

ODOR-MEDIATED HOST PREFERENCES OF WEST AFRICAN MOSQUITOES, WITH PARTICULAR REFERENCE TO MALARIA VECTORS

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Abstract. The role of odors in mosquito host preferences was studied in a village near Ouagadougou, Burkina Faso. Two odor-baited entry-traps were put beside one another and a choice of host odor-laden air was blown out of them. Odors of a human and a calf (of similar mass) were drawn from two tents in which each was separately concealed. Allowances were made for trap position, differences in human-subject attractiveness, CO₂ levels, and trap contamination with alternative host odors. Choices for the human-baited trap greater than the 0.5 random expectation were made by *Anopheles gambiae* s.l. (0.96) and *An. pharoensis* (0.68). The choices for the human-baited trap of *Culex antennatus* were significantly lower than 0.5 (0.25), whereas for the *Cx. decens* species group (0.56), the difference was not significant. Interpretation of the latter result was complicated by the significant effect of CO₂ levels on the index. Species caught in low numbers but whose trap distribution showed a bias towards the human-baited trap were *An. funestus* (total numbers in the human-baited trap to the calf-baited trap = 9:0), *Mansonia africana* (17:1), *Aedes dalzieli* (22:4), and *Ae. hirsutus* (13:1); species showing bias towards the calf-baited trap were *An. rufipes* (0:11), *Cx. duttoni* (0:17), and *Cx. nebulosus* (2:35). *Mansonia uniformis* was the only species distributed randomly between the two traps. Molecular identification of the *An. gambiae* s.l. samples revealed a marked difference in trap distribution: for the human-baited trap the ratio was 52% *An. arabiensis* to 48% *An. gambiae* s.s.; for the calf-baited trap, it was 92% *An. arabiensis* to 8% *An. gambiae* s.s.

One of the factors determining the role of a mosquito species as a vector of human disease is its degree of contact with the human host,¹ a factor whose epidemiologic importance is indicated by it being a squared term in the formulation of vectorial capacity.² The feeding pattern of field populations of mosquitoes is broadly termed host selection.¹ This depends on the genetically determined host-specific tendencies of the vector (i.e., its host preferences), on the ecology and behavior of both the host and the vector, especially with regard to concurrence in space and time, and on the effects of chance and the environment.

Mosquito host preference has traditionally been assessed by determining the blood meal origin of freshly fed specimens. This has led to the estimation of the degree of anthropophily via the human blood index (HBI), i.e., the proportion positive for humans in a sample of blood meals.³ The limits of this approach lie in the difficulty of obtaining a sample representative of the population as a whole.^{3,4} Moreover, it does not measure the inherent host preference of a mosquito, but rather the final outcome of all the above-mentioned factors.

Landing (biting) catches complement our knowledge on mosquito/host contact in the field. Partially open mosquito nets or other animal-baited devices are used to obtain the same measure. However, to provide unambiguous indications about the hierarchy of acceptability of different hosts for a given mosquito species, these measurements need to be carried out simultaneously with different pairs of alternative hosts, since some species may accept a wide range of hosts when these are presented separately, but show distinct preferences when hosts are close together.⁵

Difficulties in the interpretation of results may also arise when the sampling technique influences the behavior of the approaching insect. A classic case is known in tsetse flies, where hand net catches on oxen were used to monitor tsetse

populations until it was discovered that human collectors not only repelled tsetse flies but did so especially for female flies.⁶ Conversely, mosquito nets may attract some mosquito females in different gonotrophic conditions independently of the type of host (if any) present inside.^{7,8} The numbers of mosquitoes contacting hosts in such trials are also influenced by the size of the host,⁹ its position in relation to vegetation and breeding sites,^{9,10} its defensive movements,^{11,12} and the visual cues it provides.^{13,14} However, the development of odor-baited entry traps (OBETs) for mosquitoes¹⁵ can partly reduce some of these standardization difficulties, just as they have done for tsetse flies.

Species in the *Anopheles gambiae* Giles complex, which includes the most efficient malaria vectors in the world, show a wide spectrum of bloodfeeding patterns based on HBI analysis.¹⁶ *Anopheles quadriannulatus* (Theobald) is almost entirely zoophilic, while *An. gambiae* s.s. Giles is extremely anthropophilic throughout its distribution. The salt water species *An. melas* Theobald and *An. merus* Donitz show intermediate degrees of anthropophily, but are generally regarded as mainly zoophilic. *Anopheles arabiensis* Patton is strongly anthropophilic in many parts of its distribution, but its feeding behavior depends partly on host availability; its indoor HBI is generally high, but where cattle are abundant, it may be much lower.^{17,18} Furthermore, a general decrease in *An. arabiensis* anthropophily seems to occur from the west to east across Africa, culminating in Madagascar, where entirely zoophilic populations of *An. arabiensis* occur.^{19,20}

In the only experimental work on the host preferences of members of the *An. gambiae* complex, Gillies showed that *An. merus* consistently fed more than *An. gambiae* s.s. on a calf instead of a human when both hosts were in the same screened room and mosquitoes were released into it,²¹ and

that it was possible to shift the preference of an *An. gambiae* s.s. strain towards zoophily within six generations.²²

Little is known about the role of host odors in the determination of host preferences. Gillies⁵ remarked that mosquito species having restricted feeding habits are likely to respond at a distance more strongly to host-specific odors, rather than to common ones, such as CO₂. In a previous OBET choice-test experiment, comparing the response to whole human odor (including CO₂) and pure CO₂ (at the same concentration), Costantini and others²³ found that *An. gambiae* s.s. showed a stronger preference for whole human odor than did *An. arabiensis*, and that less anthropophilic and more generalist species were distributed at random, in agreement with Gillies' hypothesis. In the present paper, the same approach has been used to investigate for the role of whole host odors in the determination of mosquito preferences for a calf and a human.

MATERIALS AND METHODS

Study area. The experiments were conducted in the village of Nougou, 12°N, 1°30'W, in the Sudanese savanna belt 30 km northeast of Ouagadougou, Burkina Faso, during the 1994 rainy season. This village covers approximately 4 km², is composed of approximately 300 huts occupied by approximately 500 Mossi people, and is situated on the edge of a large permanent lake. Mean annual rainfall here is approximately 800 mm (with 20% variability between years) falling mainly in June–September, when the temperature ranges from 22°C to 32°C (mean = 26–27°C), with a mean relative humidity (RH) of 60–75%. The rest of the year is typically dry (< 20% of annual rainfall; mean RH = 20–60%), with mean temperatures reaching 32°C (data from Ouagadougou Airport provided by the Agence pour la Securite de la Navigation Aerienne [ASECNA], Burkina Faso). During the course of the experiment in Nougou, median temperatures at night ranged from 23°C to 29°C and the RH ranged from 72% to 100%. Winds blew from 50–249° (northeast–southwest) with a median speed of 0.08–0.79 m/sec. These variables were measured in loco by means of electronic probes and an ultrasonic anemometer linked to a data logger.

Millet and sorghum are the main crops, cultivated around and within the village, close to the family compounds. Domestic animals are mainly goats, sheep, donkeys, and poultry, with very few cattle and no pigs. Other, occasional, domestic animals include dogs and guinea fowl. Except for cattle and donkeys, animals are kept inside the compounds by night, usually in semiclosed shelters. The local small cattle herd is usually kept just outside the village, near a grazing area. Given the paucity of wildlife in the region, only birds and small mammals provide alternative warm-blooded hosts for mosquitoes to feed on.

Mosquito bionomics are well known in this area.²⁴ At Nougou in the wet season, the main malaria vectors are *An. gambiae* s.s. and *An. arabiensis*. Mopti karyotypes²⁵ represent the prevailing chromosomal form of *An. gambiae* s.s. (Merzagora L, Ph.D Thesis, University of Rome, Rome, Italy). Despite the high degree of chromosomal inversion polymorphism of *An. arabiensis*, no chromosomal forms are

known for this species in the area.²⁶ *Anopheles funestus* Giles densities increase to a peak after the end of the wet season.²⁴

Choice test experiment. The experimental arrangement was similar to the one used by Costantini and others.²³ Two OBETs, designed to catch host-seeking mosquitoes responding mainly to odor cues,¹⁵ were put beside one another inside the village on a clearing of a path between occupied compounds approximately 20 m from the nearest one and approximately 200 m from a cattle herd. Surrounding vegetation was less than 0.5 m high for approximately 30 m downwind and crosswind. Traps were not upwind or downwind of a single or major breeding site. The OBETs are similar to lobster-pot entry traps, and baited with test host odors drawn from a reservoir (a bait in a tent) to the trap by a fan. They were set on two approximately 1.5-m high wooden tables with their entrances approximately 40 cm apart facing downwind. Air coming from two tents standing approximately 7 m upwind of the traps was drawn into and blown out of the OBETs by fans via aluminium air ducts. The speed of the air stream released from the opening of the OBET was approximately 50 cm/sec, as measured at the trap entrance with a hot-wire anemometer.

A human and a calf of similar mass, each concealed in one of the tents, were used as odor baits. Two local human males (20–21 years old, 70–80 kg) volunteered to be the human odor baits; they alternated randomly on different nights to minimize differences in attractiveness effects. The calf, a zebu Egybi breed, was tethered inside a small fence and covered with the polyethylene tent. Although it would have been preferable to test several hosts as in the case of the human hosts, no other calves of suitable age and mass were available at the time in the local herd.

The concentration of CO₂ emitted by the odor baits varied with person, bait, and time, although it was relatively stable on any given night. A previous study has shown that the concentration of CO₂ released from the OBETs affects the size of the catch.²³ Therefore, to test whether the host choice of mosquitoes is affected in part by the level of CO₂ released from the traps in the presence of other host odors, the following protocol was used: each night the CO₂ concentration released by each baited OBET was measured, and, if there was a difference, on approximately half of the nights (chosen randomly) the gradient was allowed to remain while on the other nights the difference was eliminated by increasing the CO₂ concentration of the human-baited trap to the level of the calf-baited trap with CO₂ from a pressurized cylinder. The cylinder was hidden to remove any visual stimuli. A photograph depicting the experimental layout can be found in the book by Bock and Cardew,²⁷ page 314: the CO₂ cylinder is shown exposed for reference.

The CO₂ flow was controlled with pressure regulators, measured with CO₂ rotameters, and adjusted with needle valves. The CO₂ concentrations were monitored with a portable infrared gas analyzer (range of measurable concentrations = 0–2%, resolution = ± 0.01%, ADC2000; Analytical Development Co., Hoddesdon, United Kingdom). Concentrations ranged from 0.08% to 0.12% for the two human odor baits, from 0.10% to 0.15% for the calf, or from 0.09% to 0.19% when the CO₂ output was standardized. On two nights the human-baited trap released a higher CO₂ concentration than the calf-baited one.

Traps were used consistently with the same odor bait to avoid contamination, but they exchanged sites (left-right) in a random sequence on different nights. Experiments began at 9:00 PM and ended at 5:00 AM local time, when the traps were closed and all mosquitoes were collected.

Due to day-to-day variability in the availability of odor baits, zero catches for some mosquito species, and day-to-day fluctuations in CO₂ concentration, the experimental protocol was not fully balanced. The total number of replicates was 22. On two nights, towards the end of the experiment, the calf was not available, and was replaced by four goats. The CO₂ concentration was not standardized in this case, but the CO₂ difference between the two OBETs was in the opposite direction each night (± 0.02 – 0.03%). Data for these nights have been excluded from the main analysis but results are given separately.

Processing of mosquito specimens. Mosquitoes were identified using the key of Gillies and Coetzee¹⁷ for anophelines and the key of Edwards²⁸ for culicines. Among the *An. gambiae* complex, representative subsamples were tested for species identification by the polymerase chain reaction molecular technique of Scott and others.²⁹

Generally, the mosquito species were identified morphologically and classified according to their abdominal condition into three categories (unfed, partially fed/fully fed, and gravid). Culicines other than *Mansonia* were stored in test tubes containing silica gel for later identification.

Since it is difficult to distinguish females of the *Culex* (*Culex*) *decens* species group, these were pooled in the analysis. No males were available to establish the relative proportion of the three species possibly occurring in the area: *Cx. (Culex) decens* Theobald, *Cx. (Culex) invidiosus* Theobald, and *Cx. (Culex) telesilla* De Meillon and Lavoipierre.^{24, 30}

Statistical analysis. General procedures. Species were separated in three groups according to the total numbers trapped (n) and the number of replicates in which they were caught (N). Common species included those collected on most replicates ($N \geq 5$ for each CO₂ treatment combination) and for which $n > 90$. Occasional species were those trapped on six nights or more, but for which $n < 60$. Rare species included those caught on less than four replicates.

In the case of common species, a generalized linear modeling (GLM) package using the maximum likelihood method GLIM® (Numerical Algorithms Group, Ltd., Oxford, United Kingdom)³¹ was used to obtain the best fitting statistical model for the data. Differences between species and the influence of CO₂ on the mean proportion of mosquitoes caught in the human-baited trap (the index of anthropophily) was investigated by linear logistic analysis of deviance (or co-deviance).

The choice experiment was designed mainly to test for the response of mosquitoes to human versus animal odors. Although preliminary analysis could not detect any significant difference in the index of anthropophily between calf/human versus goat/human choices of *An. gambiae* s.l., *An. pharoensis* Theobald, and *Cx. decens*, because of the possibility that contamination from goat odors influenced successive responses, the replicates immediately following human odor versus goat odor ones were discarded in the calf versus human choice-test analysis.

For occasional species, the Wilcoxon signed rank test³² was used to test departures from a random distribution regardless of the CO₂ treatment combination, unless otherwise stated. In the case of rare species, the probability of having the observed distribution between the two traps (or more extreme cases) was calculated from a positive binomial distribution with $P = 0.5$.

Tests of association in two-factor contingency tables were carried out according to the guidelines given by Sokal and Rohlf³³ or by log-linear analysis in GLIM in the case of multifactor tables.

Generalized linear modeling procedures. For common species, the catch size in the human-baited OBETs represented the response variable, and a binomial error structure was assumed, specifying the total number caught in both traps for each species as the binomial denominator. Statistical significance of the experimental factors considered in the model was assessed by the change in deviance caused by the stepwise removal of each term from the full model, which can be regarded as to performing a G-test ($G = 2\ln L$, where L = the likelihood ratio).^{31, 33, 34} The deviance asymptotically approximates a χ^2 distribution for the corresponding number of degrees of freedom.

Carbon dioxide gradients between the OBETs were considered either as a factor (presence/absence: GRDF) or as a covariate (GRDC). The CO₂ concentration released from the human-baited trap was also considered as a covariate (CONC), while other factors were trap site (left/right: SIDE) and species (SPEC). First, differences between species were analyzed by fitting the model: SPEC * GRDF. Subsequently, because species differed in their response (see Results), four possible full models were considered for each species separately: a) GRDF * SIDE; b) CONC * GRDF; c) GRDC * SIDE; and d) GRDC + GRDC²; here given in a GLM notation. (The asterisks indicate the fitting of all main effects and their interactions; e.g., the model A * B implies A + B + A × B.)

After the fit of the full model, overdispersion was checked by comparing the residual deviance and degrees of freedom. If overdispersion was detected, it was corrected using Williams' algorithm.³⁴

Detransformed parameter estimates defined the index of anthropophily. These represent corrected measures because they allow for differences between the nightly sample sizes and for the nonconstant binomial variance in proportion data.³⁴ Statistical significance of departures from a random choice (i.e., index of anthropophily = 0.5) were tested for each species by Student's t -tests on the parameter estimates versus a zero estimate (which defines a 0.5 proportion on the logit scale).

RESULTS

Index of anthropophily. Figure 1 shows the index of anthropophily of common mosquitoes. Differences between species were highly significant ($G = 88.2$, degrees of freedom [df] = 3, $P < 0.001$). Species with an index greater than the 0.5 random expectation included *An. gambiae* s.l. (index = 0.96, $t_{18} = 6.59$, $P < 0.0001$) and *An. pharoensis* (index = 0.68, $t_{18} = 2.74$, $P < 0.02$). The index for *Cx. antennatus* Becker was significantly lower than 0.5, showing

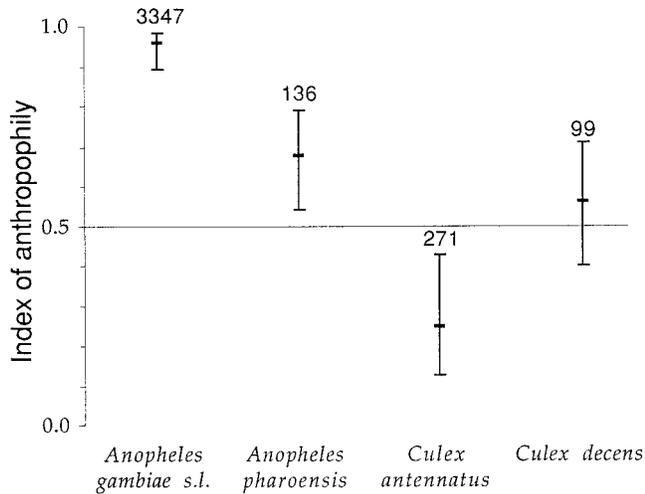


FIGURE 1. Choice test: human versus calf odor. Mean \pm 95% confidence limit proportion of mosquitoes caught in a human-baited trap versus a calf-baited trap placed side-by-side outdoors in 18 replicates (random expectation = 0.5; for a definition of the index of anthropophily, see Materials and Methods). Numbers above the bars indicate total number of mosquitoes caught.

a preference for the calf-baited trap (index = 0.25, $t_{11} = 2.95$, $P < 0.02$). The *Cx. decens* group was randomly distributed (index = 0.56, $t_{15} = 0.83$, $P < 0.45$).

Separate analyses for each of the four common species revealed that a null model gave a satisfactory fit to the data for *An. gambiae* s.l. and *An. pharoensis*. Trap site was the only variable to have a significant effect in the case of *Cx. antennatus* ($G = 4.5$, $df = 1$, $P < 0.04$), but even if considered separately for each side, the index of anthropophily was lower than 0.5 (0.13 versus 0.46).

The *Cx. decens* group differed from the previous species in that the best fit was given by model b without the interaction term (see Statistical analysis). In this case, the index of anthropophily was higher, for a given CO_2 concentration, when the CO_2 difference was eliminated ($G = 16.7$, $df = 1$, $P < 0.001$). Moreover, the index significantly decreased as

TABLE 1

Total numbers of occasional mosquito species caught in human-baited and calf-baited odor-baited entry traps placed side-by-side*

Species	Odor bait		P	N
	Human	Calf		
<i>Anopheles (Cellia) funestus</i>	9	0	0.02	6
<i>Anopheles (Cellia) rufipes</i>	0	11	0.02	6
<i>Mansonia (Mansonioides) africana</i>	17	1	<0.01	9
<i>Mansonia (Mansonioides) uniformis</i> a)	16	10	>0.50	8
b)	24	5	<0.10	6
<i>Aedes (Aedimorphus) dalzieli</i>	22	4	0.03	10
<i>Aedes (Aedimorphus) hirsutus</i>	13	1	0.01	7
<i>Culex (Culex) duttoni</i>	0	17	0.01	7
<i>Culex (Culicomyia) nebulosus</i>	2	35	0.001	10

* *Mansonia uniformis* catches are divided into a) nights when a CO_2 concentration difference was present, and b) nights when the CO_2 concentration was set to the same level in both traps. P = probability for the observed distribution according to a Wilcoxon signed rank test. N = number of nights in which at least one individual was trapped.

TABLE 2

Total numbers of rare mosquito species caught in human-baited and calf-baited odor-baited entry traps placed side-by-side*

Species	Odor bait		P	N
	Human	Calf		
<i>Aedes (Stegomyia) aegypti</i>	2	0	–	2
<i>Aedes (Aedimorphus) argenteopunctatus</i>	2	0	–	2
<i>Aedes (Aedimorphus) fowleri</i>	5	0	0.06	3
<i>Culex (Lutzia) tigripes</i>	1	1	–	2
<i>Culex (Culex) poicilipes</i>	2	0	–	2
<i>Culex (Culex) quinquefasciatus</i>	4	0	0.13	2

* P = probability for the observed distribution according to a positive binomial with P = 0.5. N = number of nights in which at least one individual was trapped. – = sample too small for P to be calculated.

the CO_2 concentration increased ($G = 14.8$, $df = 1$, $P < 0.001$).

Occasional species whose distribution showed a bias towards the human-baited trap were *An. funestus* Giles, *Mansonia africana* Theobald, *Aedes dalzieli* Theobald, and *Ae. hirsutus* Theobald (Table 1). Conversely, species showing a bias towards the calf-baited trap were *An. rufipes* Gough, *Cx. duttoni* Theobald, and *Cx. nebulosus* Theobald. *Mansonia uniformis* Theobald was equally distributed when a CO_2 gradient was present, whereas it showed a certain bias towards the human-baited trap (though not statistically significant) when the CO_2 concentration was standardized. Total numbers of rare species are shown in Table 2.

Males were only rarely caught: two of four *An. gambiae* s.l. and one of six *Ma. uniformis* were trapped in the calf-baited OBET, while all five males of *Culex* spp. were caught in the human-baited trap.

Anopheles gambiae species complex analysis. Molecular identification of the *An. gambiae* s.l. samples revealed a marked difference in trap distribution between the two members of the complex present, *An. gambiae* s.s. and *An. arabiensis*. The latter constituted 92% (*An. gambiae* s.s. = 8%) of identified specimens caught in the calf-baited trap, but only 52% (*An. gambiae* s.s. = 48%) of those caught in the human-baited trap ($n = 653$). Log-linear analysis showed that the relative frequency of the two siblings was significantly different according to the trap considered ($G = 92.79$, $df = 1$, $P < 0.0001$), but the difference was not affected by the presence or absence of a CO_2 concentration difference between the OBETs ($G = 2.73$, $df = 1$, $P > 0.05$).

Abdominal conditions. Table 3 shows the proportion of mosquitoes grouped in each of the abdominal conditions defined. Only two species groups were considered, *An. gam-*

TABLE 3

Abdominal condition of selected species groups caught in human-baited and a calf-baited odor-baited entry traps placed side-by-side*

Odor bait	Empty	Partially fed/fed	Gravid
<i>Anopheles gambiae</i> s.l.			
Calf trap	0.91	0.08	0.01
Human trap	0.89	0.09	0.02
<i>Culex</i> spp.			
Calf trap	0.79	0.03	0.19
Human trap	0.48	0.01	0.51

* Values are the proportion of the total number of mosquitoes in each category.

TABLE 4

Choice test: human goat—total numbers of mosquitoes trapped in two replicates using a human-baited odor-baited entry trap (OBET) versus a goat-baited OBET placed side-by-side*

Species	Odor bait		P
	Human	Goats	
<i>Anopheles (Cellia) funestus</i>	3	0	0.25
<i>Anopheles (Cellia) gambiae</i> s.l.	296	3	<0.001
<i>Anopheles (Cellia) pharoensis</i>	6	0	0.03
<i>Anopheles (Cellia) rufipes</i>	0	1	—
<i>Mansonia (Mansonioides) africana</i>	3	0	0.25
<i>Mansonia (Mansonioides) uniformis</i>	16	1	<0.001
<i>Aedes (Aedimorphus) dalzieli</i>	1	0	—
<i>Aedes (Aedimorphus) hirsutus</i>	2	0	—
<i>Culex (Culex) antennatus</i>	0	4	0.13
<i>Culex (Culex) decens</i>	17	8	0.06
<i>Culex (Culex) duttoni</i>	0	2	—
<i>Culex (Culicomyia) cinereus</i>	0	2	—
<i>Culex (Culicomyia) nebulosus</i>	0	6	0.03

* P = probability for the observed distribution according to a positive binomial with P = 0.5. Two *Ma. uniformis* males were caught in the human-baited trap. — = sample too small for P to be calculated.

biae s.l. and *Culex* spp., because almost all individuals of the other species collected were unfed. About 90% of the *An. gambiae* s.l. were unfed and this proportion did not change among odor baits ($n = 3,347$, $G = 1.23$, $df = 2$, $P < 0.55$). About 90% of the *Culex* species caught in the calf-baited trap (which included most of the species showing a significant preference for calf odors) were unfed, but this proportion decreased to less than 50% in the human-baited trap ($n = 493$, $G = 44.7$, $df = 2$, $P < 0.0001$). This difference was mainly due to a shift in the unfed versus gravid proportion since the partially fed/fully fed percentage remained approximately the same (Table 3).

Human versus goat odor choice test. The total numbers of those mosquito species caught during the two choice-test replicates between human and goat odors are presented in Table 4. Apart from *Cx. antennatus*, *Cx. duttoni*, *Cx. cinereus* Theobald, and *Cx. nebulosus*, all other species were caught more abundantly in the human-baited trap, although only *An. gambiae* s.l., *Ma. uniformis*, and the *Cx. decens* group had an $n > 15$. The probability of having the observed distributions between the two traps was calculated as for the rare species, but it must be stressed that this value is only indicative since the number of replicates was only two or three and the probability of a type I error is augmented by the possibility that individual mosquito choices may not be independent on each replicate.^{35, 36}

DISCUSSION

The process by which mosquitoes arrive at a host is composed of a series of responses to internal and external stimuli, in a chain of events whose outcome is an increased probability of encountering and contacting the host.³⁷ Generally, the first host cues to reach a mosquito are volatile chemicals emanating from the skin, breath, and waste products of a potential host,³⁸ carried by air currents. The probability that the mosquito responds to these cues and the strength of the response depend on the strength of the host-derived stimuli,³⁹ the strength of competing external stimuli (e.g., odors from other sources, prohibitive wind speeds, etc), the internal state

of the mosquito (e.g., circadian phase, gonotrophic status, etc),⁴⁰ and its genotype (i.e., the genetic component of the responsiveness to given stimuli).¹ Positive anemotaxis in response to host odors or alternative, odor-mediated, host-seeking strategies (e.g., klinokinesis and orthokinesis)⁴¹ can bring the mosquito nearer the host, thereby increasing the availability of additional host stimuli, such as visual cues,⁴² temperature and humidity gradients, convective currents,^{43, 44} and volatile and tactile chemical cues,⁴⁵ which may induce the mosquito to land on the host. Finally, once on the host, probing and biting are affected and initiated by the quality of the surface and the quality and quantity of stimuli such as heat and blood phagostimulants.^{46, 47}

The analysis of blood meals of a wide range of mosquito species has demonstrated that feeding patterns, i.e., the range of host species fed on and the degree of host specificity, are highly species-specific. It is likely that differences in feeding patterns reflect differences in responsiveness to stimuli present throughout the host-location process. The present study was designed to test whether or not it is possible to demonstrate a difference in the responsiveness of various mosquito species to air-borne odors from two commonly available hosts. The OBET is the least ambiguous behavioral assay used so far in the field for assessing the degree to which a species expresses an inherent preference at this early stage of the approach to a host.

These results show that the very high degree of anthropophily (as defined by the HBI) of *An. arabiensis* and *An. gambiae* s.s. in West Africa can be at least partly explained as a preference for human odors other than CO₂. They also confirm previous field observations on odor-mediated behavioral differences between *An. arabiensis* and *An. gambiae* s.s.²³

Of the proportionally few *An. gambiae* s.l. that chose the calf-baited trap, extremely few were *An. gambiae* s.s.: of an estimated 1,500 *An. gambiae* s.s. caught in the two OBETs, less than 1% were collected in the calf-baited trap. Considering the closeness of the two trap entrances, this implies an extraordinary degree of accuracy in entering the human-baited trap for this species.

It is also evident that the majority of *An. arabiensis* preferred the human-baited trap: of an estimated 1,800 collected in both OBETs, slightly more than 8% were collected in the calf-baited trap. This suggests that the West African populations of *An. arabiensis* are not generalists by nature, but rather that they may be opportunistic in their feeding habits, depending on the prevailing conditions. Alternatively, considering the outcome of selection experiments on host preference,²² environmental conditions may create selective pressures that shift the balance between anthropophilic and zoophilic genotypes according to host availability. Since non-human hosts were scarce in the experimental village, our evidence cannot distinguish between these hypotheses. Intraspecific variability in human/bovid feeding associated with carriers of alternative chromosomal 2Rb inversions have been recently found for endophilic samples of *An. arabiensis* in western Kenya.⁴⁸

The results also indicate a much higher degree of preference for humans in *An. gambiae* s.s. than reported by Gillies²¹ (namely approximately 99% versus his 18–69%). Similar selective pressures as outlined above for *An. arabiensis*

may have accounted for this difference, although extensive studies on blood meal origin in varying environmental conditions across Africa indicate substantial homogeneity in *An. gambiae* s.s. anthropophily.^{4, 16, 17} Alternatively, the difference may be explained by how cues were presented and how mosquitoes responded to them. Our experimental design was substantially different from that of Gillies. He released reared mosquitoes into a room where both hosts were present. In these conditions, there would be considerable mixing of odors and a lack of directional cues for orienting to the source (e.g., no wind). The mosquitoes may, therefore, have depended more strongly on kinetic strategies mediated by short-range cues (e.g., warm and moist convective currents, visual cues, and host movements). Gillies commented that the calf may have masked the human due to its greater surface area and greater production of short-range kairomones and physical cues.

We, on the other hand, were trapping wild mosquitoes presented with odor cues carried by the wind, thus allowing mosquitoes to make their initial response to host odors at some (unknown) distance away, probably within the mixed-odor plume in which they had the opportunity to respond by odor-mediated anemotaxis. The advantage of using OBETs to investigate host preferences is that mosquitoes are presented with odor cues in a reasonably natural way, and the confounding effects of short-range cues are eliminated by trapping mosquitoes in the OBET while they are still at a distance from the host itself.

It is interesting to note that apart from *Ma. uniformis*, none of the common and occasional mosquito species caught distributed at random between the two traps, and the presence or absence of a CO₂ differential had little effect.

Mansonia uniformis was more catholic in its preference than *Ma. africana*, which was captured on almost all occasions only in the human-baited trap. Evidence for differences in anthropophily between the two species can be extrapolated from data of Smith,⁴⁹ who collected indoor-resting blood-fed *Mansonia* in a village where the local population sleeps inside huts together with their cattle and goats, and found that *Ma. africana* were proportionally more positive for human blood than were *Ma. uniformis*, although feeds on ox predominated for both species.

Mansonia africana have also been reported biting monkeys in the forest canopy,⁵⁰ and found to be more attracted to humans than to monkeys.⁵¹ Snow⁵² in comparative catches between a human and a calf in baited stable traps, also found *Ma. africana* proportionally more abundant than *Ma. uniformis* in the human bait-attracted sample, while the reverse was true in the calf bait-attracted sample. Host preference differences between these two species have not been clearly defined,⁵³ perhaps because of their opportunistic habit of feeding on a wide variety of mammals.

Our results are consistent with Smith's general conclusions: "Anopheles gambiae and An. funestus feed almost entirely on man. An. pharoensis feed more on man than on ox. Culicines feed more on ox than on man".⁴⁹ The present results complete this picture by indicating that the *Aedes* spp. collected in the present experiment generally preferred human to calf odor. Nonhuman-biting (preferentially ornithophilic) species *Cx. cinereus* and *Cx. nebulosus*⁵⁴ were caught very rarely in the human-baited trap. *Anopheles pharoensis* was shown to be an-

thropophilic in its odor preferences: in the delta area of the Senegal River basin, blood meal analysis indicated an HBI > 90% for endophilic samples of this species.⁵⁵

Culex decens was the only other group of species for which the index of anthropophily was not significantly different from 0.5. In this case, however, the interpretation of results is complicated. First, the index was inversely correlated with the CO₂ concentration of the odor plume released from the human-baited trap. This might be explained if the samples were in fact a mixture of two or more species with different feeding habits and differential responses to CO₂. Second, the index was greater when the CO₂ difference was eliminated. This would suggest that the higher CO₂ concentrations usually released from the calf-baited trap influenced the distribution of this species group towards that trap. Third, the *Cx. decens* group, together with *Cx. antennatus*, constituted the bulk of the catches for that genus. About half of the *Culex* mosquitoes collected in the human-baited trap were gravid. If we assume that these mosquitoes were not searching for a blood meal, the effect of removing them from the analysis would be to decrease the index of anthropophily shown. Given the relatively small sample size of our catches for this group of species, and the possible confounding effect of pooling, these conclusions must be treated with caution.

One of the limiting factors when interpreting field trap-catch data is the lack of knowledge on how many mosquitoes did not respond to the stimuli presented.⁵⁶ Indeed, some species were not caught in all replicates, and we do not know if such variability was due to population densities fluctuating below the threshold detectable by our sampling technique, or by the mosquitoes failing to enter (or to respond to) the OBETs. What is of interest in this study, however, is the differential response to the two traps, and how this varied across species. The possibility that the traps were selecting for only certain fractions of the population (e.g., endophagic versus exophagic) because of the way the stimuli were presented must, of course, be kept in mind: the relative frequency of the *An. gambiae* complex siblings caught in the human-baited trap, however, was not different from that of contemporary, single OBET catches using human odor baits only (Costantini C and others, unpublished data).

Obviously, we did not have any control over the way the odor plumes dispersed and the mosquitoes approached the traps, as in laboratory wind-tunnel, choice-test bioassays. From our unpublished infrared video recording field observations, however, mosquitoes flying in front of the OBET appear to move across the odor plume to a considerable extent. We exchanged the traps to allow for any unknown environmental variance between the left and right positions. Additionally, the conditions in which the experiment was performed changed through the course of the trapping season without appreciable effects on the differential trap response, for *An. gambiae* s.l. at least (see 95% CL in Figure 1). Although this field experiment cannot be regarded as being as precise as a laboratory bioassay, it is likely that many of the mosquitoes approaching the trap had the opportunity to sample both odor plumes, and therefore did make a choice between them.

It is difficult to decide from the data if a preferential response was due to attractive or repellent effects from one of the traps as compared with the other. Moreover, it is not possible to distinguish which behavioral traits were affected

by the attractive and/or repellent effects. The dilemma is exemplified by the interpretation of the evidence that some human subjects attract different numbers of mosquitoes:^{57, 58} a complementary view coming from experimental results with dipteran pests of cattle is that certain subjects within a herd produce substances that mask their normal attractiveness, so that some subjects are more repellent than others.⁵⁹

A comparison between our present choice test results and previous ones obtained in similar (but not identical) experimental conditions points to the possibility that a phenomenon of avoidance of unsuitable hosts may take place in the case of mosquitoes as well. In particular, *An. gambiae* s.l. preference for human odors was about 2:1 against a human-equivalent amount of CO₂,²³ but about 20:1 against calf odor at the same CO₂ concentration. Similarly, *An. pharoensis* chose at random or possibly with a slight bias towards the CO₂-baited trap in the above human-CO₂ choice test, but preferred the human odor in the present human-calf choice test. The final response of a hematophagous insect to its host may thus be the result of a push-pull system of attractive and repellent components, as for example in tsetse flies, in which human skin odors are repellent but breath odors are highly attractive.⁵⁶

In spite of these interpretation difficulties, the use of the OBET, as in the field approach presented in this paper, can contribute to the understanding of the determination of mosquito feeding patterns and the evaluation of differences across mosquito species.

It remains to be seen how far these results can be generalized in time and space. We examined only one stage of the chain of events that lead mosquitoes into contacting the host, and we tested only a limited number of alternative hosts. Under natural conditions, of course, the host preferences of a mosquito (as indicated by its odor choice) may not be entirely represented in the final host selection, since a number of additional factors come into play: relative abundance and accessibility of suitable hosts,⁶⁰ defensiveness of different species of hosts,¹² and mosquito feeding success, among others. Moreover, feeding preferences may show patterns of temporal organization, e.g., *Cx. nigripalpus* and *Cx. tarsalis* shift their host selection from birds to mammals according to the season.⁶¹ Host preference, as it is applied to field populations of mosquitoes, remains a subtle concept: is it a homogeneous or heterogeneous property of the population?

It is clear, however, that at least for *An. gambiae* s.l., host odors other than CO₂ play an important role in the feeding preference for humans, and that the response of *An. gambiae* s.s. to human odors is stronger than in its sibling species *An. arabiensis*.

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