not considered a reliable means for estimating malaria vector outdoor biting densities in this area. No difference was found in the parous rate of *A. gambiae* s.l. samples obtained with CDC light-traps and human landing collections.

Introduction

Several studies have analysed, with somewhat conflicting results, the usefulness of light-traps in epidemiological studies of mosquito-borne diseases. Some of the variability is no doubt due in part to the differing conditions in which the studies were conducted. There are, however, known sources of experimental variability. For example, the efficiency of light-traps in sampling mosquitoes has been related to light intensity (Barr *et al.*, 1960) and wavelength (Sexton *et al.*, 1986), or the use of light at all (Barr *et al.*, 1960). Similarly,

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olfactory baits such as carbon dioxide (Carestia & Savage, 1967; Carestia & Horner, 1968), or other 'attractants' (Takken & Kline, 1989; Kline *et al.*, 1990), and their interaction with the light (Stryker & Young, 1970), or wavelength (Wilton, 1975b), affect both numbers caught and species composition. Light-trap effectiveness is also affected by trap position (Odetoyinbo, 1969; Wilton & Fay, 1972a), or the protection of a host with a mosquito-net (Garrett-Jones & Magayuka, 1975; Lines *et al.*, 1991), while other variables affect the trap function more directly: vertical or horizontal screens, air flow and direction, trap colour, screen mesh size, etc. (Barr *et al.*, 1963; Wilton & Fay, 1972a).

The early version of the CDC light-trap (hereafter 'light-trap', or 'trap') described by Sudia & Chamberlain (1962) has been progressively modified to fit specific research needs and to improve its efficiency (e.g. the updraft version using UV light to catch *Anopheles albimanus* Wiedemann (Diptera: Culicidae) (Wilton & Fay, 1972a; Wilton, 1975a; Sexton *et al.*, 1986)). Nevertheless, most studies employing light-traps to capture mosquitoes are still made using the basic design (see Service, 1993).

Several authors have regressed mosquito light-trap catches on standard human biting collections, in an attempt to find a functional relationship which may be used to infer biting rates from the number of mosquitoes caught in the traps (Rubio-Palis & Curtis, 1992; Githeko *et al.*, 1994). Lines *et al.* (1991), however, have suggested that, on theoretical grounds, inference based on regression analysis may be misleading, even though light-trap figures, when they correlate significantly with biting catches, may be useful in assessing relative changes in the biting fraction of the mosquito population. In their study, three light-traps caught approximately equal numbers of vectors as two human collectors.

Clearly, it is important to be able to estimate mosquito biting rates in any epidemiological study. The problem is the ethical one of asking catchers to expose themselves to transmission of drug-resistant malaria and other vector-borne diseases. Moreover, human biting catches are difficult to standardize, and too demanding for large-scale sampling. The possibility of making valid biting estimates from light-traps (or any other trapping device) is therefore highly desirable. What is required is that the relationship between traps and biting catches be calibrated locally (Lines *et al.*, 1991), since most of the relevant variables affecting trap catch variability are still poorly understood.

While light-traps have been routinely used in studies of temperate culicine species, relatively little use of them has been made until recently to collect tropical anophelines (for a review, see Service, 1993). Most published work evaluating light-traps to sample afrotropical malaria vectors of the *A. gambiae* Giles (Diptera: Culicidae) complex, has not presented results for each separate sibling species. In view of this, interpretation of results from these studies must be gauged according to the known prevailing species composition of each author's study area. Light-traps have been utilized in rain forest habitats (Carnevale & Le Pont, 1973; Le Goff *et al.*, 1993) where only *A. gambiae* s.s. Giles occurs, in mangrove swamp areas where *A. melas* Theobald prevails (Odetoyinbo, 1969), in Madagascar (Fontenille & Rakotoarivony, 1988) where behaviourally-distinct populations of *A. arabiensis* Patton exist (Ralisoa Randrianasolo & Coluzzi, 1987), and in East African savannas (Chandler *et al.*, 1975; Mbogo *et al.*, 1993) where either *A. gambiae* s.s. (Lines *et al.*, 1991), or *A. arabiensis* (Mukiama & Mwangi, 1990; Githeko *et al.*, 1994) predominate.

West African savanna populations of *A. gambiae* and *A. arabiensis* are peculiar in the extent of their genetic variability (Coluzzi *et al.*, 1979), leading, in *A. gambiae*, to incipient speciation in the 'Mopti', 'Bamako' and 'Savanna' chromosomal forms (Coluzzi *et al.*, 1985). In some cases it has been possible to associate behavioural differences with genotypes (Coluzzi *et al.*, 1977), and chromosomal heterogeneities have been related to vector behaviour in *A. funestus* Giles from Burkina Faso (Boccolini *et al.*, 1994). Although several studies evaluated light-trapping of West African savanna populations of *A. gambiae* s.l., they were carried out either without using bednets to protect the human bait (Coz *et al.*, 1971; Faye *et al.*, 1992), or using the modified Monks Wood model developed by Service (1970). For these reasons, a reappraisal for Western Africa of CDC light-traps used as a malaria vector sampling technique in combination with bednet-protected baits seemed especially important.

Useful results when sampling afrotropical anophelines with light-traps have been obtained by hanging the traps inside dwellings when humans are protected by mosquito-nets (Odetoyinbo, 1969; Lines *et al.*, 1991). Because the range of action of the trap is limited (less than 5 m, Odetoyinbo, 1969), mosquitoes that persistently attempt to penetrate the bednet and explore all their way around it, thus increase the chances of coming close enough to the trap to be caught. If this explanation is correct, one would expect that putting the trap as close to the net as possible would increase the probability of capture even further.

In this work, different combinations of light and bednet models were tested, both indoors and outdoors, in order to assess the relative contribution of the light and the bednet, and to develop a more efficient use of the trap for catching malaria vectors in the prevailing conditions of the Sudan savanna areas of West Africa.

Materials and methods

Study area

Experiments were carried out in Noungou (12°31′N, 1°25′W), a village 35 km northeast of Ouagadougou, Burkina Faso, during the 1992–1993 rainy seasons. The general climate and vegetation of the area, the village and its malaria vectors have been described in detail elsewhere (Costantini *et al.*, 1996). During the 1993 collections, the relative frequency of the members of the *A. gambiae* s.l. (Diptera: Culicidae) complex present in the area was about 70% *A. gambiae* s.s. Giles and 30% *A. arabiensis* Patton, as estimated by indoor landing catches on human bait (Costantini *et al.*, 1996). In Noungou, Mopti represents the prevailing (80–90%) chromosomal form of *A. gambiae* s.s. (Merzagora, 1993).

CDC light-trap collections

Miniature CDC light-traps (Hausherr's Machine Works, Toms River, New Jersey and John W. Hock Company, Gainesville, Florida) operated by disposable or rechargeable batteries, were fitted with 150 mA incandescent bulbs and 0.7 cm mesh grids to exclude larger insects. In 1992, two experimental treatments involved 4 W fluorescent UV 'Blacklight-blue' tubes. Traps were operated either by four

zinc-carbon type D batteries, or by one 6 V lead-acid battery. Disposable batteries were replaced every night before the traps were switched on, while rechargeable batteries were charged daily. No significant difference was found in the trap composition and abundance using these two different power sources (Costantini, 1996).

In all experiments, traps were set up close (less then 30 cm) to one side of a sleeping male local human volunteer protected (according to the treatment) by an insecticide-free mosquito-net, or otherwise. The inlet of the trap was set up at the same height as the man's bed. When the man was sleeping on the floor, the light-trap was hung so that the bottom of the collecting bag almost touched the ground. During all indoor catches no other sleepers were present inside the hut. In 1993, light-trap collections were performed outdoors too. The trap and the net-protected human bait were under a small shelter of *c.* 2 m high wooden poles sustaining a thatched or corrugated metal roof. In this way catches were not interrupted when light rains without strong winds occurred. Sites were chosen from those naturally utilized by local villagers for outdoor activities during the early night.

Traps were switched on at 21:00 h local time, and sleepers were instructed to switch them off at 05:00 h, after having tied the neck of the collecting bag. Data for those traps which had battery and/or light-bulb failures during the course of a night were discarded.

Human biting collections

Collections were made of the mosquitoes landing (whether or not the mosquito was probing or feeding) on the exposed legs of one sitting catcher, by means of an electrical aspirator (based on the design of Coluzzi & Petrarca (1973)) which aspirated mosquitoes directly into a paper cup. A torch was intermittently switched on to assist collection. Hourly batches of mosquitoes were provided with a 5% sugar solution until processed. Although the catchers attempted to collect mosquitoes as they landed, about 9% of the catch were partially fed or fully fed mosquitoes. Bloodmeal analysis showed that some of these mosquitoes had fed on other hosts and some may have fed on another human before landing on the catcher.

In order to keep up the efficiency of the catchers during whole-night sessions, collectors were changed every 2 h. Collections started at 21:00 h and ended at 05:00 h, so that four time-sets were established for each site-night. To avoid any individual bias due to the allocation of collectors, each catcher was assigned to different time sets on successive nights according to independently randomized 4×4 latin squares.

Light and bednet effect indoors

Different combinations of light and mosquito-nets were employed to investigate the influence of the light and the bednet on the trap yield. Light was either provided by incandescent bulbs or UV fluorescent 'Blacklight-blue' tubes, or was eliminated by removing the bulb from the trap. Insecticide-free mosquito-nets were used to protect the bait according to the treatment. Moreover, a special model of double bednet was developed. This consisted of an internal mosquito-net, which fully protected a human bait, enveloped by an external one, with one side raised *c.* 30 cm from the floor, which allowed mosquitoes to enter into a 'chamber' formed by the walls of the internal and external net. In order to have the light-trap surrounded by the bednet walls, the trap was hung inside this 'chamber' close to the external net ceiling; therefore, this was the only treatment where the light-trap was at a height higher than the sleeping human bait.

Overall, eight treatments were established: A = no light + no bednet; B = incandescent bulb + no bednet; C = no light + single bednet; D = incandescent bulb + single bednet; E = no light + double bednet; F = incandescent bulb + double bednet; G = UV fluorescent tube + no bednet; H = UV fluorescent tube + single bednet.

Treatments were allocated inside different huts on different nights according to a 9×9 randomized latin square design. The ninth treatment was an indoor human biting catch. The experiment, performed in the 1992 rainy season, ceased for one month as torrential rains and flooding made the village inaccessible. During this period one of the huts included in the latin square collapsed, so that it was replaced by the one next to it in the same compound.

Samples of live mosquitoes from some of the light-trap treatments were dissected for parity using the method of Detinova (1962). A few specimens collected during occasional outdoor light-trap catches and human biting catches performed from midnight to 05:00 h in a nearby village were also dissected for parity.

Light and bednet effect outdoors

Three of the above treatments, namely, incandescent bulb + no bednet, no light + single bednet, and incandescent bulb + single bednet (i.e. B, C, and D, respectively), were employed for a series of outdoor collections during the 1993 rainy season. Each treatment was assigned to a given compound on a given night according to a series of replicated 4×4 latin square designs. The fourth treatment was an outdoor human biting catch. One of the sites was of a more 'closed' nature: the shelter was surrounded by loosely interwoven thatched walls, leaving one side open. Because of the general 'openness' of the walls and the exclusive use of the site in outdoor activities, this was initially regarded as an outdoor position, but it became apparent that results from this shelter were heterogeneous as compared to the other sites, so its data have been excluded from the analysis.

A series of indoor collections following the same experimental protocol and three light-trap catch treatments as above, were interspersed in a 2 : 2 regular succession with the outdoor light-trap catch protocol. The indoor protocol was not fully completed, but data were analysed where appropriate.

Statistical analysis

A Generalized Linear Modelling package using the maximum likelihood method, GLIM® (Payne, 1987), was employed. This software allows the user to specify the function linking the experimental data with a linear predictor, and the error distribution which best fit the structure of the data. For details about generalized linear modelling in GLIM, see Crawley (1993). Means were calculated as the antilogarithm of mean $\log(x+1)$-transformed counts (i.e. Williams' means). The probability level of non-orthogonal contrasts in ANOVA was adjusted by comparing the *P*-value with an experimentwise error rate calculated by the

Dunn-Sidák method (Sokal & Rohlf, 1981). Correlation analysis was carried out according to the guidelines given in Sokal & Rohlf (1981).

Results

Light and bednet effect indoors

The mean number of mosquitoes trapped with each combination of light and bednet is shown in table 1. The UV 'Blacklight-blue' fluorescent tube caught the most *A. gambiae* s.l., while the incandescent bulb caught the most *A. funestus*. Traps without light caught the least mosquitoes for both species. The single bednet increased the catch about three times in both species, as compared to the unprotected bait. The double bednet caught intermediate numbers.

The analysis of deviance (ANODEV) was carried out on log(x+1)-transformed counts, by assuming normal errors (table 2). Two degrees of freedom were removed from the error term because missing data for one hut night were estimated from the formula appropriate for latin square designs given in Lison (1961).

Table 2 shows that, among the 3- and 2-way interaction terms, only SPECIES × DAY was statistically significant, as expected from the different population dynamics of *A. gambiae* s.l. and *A. funestus*. The lack of other significant interaction terms indicates that both species responded in a similar way to the experimental treatments (i.e. SPECIES × LIGHT, and SPECIES × BEDNET), and that the effect of the light and the bednet was additive in these experimental conditions (i.e. LIGHT × BEDNET).

Main effects (i.e. DAY, HUT, SPECIES, LIGHT, and BEDNET), on the other hand, were all statistically significant (table 2). Temporal and spatial heterogeneities in vector densities were reflected in significant DAY and HUT factors, respectively. Higher mean numbers of *A. gambiae* s.l. caught were reflected in the statistical significance of the SPECIES factor. After having verified that treatments for both factors LIGHT and BEDNET were significantly different, each factor variance was partitioned to assess differences between treatment levels by *a priori* orthogonal contrasts. The addition of light increased the catch (factor LIGHT, contrast *a*), but no difference could be demonstrated between incandescent and UV 'Blacklight-blue' bulbs (factor LIGHT, contrast *b*). The addition of both bednet models increased the catch (factor BEDNET, contrast *a*), but the double bednet was less efficient than the single bednet (factor BEDNET, contrast *b*).

Table 1. Williams' means and 95% confidence limits for different light and bednet treatments used in CDC light-trap indoor collections carried out during August–October 1992 near Ouagadougou, Burkina Faso.

Treatment	Species			
	A. gambiae s.l.		*A. funestus*	
	Mean	95% C.L.	Mean	95% C.L.
LIGHT				
absent	9.1	4.8–16.5	1.7	0.7–3.2
incandescent	24.6	10.7–54.8	3.7	1.7–7.1
UV Blacklight blue	27.1	10.1–70.0	2.3	1.1–4.4
BEDNET				
absent	11.2	5.0–23.8	1.3	0.6–2.3
double	13.0	4.0–38.0	2.6	1.0–5.5
single	32.5	17.7–58.8	4.3	2.1–8.0

Table 2. Analysis of deviance for the effects of light and bednet on the mean number of *Anopheles gambiae* s.l. and *Anopheles funestus* collected in indoor CDC light-trap catches.

Source	Deviance	d.f.	F	*P*
DAY	26.98	8	3.10	0.004***
HUT	18.08	8	2.08	0.05 *
SPECIES	99.06	1	91.08	<0.001 ***
LIGHT	16.05	2	7.38	0.001 ***
(a) absent vs. present	15.55	1	14.30	<0.001 ***
(b) incandescent vs. UV	0.55	1	0.50	0.48 ns
BEDNET	23.39	2	10.75	<0.001 ***
(a) absent vs. present	17.22	1	15.83	<0.001 ***
(b) single vs. double	6.07	1	5.58	0.02 *
SPECIES × DAY	124.30	8	14.29	<0.001 ***
SPECIES × HUT	3.18	8	0.37	0.94 ns
SPECIES × LIGHT	2.78	2	1.28	0.28 ns
SPECIES × BEDNET	0.45	2	0.21	0.81 ns
LIGHT × BEDNET	0.89	3	0.27	0.85 ns
SPECIES × LIGHT × BEDNET	1.87	3	0.57	0.63 ns
Error	102.24	94		

*** P<0.001; ** P<0.01; * P<0.05; ns = not significant.

Light and bednet effect outdoors

Table 3 shows the mean of the treatment combinations tested during the 1993 outdoor collections. Catch variability was high, especially for the incandescent bulb + single bednet combination (i.e. treatment D), both in *A. gambiae* s.l. and *A. funestus*. A model using untransformed counts, Poisson errors, and a logarithmic link gave a satisfactory fit to the data, and hypothesis testing was carried out accordingly (Crawley, 1993).

Differences between treatments were statistically significant (table 4). Non-orthogonal, *a priori* contrasts for factor TREATMENT in table 4 showed a significant increase in the number of mosquitoes caught when a bednet was employed (i.e. B vs. D in table 3, contrast *b* in table 4), but not when the light was added (i.e. C vs. D in table 3, contrast *a* in table 4). As both *A. gambiae* s.l. and *A. funestus* responded in the same

Table 3. Williams' means and 95% confidence limits for different light and bednet treatments used in CDC light-trap outdoor collections carried out during September–October 1993 near Ouagadougou, Burkina Faso. Treatment codes as reported in text (p. 505) are given in parentheses.

Treatment	Species			
	A. gambiae s.l.		*A. funestus*	
	Mean	95% C.L.	Mean	95% C.L.
Incandescent bulb + no bednet (B)	1.9	0.7–4.0	0.2	0.0–0.7
No light + single bednet (C)	3.5	1.4–7.4	0.5	0.1–0.9
Incandescent bulb + single bednet (D)	2.4	0.1–9.2	0.9	0.2–2.1

way to the experimental treatments (i.e. the SPECIES × TREATMENT interaction in table 4 is not statistically significant), this result emphasizes the substantial role of the bednet as compared to the light in determining the light-trap yield, casting doubts on the usefulness of the incandescent bulb in outdoor sampling of both malaria vectors. As for the 1992 indoor collections, different specific population dynamics, absolute numbers caught, and spatial and temporal heterogeneities were reflected in the statistical significance of the relevant factors (i.e. SPECIES × DAY, SPECIES, HUT, and DAY, respectively).

Light-trap efficiency

Data for the 1992/93 indoor and outdoor collections of the incandescent bulb + single bednet treatment were analysed to estimate the proportion of mosquitoes caught by light-traps as compared to a human biting catch following similar procedures as in Lines *et al.* (1991). The aims were: (i) to establish whether the two sampling methods were correlated; (ii) to verify that efficiency was not density-dependent; and (iii) to test for differences between years, species and indoor/outdoor collections.

First, Pearson correlation coefficients for the relationship between log(x+1)-transformed light-trap catches and human biting collections were calculated for each combination of species/sites (table 5 and fig. 1). *Anopheles gambiae* s.l. outdoor catches was the only case in which the correlation was not significantly different from zero. Then, two new variates, i.e. the log[(light-trap catch+1)/(human biting catch+1)] ratio and the Williams' mean of light-trap and human biting catches, were submitted to correlation analysis as above (table 5 and fig. 2). No correlations were statistically significant except for *A. funestus* outdoor collections.

In view of the above results and the small sample size for outdoor catches, the subsequent analysis was limited to the indoor data. ANODEV in GLIM did not detect significant differences in the log-ratio either between years ($F_{1,35}$ = 0.13; P>0.72) or among species ($F_{1,35}$ = 2.30; P>0.13). The mean log-ratio was 0.078 (s.e. 0.179), whose antilog, 1.08 (0.76–1.53; 95% C.L.), is remarkably similar to that found by Lines *et al.* (1991) (fig. 2). As for that study, this result means that, on average, the catch from one indoor light-trap was 1.08 times that from a human landing collection (see also fig. 1). It is to be stressed, however, that in this study the relationship has been established between one light-trap and one human collector, whereas the ratio in the study of Lines *et al.* (1991) derived from the total catch from three light traps vs. that on two human collectors. Thus, our light-trapping was relatively more efficient than that of Lines *et al.* (1991).

Table 4. Analysis of deviance for the effects of light and bednet on the mean number of *Anopheles gambiae* s.l. and *Anopheles funestus* collected in outdoor CDC light-trap catches. In this case (Poisson model), the deviance approximates a chi-square distribution for the corresponding number of degrees of freedom, and therefore it is compared to chi-square values for hypothesis testing.

Source	Deviance	d.f.	*P*
DAY	165.30	11	<0.001 ***
HUT	16.91	2	<0.001 ***
SPECIES	113.70	1	<0.001 ***
TREATMENT	23.51	2	<0.001 ***
(a) light: absent vs. present	1.51	1	0.219 ns
(b) bednet: absent vs. present	12.21	1	<0.001 ***
SPECIES × DAY	54.17	11	<0.001 ***
SPECIES × HUT	4.62	2	0.099 ns
SPECIES × TREATMENT	1.94	2	0.379 ns
Error	25.08	22	

Probability values are approximate and coded as in table 2.

Table 5. Pearson correlation coefficients for the relationship between CDC light-trap catches (LTC) and human biting collections (HBC), and between the log-ratio (LTC+1)/(HBC+1) and the Williams' mean of light-trap catches and human biting catches (see text for further details; cf. also figs 1 and 2).

Correlation	Species	
	A. gambiae s.l.	*A. funestus*
LTC vs. HBC		
Indoors	0.62 **	0.65 **
Outdoors	0.32 ns	0.82 **
Log-Ratio vs. Williams' Mean		
Indoors	0.18 ns	0.16 ns
Outdoors	0.26 ns	−0.77 *

Significance levels as for table 2.

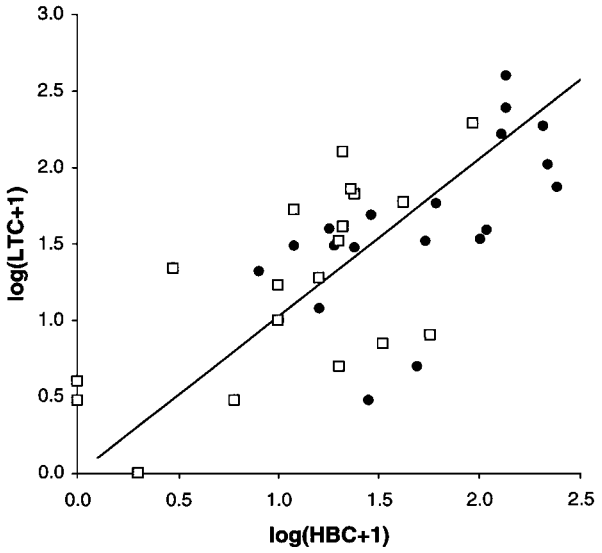

Fig. 1. Scatter distribution for the relationship between indoor CDC light-trap catches (LTC) and human biting collections (HBC) of *Anopheles gambiae* s.l. (closed circles) and *Anopheles funestus* (open squares). The Pearson correlation coefficient for the pooled data was r = 0.69 (P<0.0001). The principal (or major) axis regression line (Sokal & Rohlf, 1981) is shown. The slope (1.030) and the intercept (−0.008) of the major axis are close to, respectively, one and zero, confirming the result from the log-ratio analysis (cf. fig. 2) by which one light-trap caught on average the same number of malaria vectors as one human collector independently of the mean density (see text for further details).

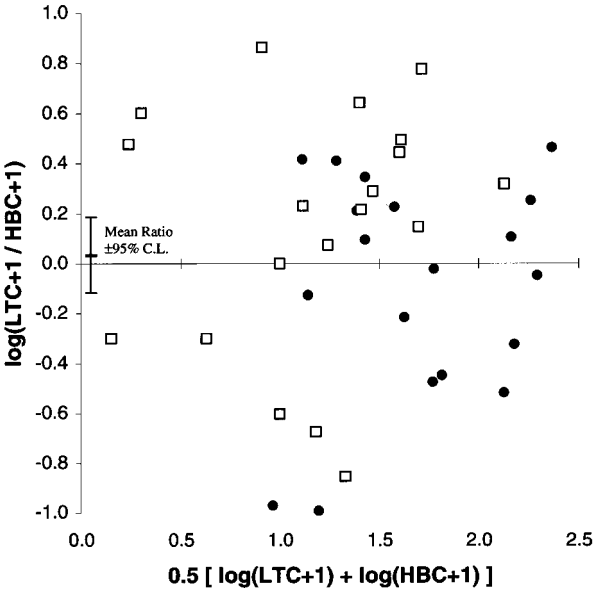

Fig. 2. The same data points as in fig. 1 plotted as the log-ratio between light-trap (LTC) and human biting catches (HBC) on the ordinate, and the Williams' mean (on a logarithmic scale) of light-trap and human biting catches on the abscissa; the former is an estimate of the light-trap efficiency on the log scale, whereas the latter gives an estimate of mean vector abundance. The Pearson correlation coefficient was, in this case, not significantly different from zero (r = 0.03, P>0.87).

Both indoor and outdoor catches were carried out during nights spanning different lunar phases. No effect of the phase of the moon on the light-trap efficiency was evident for indoor collections, whereas there was some indication that *A. gambiae* s.l. outdoor light-trap efficiencies were lower at full moon and, conversely, higher at new moon. A more extensive data set confirming these results will be presented in a companion paper.

Parity

Too few specimens of *A. funestus* were dissected for parity, so the analysis is restricted to *A. gambiae* s.l. Because of the limited number of live specimens available for dissection in the traps on each sampling date, data were pooled for different light-trap treatments, as no differences could be detected among the parous rates of samples collected with different light sources (G = 0.04; P>0.97; table 6). For the same reason it was not possible to compare different bednet treatments.

During the experiment, parous rates ranged widely between sampling occasions (19–68% when n>50 in the human biting samples; 34–48% when n>30 in the light-trap samples). Allowing for this temporal variability in the analysis (i.e. keeping dates as a stratifying factor), it was not possible to demonstrate significant differences between the two sampling methods (table 6). The factor DATE in table 6 contains one degree of freedom more than expected from the latin square design, due to the addition of specimens collected a night before the start of the protocol. The number of outdoor samples dissected was limited, but, again, pooled parous rates were not different (49 / 76 = 64% in light-trap catches vs. 89 / 138 = 64% in human biting catches).

Table 6. Analysis of deviance for differences in parous rate estimates of *Anopheles gambiae* s.l. obtained with different CDC light-trap (LTC) treatments and contemporary human biting catches (HBC) carried out indoors.

Source	% Parous rate (95% C.L.)	n	d.f.	χ^2	P
DATE			9	50.34	<0.0001
SAMPLING METHOD			1	1.70	ns
HBC	46.6 (40–53)	324			
LTC	45.6 (38–53)	270			
no light	45.6 (32–59)	90			
incandescent bulb	45.1 (35–56)	133			
UV Blacklight-blue	46.8 (27–67)	47			

n = number of dissected females; ns = not significant.

Discussion

Both light and human-baited bednets have been shown to increase the catch of afrotropical malaria vectors in CDC light-traps (Carnevale & Le Pont, 1973; Garrett-Jones & Magayuka, 1975). The present results extend these observations to the genetically-distinct West African populations of *A. gambiae* s.l. and *A. funestus*, and show that their relative contribution to the probability of these vectors being captured is similar (about 2.5- and 3-fold increase for the light and net, respectively) and additive.

It seems likely that the light exerts its action at only very close range (i.e. much less than 5 m as estimated by Odetoyinbo, 1969), once these malaria vectors are brought close to it by their olfactory responses to the bait. In fact, although mosquitoes were unlikely to be attracted into huts by the CDC light, the trap caught a very similar proportion of those entering when operated with its light on (treatment B) as it did with its light off but with a bait under a bednet (treatment C) – a similarity that was even reversed outdoors (cf. treatments B vs. C in table 3). Considering (i) that the white light threshold for vision in *A. gambiae* is less than 10^{-5} W m^{-2} (Gibson, 1995), i.e. they could certainly see the CDC light as soon as they entered inside the huts present in this area of West Africa; (ii) the proximity of the light to the bait (less than half a metre); and (iii) the importance of the bait for attracting mosquitoes; these results suggest that *A. gambiae* s.l. and *A. funestus* were primarily responding to the host cues, and therefore the bednet was more important for increasing the catch than the light, perhaps because the net arrested the mosquitoes in the vicinity of the trap as they explored round it to 'get at' the bait. If both species were attracted and diverted to the light-trap once indoors, one would expect a great difference in the catch with and without the light on, independent of the presence of the bednet. This effect would be even more obvious outdoors, due to the recruitment effect of the light in addition to the host cues. The fact that this was not the case, implies a very high intensity threshold for the response of *A. gambiae* s.l. and *A. funestus* to light in the presence of host stimuli.

Both *Aedes aegypti* (Linnaeus) and *Anopheles stephensi* Liston (Diptera: Culicidae) have been shown to respond

strongly to near-UV light (323–365 nm) by means of, respectively, electroretinograms (Muir *et al.*, 1992), and a behavioural assay (Wilton & Fay, 1972b). 'Blacklight-blue' tubes emit radiation in a range between 300 and 400 nm, with a peak in the region of 360 nm (Wilton & Fay, 1972b). The employment of UV light, however, did not sufficiently increase the number of females caught (in the conditions tested) to justify its (more inconvenient) use. The addition of a bait (in the form of dry ice) did not increase the efficiency of an experimental updraft UV light-trap as dramatically as for standard CDC light-traps (Wilton, 1975b). However, the enhancing effect of UV light in trap catches of some anophelines has been demonstrated using modified versions of the miniature CDC model (Service, 1970; Wilton, 1975a; Sexton *et al.*, 1986). Therefore, the possibility that CDC light-traps fitted with UV tubes may still be useful where suitable baits are not available or when sampling of afrotropical malaria vectors has to be carried out outdoors, still need to be evaluated.

It is difficult from field studies to assess which aspect of the light mosquitoes were responding to more strongly. UV 'Blacklight-blue' tubes emit some visible light, and the physiologically-functional light intensities were not necessarily comparable. Indeed, this study was primarily devised to give an empirical answer to what is the best tool to sample West African malaria vectors.

Accordingly, the double bednet was devised with the idea that restraining vectors attempting to penetrate the mosquito-net would increase the chances of catching them with a light-trap. The reasons why this design worked less efficiently than the single bednet are unclear but probably manifold. The external net obviously constituted an obstacle to the free access of mosquitoes into the 'chamber' and to the light-trap, and the trap itself was higher than the level at which the human bait slept.

Therefore, from these results, we infer that a human bait protected by a single bednet close to a miniature CDC light-trap fitted with an incandescent bulb give the best combination in terms of malaria vector yields for sampling purposes. The following discussion, therefore, refers to this particular application.

As for the studies of Lines *et al.* (1991) and Faye *et al.* (1992), the *A. gambiae* s.l. parous rate of indoor light-trap samples was not biased as compared to that estimated from human biting catches. This is important from an epidemiological point of view, as light-traps could be used to estimate parous rates without resorting to catches off human baits. Githeko *et al.* (1994), however, found lower parity in light-trap samples. This discrepancy may be explained by differences in the genetic composition of the two vector populations with respect to the members of the *A. gambiae* complex: *A. gambiae* s.s. chromosomal form Mopti was likely to be the most abundant during our 1992 collections, while *A. arabiensis* was reported as the only species of the complex present in one of the Kenyan villages of Githeko *et al.* (1994) study area.

In outdoor collections, the correlation with biting catches was not significant for *A. gambiae* s.l., and trap efficiency was density-dependent in the case of *A. funestus*. The use of CDC light-traps in estimating outdoor biting rates of malaria vectors is, therefore, not warranted on the basis of this, admittedly limited, data set. Conversely, indoor catches with light-traps caught, on average, about the same number of vectors as one human collector working indoors. As found in other similar studies, however, there was large variability in the relative numbers caught by either method.

It is difficult to compare results from different sites because the operational conditions in which traps and biting catches are employed vary widely. Thus, for entomological sampling techniques to be useful in comparative epidemiological work, standardized modes of operation should be used. Environmental variability, however, may still confound interpretation of results. Our light-trap efficiency estimates for *A. gambiae* s.l. were similar to those of Lines *et al.* (1991), Githeko *et al.* (1994) and Davis *et al.* (1995). This similarity, however, might be partially spurious. Our efficiencies were estimated with respect to one human collector, while two catchers working in the same hut were employed by Lines *et al.* (1991) and Davis *et al.* (1995). If the number of mosquitoes entering a hut and attempting to bite is not twice as much when using two collectors instead of one, the mean number of bites per man would be lower, leading therefore to higher efficiency estimates. If this is the case, moving the trap closer to the bait might have been responsible for the higher trap efficiency of this study.

Such variability reiterates the need for local calibration of light-trap efficiency estimates, or, better still, for deeper understanding of vector behaviour and its heterogeneities in response to light and other environmental variables relevant to the sampling process.

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