

Beyond Nature and Nurture: Phenotypic Plasticity in Blood-Feeding Behavior of *Anopheles gambiae* s.s. When Humans Are Not Readily Accessible

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Abstract. To test for the effects of host accessibility on blood-feeding behavior, we assessed degrees of anthropophily of the malaria mosquito *Anopheles gambiae* at two stages of the behavioral sequence of host foraging, in a rice growing area near Bobo-Dioulasso, Burkina Faso, where humans are not readily accessible because of years of generalized use of (mostly non-impregnated) bed nets. First, patterns of host selection were assessed by the identification of the blood meal origin of indoor-resting samples. Inherent host preferences were then determined by two odor-baited entry traps, set side by side in a choice arrangement, releasing either human or calf odor. The proportion of feeds taken on humans was around 40%, whereas 88% of trapped *An. gambiae* “chose” the human-baited trap, indicating a zoophilic pattern of host selection despite a stronger trap entry response with human odor. This paradox can be interpreted as the evolution of a plastic strategy of feeding behavior in this field population of *An. gambiae* because of the greater accessibility of readily available, although less-preferred, hosts.

INTRODUCTION

The degree of vector-host association is a key predictor of vectorial capacity and transmission intensity of vector-borne diseases.¹ Understanding the blood-feeding behavior of mosquitoes of medical importance is thus of great interest because it can contribute to the design of vector control and disease prevention measures. A detailed mechanistic understanding of the proximate and ultimate causes and processes modulating the host choice of mosquitoes, however, is still in its infancy.²

To obtain a successful blood meal, a female mosquito is confronted by a series of trade-offs. For instance, the mosquito must balance the risk of death caused by host defensive behavior³ against the benefits to feed on a host species that maximize fertility.⁴ Such trade-offs, along with other selective forces (e.g., host diversity, density, and distribution in the insect environment) have likely shaped in the course of evolution the level of host specificity and the innate host preference of mosquitoes⁵. However, many environmental factors, acting in combination with the innate preferences, influence the final host selection. These factors include host availability,⁶ host accessibility,⁷ or mosquito previous experience.⁸ For instance, it has been shown that in late summer North American *Culex pipiens* (the dominant vector of West Nile virus) shift their feeding pattern from birds to humans. This change in host selection coincides with the migration of its preferred host (the American Robin) and the rise in human West Nile virus infections.⁹ Another well-known source of behavioral variation is that induced by house spraying of insecticides for vector control, which makes humans less accessible to mosquitoes. For example, Sharp and le Sueur¹⁰ showed that the proportion of indoor resting *Anopheles arabiensis* that had fed on humans was significantly higher in areas that had not been sprayed with DDT.

At least two processes can explain variations in blood-feeding behavior. First, the decrease in one host availability or accessibility may favor individual mosquitoes that have an innate preference for alternative, more abundant, or accessible hosts, thereby changing the genetic composition of the population (i.e., selection for host preference). Second, the population may express an environmentally induced phenotypic plasticity, so that changes in host availability or accessibility modify host selection patterns without changes in innate host preferences.

Of the seven sibling species forming the *Anopheles gambiae sensu lato* complex, *Anopheles gambiae sensu stricto* (hereafter, *An. gambiae*) is regarded as the most efficient vector of malaria. The key element that defines *An. gambiae* as a highly efficient malaria vector is its anthropophilic behavior, i.e., its preference for humans as a source of blood, its indoor resting habits (endophily), and exploitation of breeding habitats created by human activities.¹¹ Although it is widely accepted that *An. gambiae* exhibits an extreme form of specialization for human hosts as a source of blood, whereas its sibling *Anopheles arabiensis*, for example, is more opportunistic,^{11–13} this categorization is probably too simplistic given that spatial and temporal variations in blood-feeding behavior of *An. gambiae* have been at times reported; e.g., for highly anthropophilic/anthropophagic behavior^{14–17} and for lower anthropophilic/anthropophagic behavior.^{18–23} It is perhaps more appropriate to characterize the behavior of populations rather than whole taxonomic units, especially in the case of species that occur in a wide range of environmental conditions, over large spatial extents, and with genetically highly structured populations, as is the case for *An. gambiae*.^{24–27}

The purpose of this study was to investigate patterns of blood-feeding behavior of *An. gambiae* in a rice growing area of Burkina Faso, where the availability of human hosts is relatively poor because of the widespread and generalized use of (mostly non-impregnated) bed nets for > 30 years.²⁸ More specifically, we addressed the following questions. First, can restricted access to humans divert a strongly anthropophagic mosquito such as *An. gambiae* to feed on hosts other than humans? Second, could the long-term use of bed nets eventu-

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ally select for an inherent tendency to feed on animals other than humans in this natural population? To answer these questions, we first studied patterns of host selection by identification of the origin of blood meals of field-collected mosquitoes. We then assessed the degree of inherent host preference of this population by a field choice test using either human or calf odor as alternative stimuli, following previously established experimental protocols.^{14,22,29,30}

MATERIALS AND METHODS

Study area. Field studies were carried out in VK5 and VK7, two villages that are located in a rice growing area in the Kou Valley, 30 km north of Bobo-Dioulasso, southwestern Burkina Faso.³¹ The rainy season here lasts from May to October with an annual rainfall of ~1200 mm. Domestic animals found in the villages include cattle, goats, sheep, pigs, donkeys, dogs, cats, and poultry. Animals are usually kept during the night within the village, either outdoors, in shelters within family compounds, or in nearby pens. *Anopheles gambiae* and *Anopheles funestus* are the major malaria vectors in this area, with *An. gambiae* molecular form M as the predominating species for most of the year.^{32,33}

Mosquito collections for host selection studies. Indoor resting mosquitoes were collected in January–February 2007 between 07:00 and 09:00 hours, either manually using hemolysis tubes or by insecticide pyrethroid spray catches.³⁴ Blood-fed mosquitoes that were morphologically identified as *Anopheles gambiae* s.l. using the identification keys of Gillies and De Meillon³⁵ were selected for further processing (see later), stratifying by location of capture (animal sheds versus human dwellings) and sampling technique (manual collections versus spray catches).

Field choice tests of host preference. Two odor-baited entry traps (OBETs; described in Costantini and others³⁶) baited with calf and human odors were used to assess the host preference of field populations of mosquitoes in VK5 and VK7. The two OBETs were placed adjacent to one another in a choice arrangement, and each was connected to a tent (2.5 × 1.5 × 1.5 m for the tent containing a calf and 2 × 1.5 × 1.5 m for the tent containing a human) by lay-flat tubing (5 m long and 15 cm in diameter).^{14,22,30} The odors of the two hosts were drawn by a 12-V fan from the tents and into the OBETs by the lay flat tubing, coming out of the traps at a speed of 0.5 m/s, as measured with a 435-4 Testo multi-functional meter (Testo AG, Lenzkirch, Germany) equipped with a probe for degree of air turbulence (range: 0 to +5 m/s, accuracy: ±0.03 m/s +4% of mv). Host-seeking mosquitoes responding to the host cues flew up the odor-laden streams and entered one of the two traps. To rule out any trap positional effect, the relative left/right position of each host odor on a single test night was swapped on two consecutive nights, with the initial position in a series of two chosen at random. In addition, to obviate any host effect, different combinations of calves and humans were presented on subsequent nights (a total of 5 calves and 14 human volunteers acted as odor baits through the experiments). The traps were operated from 19:00 to 05:30 hours, for eight nights in February 2007 (four nights in VK5 and four nights in VK7), and six nights in June 2007 in VK5. Trapped mosquitoes were retrieved from each OBET in the morning using mouth aspirators, and brought to the laboratory for further processing.

Laboratory processing of samples. Female mosquitoes were dissected in a drop of phosphate buffered saline (PBS) (pH 7.2). First, ovaries were dissected to determine parity based on the condition of ovarian tracheoles.³⁷ Midguts were then gently squeezed to get the blood, which was mixed with PBS, absorbed on filter paper, and then kept at 4°C until identification by an enzyme-linked-immunosorbent assay (ELISA), as described by Beier and others.³⁸ Each blood meal was tested for reaction against human, cattle, goat/sheep, pig, and horse/donkey antibodies. The extracted midguts were then stained with 2% mercurochrome to detect with a microscope (10× magnification) the presence and number of *Plasmodium* spp. oocysts.³⁹ The head and thorax of individual mosquitoes were stored at –20°C in 1.5 mL microtubes to detect the circumsporozoite protein epitopes of *Plasmodium falciparum* with specific monoclonal antibodies by ELISA.⁴⁰ The legs and wings of each tested mosquito were placed in individual microtubes containing a desiccant (silica gel) for molecular identification of the siblings of the *An. gambiae* complex. Polymerase chain reactions (PCR) without prior DNA extraction were performed according to the procedure of Favia and others⁴¹ to determine the molecular form status (M or S) of *An. gambiae*.

This protocol allowed us to gather the following information for each tested mosquito: 1) parity (parous versus nulliparous); 2) immature *Plasmodium* infection status (presence of oocysts in the midgut); 3) mature *P. falciparum* infection status (presence of sporozoites in salivary glands); 4) molecular form status of *An. gambiae* (M or S); and 5) source of blood meal, if engorged. This information was related to the location of capture and sampling technique in the case of mosquitoes collected for the host selection study, and to the trap relative position and host chosen in the case of the choice test of host preference (except for mosquitoes trapped in July, for which parity and oocyst infection status were not determined).

The institutional ethical committee of Institute for Resource and Security Studies (IRSS)/Center Muraz from Burkina Faso approved the experimental protocol.

Statistical analysis. Each collected mosquito was treated as a binary variable: samples of the host selection study were encoded as human-fed or not human-fed; samples of the host preference study were treated as collected in the human-baited trap or in the calf-baited trap. We fitted logistic regression models (Generalized Linear Modeling with binomial errors and logit link function, analyzed with the software R, version 2.5.1) to the number of mosquitoes that were human-fed or collected in the human-baited trap, using the total sample size as the binomial denominator. In this way, we studied the effect of explanatory variables locality, type of shelter, collection technique, and mosquito parity on the proportion of mosquitoes that fed on humans. Similarly, we fitted season, trap relative position, and mosquito parity (February samples only) as explanatory variables in the host preference study, and verified whether the proportion of mosquitoes collected in the human-baited OBET p was compatible with a random choice (null hypothesis: $p = 0.5$).

RESULTS

Host selection. A total of 1,065 *An. gambiae* s.l., of which 892 engorged, were collected in January and February 2007. A sub-sample of 100 *An. gambiae* s.l. was successfully identified:

97% were *An. gambiae* molecular form M, and the remaining 3% were *An. gambiae* molecular form S, confirming previous results of *An. gambiae* s.l. complex species abundance obtained in the same area.³³ Of 1,065 mosquitoes, only 0.75% (a few blood-fed, most unfed) and 0.19% (all unfed) were found parasitized with *Plasmodium* oocysts (likely *P. falciparum*) or *P. falciparum* sporozoites, respectively. Of those specimens found positive for oocysts, two were unfed, three were engorged with cattle blood, and three with human blood; only one oocyst per midgut was detected each time.

The source of blood meals was studied in a sub-sample of 349 mosquitoes, and in another sub-sample of 120 individuals using only human and cattle antibodies (Table 1). In the first sample, 8% of tested mosquitoes returned a negative reaction. We considered that most of these negative reactions resulted from technical issues rather than from the meals being taken off untested hosts, because very few alternative unconventional and/or scarce hosts were not tested. These include dogs and cats, which were not abundant in the study area relative to other domestic animals, and poultry, which have been rarely reported as significant hosts for *An. gambiae*.² Therefore, if we exclude negative reactions from the sample, half of the meals were taken off cattle alone; 14% of positive reactions were mixed meals, of which human/cattle meals accounted for 71% of the mixed meals. If we correct the figures in the second sample based on these estimates, we arrive at estimates of the human blood index (HBI, i.e., the proportion of meals taken off humans) that are comparable between the two samples 41% versus 45% (Table 1).

The HBI of mosquitoes collected in human dwellings was consistently higher (46%, 115/250) than that of mosquitoes collected in animal sheds (31%, 68/219). The odds ratio (OR) of this difference is statistically significant (OR = 1.9; 95% confidence interval [CI] = [1.5, 2.2]; *P* < 0.001). We found no significant effect of parity or collection method on the HBI. We found, however, a significant effect of locality on HBI (OR = 1.8, CI = [1.5, 2.4], *P* = 0.001). This effect resulted because of a higher prevalence of mixed cattle-human meals in VK5 compared with VK7.

Host preference. Over 14 nights, the two OBETs baited with human and calf odor caught a total of 3,087 mosquito females belonging to three genera and 12 species or species groups (Table 2 and Figure 1).

Human odor attracted significantly more *An. gambiae* s.l. than calf odor (OR = 7.2; 95% CI = [6.2, 8.4]; *P* < 0.001; Figure 1). Among the 1,490 trapped *An. gambiae*, only 4 (0.27%) were

found parasitized with *P. falciparum* sporozoites, all of which were found in the human trap (three in June and one in February), and 107 (7.2%) were engorged. Among the 182 *An. gambiae* caught in the calf-baited trap, 15 (8.2%) were engorged, whereas among the 1,308 caught in the human-baited trap, 92 (7.0%) were engorged. It is likely that these mosquitoes had taken only incomplete meals on a previous host and were returning to complete blood intake on a new host on the same night of capture, or they were otherwise pre-gravid individuals (*sensu* Gillies⁴²) needing a second blood meal to complete the first gonotrophic cycle. We analyzed, by ELISA, the blood source of all these mosquitoes responding to host cues despite the presence in their guts of a previous meal (Table 1). Because some of the blood meals tested were partially digested, we found that more ELISA reactions returned a negative result, as compared with the rates that could be achieved for the indoor-resting samples (Table 1). Among the mosquitoes retrieved from the human-baited trap, 25 had fed on cattle and 11 on humans. If we discard negative reactions and take into account mixed meals, we obtained a HBI of 34%, of which more than one-third was represented by mixed meals, a result that is consistent with that found for the host selection samples (see above). Similarly, of the nine positive reactions obtained from the calf-baited trap sample, 44.4% had previously fed on humans, a difference with the human-baited trap that is not statistically significant by the Fisher exact test (*P* = 0.71).

No effects of season or trap side were found on the proportion of mosquitoes from the human-baited OBET. A sub-sample of 280 *An. gambiae* s.l. trapped in the OBETs was molecularly identified; of these, 21 were collected from the calf-baited and 50 from the human-baited trap in February, whereas 63 were collected from the calf-baited and 146 from the human-baited trap in June. We found 96.8% *An. gambiae* molecular form M, 2.1% *An. gambiae* molecular form S (three in the calf-baited trap and three in the human-baited trap), and 1.1% *An. arabiensis* (all in the human-baited trap).

The second most abundant species caught in the OBETs was *Culex quinquefasciatus* (Figure 1). Overall, calf odor attracted significantly more *Cx. quinquefasciatus* than human odor (OR = 0.48; CI = [0.43, 0.54] *P* < 0.001; Figure 1). However, unlike *An. gambiae*, we found a strong effect of season on odor choice for this species (OR = 5.5; CI = [4.2, 7.2]; *P* < 0.001) with a marked bias toward the calf-baited trap in February (OR = 0.30; CI = [0.26, 0.36]; *P* < 0.001), and a slight but significant bias in distribution toward the human-baited trap in June (OR = 1.6; CI = [1.3, 2.1]; *P* < 0.001).

TABLE 1

Blood meal origin of *Anopheles gambiae* s.l. (> 95% *An. gambiae* s.s. molecular form M) collected resting in human dwellings or animal sheds*

Sample	Human	Bovine	Equine	Ovine	Porcine	Mixed										-ve	Total	HBI
						H/B	H/E	H/P	H/B/O	H/B/E	B/E	B/O	B/P	E/P				
IRC-1	97	160	8	3	5	31	1	2	1	1	3	7	2	0	28	349	132	
	30%	50%	2%	1%	2%	10%	0.3%	0.6%	0.3%	0.3%	0.9%	2.2%	0.6%	0.0%	8%		41%	
IRC-2	30	44	-	-	-	20	-	-	-	-	-	-	-	-	26	120	50	
	32%	47%	-	-	-	21%	-	-	-	-	-	-	-	-	22%		53%	
OBT-H	11	25	6	0	1	4	2	0	0	0	0	0	0	1	42	92	17	
	22%	50%	12%	0%	2%	8%	4.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.0%	46%		34%	
OBT-C	2	4	0	0	0	2	0	0	0	0	0	0	1	0	6	15	4	
	22%	44%	0%	0%	0%	22%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	11.1%	0.0%	40%		44%	

* (IRC sample. IRC-1: using human, cattle, goat/sheep, pig, and horse/donkey antibodies; IRC-2: using only human and cattle antibodies) or in odor-baited entry-traps (OBET) baited with human (-H) or calf (-C) odor. The table shows the absolute frequency of identified meals (above) and the percent relative frequency, excluding negative (-ve) reactions. H = human; B = bovine (cattle); E = equine (horses and donkeys); O = ovine (sheep and goats); P = porcine (pigs) blood; - = not tested. HBI is the human blood index, the proportion of meals taken off humans (including mixed meals).

TABLE 2

Number of less abundant female mosquitoes collected in 14 nights with traps baited with human or calf odor (*N*: number of testing nights, i.e., number of replicates)*

Species	February (<i>N</i> = 8)		June (<i>N</i> = 6)		Total (<i>N</i> = 14)		OR	CI	<i>P</i>	<i>n</i>
	Calf	Human	Calf	Human	Calf	Human				
<i>Anopheles pharoensis</i>	4	7	2	2	6	9	1.5	[0.5, 2.5]	0.4	6
<i>An. rufipes</i>	3	1	0	0	3	1	0.3	[1.9, 2.6]	0.3	4
<i>An. coustani</i>	7	7	1	0	8	7	0.9	[0.1, 1.9]	0.8	6
<i>Mansonia uniformis</i>	6	8	19	28	25	36	1.4	[0.9, 2]	0.16	10
<i>Ma. africana</i>	11	26	0	0	11	26	2.4	[1.7, 3]	0.017	6
<i>Culex univittatus</i>	11	20	47	17	58	37	0.6	[0.2, 1.1]	0.3	8
<i>Cx. poicilipes</i>	5	10	3	20	8	30	3.8	[3, 4.5]	< 0.001	10
<i>Cx. gr. decens</i>	5	1	0	3	5	4	0.8	[0.5, 2.1]	0.7	4
<i>Culicomyia</i>	56	1	0	0	56	1	0.02	[2, 2]	< 0.001	5

* *P* = value of the probability that the proportion of mosquitoes collected in the human-baited odor-baited entry traps (OBET) was compatible with a random choice; *n* = number of nights in which at least one individual was trapped.

Of the remaining species collected in the OBETs (Table 2), the pattern of host preference was consistent with previously reported findings¹⁴: species showing a bias in distribution toward the calf-baited trap were *Culicomyia* mosquitoes; species with a bias toward the human baited trap were *Mansonia africana* and *Culex poicilipes*. Species that overall did not show a clear bias toward any of the two traps were *Anopheles pharoensis*, *Anopheles coustani*, *Mansonia uniformis*, *Culex gr. decens* and *Culex univittatus*. However, *Cx. univittatus* exhibited significant temporal variation in trap bias across the two surveys (OR = 5; CI = [4.1, 5.9]; *P* < 0.001).

DISCUSSION

This study investigated the blood-feeding behavior of *An. gambiae* in a rice growing area of Burkina Faso where humans are not readily accessible to endophagic vectors because of the long-standing and widespread use of bed nets by the local population to evade the serious mosquito nuisance. To study whether enduring reduced accessibility of humans in this area might have induced changes in the blood-feeding behavior of the strongly anthropophilic malaria vector *An. gambiae* s.s., we used two techniques to measure levels of anthropophily at

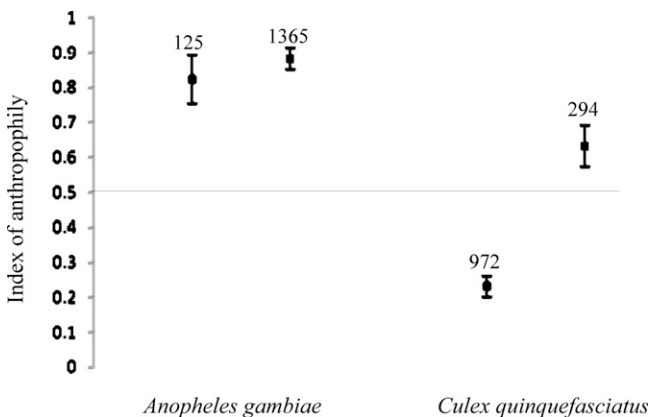


FIGURE 1. Choice test: human vs. calf odor. Mean proportion of mosquitoes ($\pm 95\%$ confidence limits) caught in a human-baited trap vs. a calf-baited trap placed side-by-side outdoors. Numbers above the bars indicate the total number of mosquitoes caught. The shape of symbols indicates the month of collection (circles in February and rhombus in June 2007).

different stages of the behavioral sequence of host foraging. First, we studied patterns of host selection by analyzing the origin of blood meals of indoor-resting mosquitoes. We then assessed the degree of inherent host preference of this field population by using two odor-baited entry traps, set side by side like a field olfactometer, baited with either human or calf odor. The findings indicate that, *An. gambiae* s.s. has an innate preference for humans but the weak accessibility of this host species, due to the use of bed net, force the mosquitoes to feed on cattle, an available, less-preferred host.

The proportion of feeds taken on humans in indoor-resting samples of *An. gambiae* was around 40%, confirming results of previous studies carried out in the same site more than 20 years ago.²⁸ These authors suggested that such a low HBI for an anthropophilic species, such as *An. gambiae*, was probably a result of the generalized use of bed nets throughout the year, coupled with the greater availability of alternative hosts such as cattle. The same authors argued that, in this area, the HBI varied in relation to the seasonal abundance of nuisance mosquitoes: the HBI was lower when mosquito densities were higher, indicating that humans were less accessible, presumably because of better protection, when nuisance was greater.²⁸ Because we measured the HBI in the dry season, when mosquito densities are lower, from samples that are bound to return a higher than average proportion of feeds off humans, it can be expected that our estimates of HBI represent figures that are nearer the ceiling HBI for *An. gambiae* in this area. Reduced anthropophagy of *Anopheles* mosquitoes in rice scheme areas has been reported by several studies, and has often been associated with a decrease in malaria transmission.⁴³⁻⁴⁵ This suggests that keeping cattle near human dwellings coupled with effective mass coverage of bed nets can divert even strongly anthropophilic species like *An. gambiae* in areas where densities of this vector are exceptionally high. However, despite the low sporozoite rates, malaria transmission still occurred in the study area. During June 2007, in VK5 and VK7 the malaria prevalence (asexual stages of *P. falciparum*) among children < 10 years of age was 29% (2% prevalence for sexual stages) from 1,334 slides examined (Lefèvre T and others, unpublished data).

Although the HBI is a crucial epidemiologic statistic assessing the degree of contact between humans and insect disease vectors, it does not evaluate inherent host preferences, because it is a measure of the final endpoint in the behavioral

sequence leading vectors to their hosts, i.e., the final host selection, which is influenced by many environmental factors such as host accessibility—among others.⁴⁶

Field choice tests with odor-baited entry traps can obviate some of the limitations caused by environmental variations when trying to determine the host preferences of natural mosquito populations. Using this technique, we found that the index of anthropophily (the proportion of mosquitoes retrieved from the human-baited trap) of *An. gambiae* in the Kou Valley was 88%, indicating a marked preference for human over calf odor. This figure is consistent with that found in a laboratory choice test using a Y-tube olfactometer (ARS, Inc.) for this same field population of *An. gambiae*.⁴⁷ In another host preference study near Ouagadougou, in a village of central Burkina Faso where bed net usage was only occasional, Costantini and others¹⁴ using the same OBET choice test protocol of this study found that 99% *An. gambiae* had been caught in the human-baited trap, indicating an extreme degree of inherent anthropophily for this population. The slight, but biologically meaningful, difference in the index of anthropophily between these neighboring populations may be related to the degree of accessibility of humans: in Burkina Faso, mass coverage with bed nets is generally limited to local, exceptional situations where mosquito nuisance is particularly high. Any selection for less anthropophilic behavior in these locales, therefore, is counteracted by migration from nearby populations where inherent anthropophily can be expressed without constraints in human accessibility.

In our study area, *An. gambiae* fed mainly on cattle despite the fact that it clearly showed a preference for human as compared with cattle odor. We can propose at least four hypotheses to explain these apparently contradictory results.

First, it can be argued that the two collection techniques used in this study sampled two different sub-populations of *An. gambiae*. One could conceive that the OBET might have been selected for the most anthropophilic (endophagic) fraction of the population, whereas the blood meal analysis of indoor-resting samples returned a mix of both the zoophilic (exophagic) and anthropophilic (endophagic) fractions in the population.

Second, it might be proposed that cues other than host odors influenced the final host selection, thereby determining alternative patterns from host preference. Indeed, OBETs obviate physical stimuli such as visual cues and short-range stimuli such as warm, moist convective currents and host movement. Under this scenario, *An. gambiae* would prefer human odor in the early stages of the host-seeking process, when it mostly responds to host odors, and then prefer cattle at a shorter range, when other cues become more important. Third, because we used only calves as a source of host odors in the choice test experiment, whereas adult cattle were more abundant in the village, one could argue that the host preference might depend on the age of the host,⁴⁸⁻⁵¹ with adult cattle preferred over humans and humans preferred over calves. Although all these hypotheses are not mutually exclusive, the fact that a consistent proportion of engorged mosquitoes retrieved from OBETs had fed previously on a host other than that used to bait the trap is not particularly consistent with these explanations.

Finally, it could be that *An. gambiae* have an inherent preference for humans, but the relative poor accessibility of this host because of the generalized use of bed nets induced most of the mosquitoes to feed on more readily available, although

less-preferred, hosts. A similar explanation was proposed by Tirados and others³⁰ for *An. arabiensis* in southern Ethiopia, where this species preferred human to calf odor in OBET choice tests, but it fed mainly off cattle because of the lower accessibility of humans sleeping indoors. Despite the fact that the reasons of reduced human accessibility are fundamentally different between these studies, both indicate the existence of plasticity in feeding behavior in both *An. gambiae* and *An. arabiensis*. Evidence of plastic feeding behavior is well documented in *An. arabiensis*,⁵²⁻⁵⁴ but it has rarely been reported in *An. gambiae*.⁵⁵ Host accessibility could have contributed to select for a plastic strategy of host foraging in *An. gambiae*. Ultimately, a successful blood meal could be more important for fitness than the origin of the blood meal itself. In other words, when host availability vary in space or time, it is better to be a “gourmand rather than a gourmet.”⁵⁶

More than 20 years of mass (non-impregnated) bed net coverage²⁸ did not select for an inherent preference for cattle in *An. gambiae*, suggesting that in this area a plastic foraging strategy could provide greater benefits than a specialist strategy for this species. How is it so? First, the benefits of feeding on humans may be greater than those gained in feeding on cattle (e.g., lower risk as a result of the defensiveness of the host, higher nutritious value of the meal). It would be interesting to compare the lifetime reproductive success of mosquitoes fed on cattle blood with that of mosquitoes fed on human blood. Second, attempts to seek the preferred host (i.e., humans) despite lower accessibility, with shift feeding to less-preferred hosts (i.e., cattle) when attempts are unsuccessful beyond a threshold, may not be very costly because cattle were kept close to human dwellings in our study area. Therefore, temporal and/or spatial variation in host availability and/or accessibility could account for the lack of selection for more zoophilic tendencies in this population of *An. gambiae*, as previously explained.

Our study also provides data on the feeding behavior of *Cx. quinquefasciatus*, the most common urban mosquito in tropical Africa and an important vector of lymphatic filariasis, a species for which there is little information on inherent host preferences. On the basis of blood meal identifications, this mosquito is generally classified as anthropophagic,^{6,57} (but see Muturi and others⁵⁸ for strong zoophagy). Using odor-baited tents, Mboera and Takken⁵⁹ showed that *Cx. quinquefasciatus* exhibited a preference for humans. Our study provides evidence that in our study area *Cx. quinquefasciatus* preferred calf to human odor in February, and inversely in June. This change in feeding preference cannot be explained by the difference in sampling effort between February and June across villages. Shifts in the selection of hosts from birds to mammals are known to occur in *Culex* mosquitoes, and are presumably driven by environmental factors, e.g., bird migration.^{8,60} Here, because the OBETs measured the odor-mediated host preference under equal accessibility of cues, this variation is less likely the cause of environmental factors. Shifts in feeding preference could result from intrinsic factors such as, for instance, a change in age structure of the mosquito population between February and June or from genetic factors such as, for instance, temporal variation in the abundance of alternative genotypes. These results clearly advocate for more research to understand these heterogeneities.

This study showed, using two different techniques measuring anthropophily at different stages in the behavioral

sequence of host foraging that, in the study area *An. gambiae* fed mainly on cattle despite a strong preference for human odor. We argue that long-term reduced accessibility of the preferred host determined the observed behavioral pattern, as a consequence of the selection for a plastic rather than specialist strategy of host foraging under the prevailing conditions available in this area (e.g., marked spatial variation in host accessibility). When measuring epidemiologic parameters modulated by vector behavior, it is important to use complementary techniques to understand the behavioral determinants that influence variations in these parameters.

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REFERENCES

- Dye C, 1992. The analysis of parasite transmission by blood-sucking insect. *Annu Rev Entomol* 37: 1–19.
- Lehane M, 2005. *The Biology of Bloodsucking in Insects*. Second edition. Cambridge: Cambridge University Press.
- Charlwood JD, Smith T, Kihonda J, Heiz B, Billingsley PF, Takken W, 1995. Density independent feeding success of malaria vectors (Diptera: Culicidae) in Tanzania. *Bull Entomol Res* 85: 29–35.
- Edman JD, Spielman A, 1988. Blood feeding by vectors: physiology, ecology, behavior, and vertebrate defense. Monath T, ed. *Epidemiology of Arthropod-Borne Viral Diseases*. Volume 1, 153–89.
- Lyimo IN, Ferguson HM, 2009. Ecological and evolutionary determinants of host species choice in mosquito vectors. *Trends Parasitol* 25: 189–196.
- Killeen GF, McKenzie FE, Foy BD, Bogh C, Beier JC, 2001. The availability of potential hosts as a determinant of feeding behaviours and malaria transmission by African populations. *Trans R Soc Trop Med Hyg* 95: 469–476.
- Bøgh C, Pedersen EM, Mukoko DA, Ouma JH, 1998. Permethrin-impregnated bed net effects on resting and feeding behavior of lymphatic filariasis vector mosquitoes in Kenya. *Med Vet Entomol* 12: 52–59.
- Mwandawiro C, Boots M, Tuna N, Suwonkerd W, Tsuda Y, Takagi M, 2000. Heterogeneity in the host preference of Japanese encephalitis vectors in Chiang Mai, northern Thailand. *Trans R Soc Trop Med Hyg* 94: 238–242.
- Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P, 2006. West Nile Virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biol* 4: e82.
- Sharp LB, le Sueur LD, 1991. Behavioural variation of *Anopheles arabiensis* (Diptera: Culicidae) population in Natal, south Africa. *Bull Entomol Res* 81: 107–110.
- Besansky N, Hill CA, Costantini C, 2004. No accounting for taste: host preference in malaria vectors. *Trends Parasitol* 20: 250–251.
- Coluzzi M, Sabatini A, della Torre A, Di Deco MA, Petrarca V, 2002. A polytene chromosome analysis of the *Anopheles gambiae* species complex. *Science* 298: 1415–1418.
- Day JE, 2005. Host-seeking strategies of mosquito disease vectors. *J Am Mosq Control Assoc* 21: 17–22.
- Costantini C, Sagnon N, della Torre A, Diallo M, Brady J, 1998. Odor-mediated host preferences of west African mosquitoes, with particular reference to malaria vectors. *Am J Trop Med Hyg* 58: 56–63.
- Wanjji S, Tanke T, Atanga SN, Ajonina C, Nicholas T, Fontenille D, 2003. *Anopheles* species of the mount Cameroon region: biting habits, feeding behaviour and entomological inoculation rates. *Trop Med Int Health* 8: 643–649.
- Cohuet A, Simard F, Wondji CS, Antonio-Nkondjio C, Awono-Ambene P, Fontenille D, 2004. High malaria transmission intensity due to *Anopheles funestus* (Diptera: Culicidae) in a village of Savannah-forest transition area in Cameroon. *J Med Entomol* 41: 901–905.
- Mwangangi JM, Mbogo CM, Nzovu JG, Githure JI, Guiyun Y, Beier JC, 2003. Blood-meal analysis for anopheline mosquitoes sampled along the Kenyan coast. *J Am Mosq Control Assoc* 19: 371–375.
- Lemasson JJ, Fontenille D, Lochouart L, Dia I, Simard F, Ba K, Diop A, Diatta M, Molez JF, 1997. Comparison of behaviour and vector efficiency of *Anopheles gambiae* and *An. arabiensis* (Diptera: Culicidae) in Barkedji, a sahelian area of Senegal. *J Med Entomol* 34: 396–403.
- Diatta M, Spiegel A, Lochouart L, Fontenille D, 1998. Similar feeding preferences of *Anopheles gambiae* and *Anopheles arabiensis* in Senegal. *Trans R Soc Trop Med Hyg* 92: 270–272.
- Bøgh C, Clarke SE, Pinder M, Sanyang F, Lindsay SW, 2001. Effect of passive zoophylaxis on malaria transmission in the Gambia. *J Med Entomol* 38: 822–828.
- Sousa CA, Pinto J, Almeida APG, Ferreira C, do Rosário VE, Charlwood JD, 2001. Dogs as a favoured host choice of *Anopheles gambiae sensu stricto* (Diptera: Culicidae) of São Tomé, west Africa. *J Med Entomol* 38: 122–125.
- Duchemin JB, Tsy JM, Rabarison P, Roux J, Coluzzi M, Costantini C, 2001. Zoophily of *Anopheles arabiensis* and *An. Gambiae* in Madagascar demonstrated by odour-baited entry traps. *Med Vet Entomol* 15: 50–57.
- Caputo B, Nwakanma D, Jawara M, Adiamoh M, Dia I, Konate L, Petrarca V, Conway DJ, della Torre A, 2008. *Anopheles gambiae* complex along the Gambia river, with particular reference to the molecular forms of *An. Gambiae* s.s. *Malar J* 7: 182.
- Lanzaro GC, Touré YT, Carnahan J, Zheng L, Dolo G, Traoré S, Petrarca V, Vernick KD, Taylor CE, 1998. Complexities in the genetic structure of *Anopheles gambiae* populations in west Africa as revealed by microsatellite DNA. *Proc Natl Acad Sci USA* 95: 14260–14265.
- Lehman T, Licht M, Elissa N, Maega BTA, Chimumbwa JM, Watsenga CS, Wondji CS, Simard F, Hawley WA, 2003. Population structure of *Anopheles gambiae* in Africa. *J Hered* 94: 133–147.
- della Torre A, Tu Z, Petrarca V, 2005. On the distribution and genetic differentiation of *Anopheles gambiae* s.s. molecular forms. *Insect Biochem Mol Biol* 35: 755–769.
- Esnault C, Boulesteix M, Duchemin JB, Koffi AA, Chandre F, Dabiré R, Robert V, Simard F, Tripet F, Donnelly MJ, Fontenille D, Biémont C, 2008. High genetic differentiation between the M and S molecular forms of *Anopheles gambiae* in Africa. *PLoS One* 3: 1–7.

28. Robert V, Petrarca V, Coluzzi M, Boudin C, Carnevale P, 1991. Etude des taux de parturité et d'infection du complexe *Anopheles gambiae* dans la rizière de la vallée du Kou, Burkina Faso. Robert V, Chippaux JF, Diomandé L, eds. *Le Paludisme en Afrique de l'Ouest: Études Entomologiques et Épidémiologiques en Zone Rizicole et en Milieu Urbain*, 17–35.
29. Costantini C, Birkett MA, Gibson G, Ziesman J, Sagnon NF, Mohamed HA, Coluzzi M, Pickett JA, 2001. Electroantennogram and behavioural responses of the malaria vector *Anopheles gambiae* to human-specific sweat components. *Med Vet Entomol* 15: 259–266.
30. Tirados I, Costantini C, Gibson G, Torr S, 2006. Blood-feeding behaviour of the malarial mosquito *Anopheles arabiensis*: implications for vector control. *Med Vet Entomol* 20: 425–437.
31. Dabire RK, Diabate A, Baldet T, Pare-Toe L, Guiguemde RT, Oudreaogo JB, Skovmand O, 2006. Personal protection of long lasting insecticide-treated nets in areas of *Anopheles gambiae* s.s. resistance to pyrethroids. *Malar J* 5: 12.
32. Baldet T, Diabaté A, Guiguemde TR, 1999. Etude de la transmission du paludisme en 1999 dans la zone rizicole de la vallée du Kou (Bama), Burkina Faso. *Cahiers Santé* 13: 55–60.
33. Diabaté A, Baldet T, Chandre F, Dabiré KR, Kengne P, Guiguemde TR, Simard F, Guillet P, Hemingway J, Hougard JM, 2003. Kdr mutation, a genetic marker to assess events of introgression between the molecular M and S forms of *Anopheles gambiae* (Diptera: Culicidae) in the tropical savannah area of west Africa. *J Med Entomol* 40: 195–198.
34. Service WM, 1993. *Mosquito Ecology: Field Sampling Methods*. Second edition. London, UK: Elsevier Applied Science Publisher Ltd, 1–954.
35. Gillies MT, De Meillon B, 1968. *Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region)*. Second edition. Johannesburg: South African Institute for Medical Research. Publication of the South African Institute for Medical Research no. 54.
36. Costantini C, Gibson G, Brady J, Merzagora L, Coluzzi M, 1993. A new odour-baited trap to collect host-seeking mosquitoes. *Parassitologia* 35: 5–9.
37. Detinova TS, 1962. Age-grouping methods in Diptera of medical importance importance with special reference to some vectors of malaria. *Monogr Ser WHO* 47: 13–191.
38. Beier JC, Perkins PV, Wirtz RA, Koros J, Diggs D, Gargan TP, Koech DK, 1988. Bloodmeal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. *J Med Entomol* 25: 9–16.
39. Gouagna LC, Mulder B, Noubissi E, Tchuinkam T, Verhave JP, Boudin C, 1998. The early sporogonic cycle of *Plasmodium falciparum* in laboratory-infected *Anopheles gambiae*: an estimation of parasite efficacy. *Trop Med Int Health* 3: 21–28.
40. Wirtz RA, Zavala F, Charoenvit Y, Campbell GH, Burkot TR, Schneider I, Esser KM, Beaudoin RL, Andre RG, 1987. Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. *Bull World Health Organ* 65: 39–45.
41. Favia G, Lanfrancott A, Spanos L, Side'n-Kiamos I, Louis C, 2001. Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of *Anopheles gambiae* s.s. *Insect Mol Biol* 10: 19–23.
42. Gillies MT, 1954. The recognition of age-groups within populations of *Anopheles gambiae* by the pre-gravid rate and the sporozoite rate. *Ann Trop Med Parasitol* 48: 58–74.
43. Bruce-Chwatt LJ, Garreett-Jones C, Weitz B, 1966. Ten year study (1955–1964) of host selection by anopheline mosquitoes. *Bull World Health Organ* 35: 405–439.
44. Robert V, Gazin P, Boudin C, Molez JF, Ouédraogo V, Carnevale P, 1985. La transmission du paludisme en zone de savane arborée et en zone rizicole des environs de Bobo-Dioulasso (Burkina Faso). *Ann Soc Belg Med Trop* 65: 201–214.
45. Muriu SM, Muturi EJ, Shililu JI, Mbogo CM, Mwangangi JM, Jacob BG, Irungu LW, Mukabana RW, Githure JI, Novak RJ, 2008. Host choice and multiple blood feeding behaviour of malaria vectors and other anophelines in Mwea rice scheme, Kenya. *Malar J* 7: 43.
46. Costantini C, Sagnon NF, della Torre A, Coluzzi M, 1999. Mosquito behavioral aspects of vector-human interactions in the *Anopheles gambiae* complex. *Parrasitologia* 41: 209–217.
47. Lefèvre T, Gouagna LC, Dabiré R, Elguero E, Fontenille D, Costantini C, Thomas F, 2009. Evolutionary lability of odour-mediated host preference by the malaria vector *Anopheles gambiae*. *Trop Med Int Health* 14: 1–9.
48. Scott TW, Lorenz LH, Edman JD, 1990. Effects of house sparrow age and arbovirus infection on attraction of mosquitoes. *J Med Entomol* 27: 856–863.
49. Muirhead-Thomson RC, 1951. The distribution of anopheline mosquito bites among different age groups: a new factor in malaria epidemiology. *BMJ* 15: 1114–1117.
50. Spencer M, 1967. Anopheline attack on mother child pairs, Fergusson Island. *Papua New Guinea Med J* 10: 75.
51. Carnevale P, Frezil JL, Bosseno MF, Le Pont F, Lancien J, 1978. The aggressiveness of *Anopheles gambiae* A in relation to the age and sex of the human subjects. *Bull World Health Organ* 56: 147–154.
52. Coluzzi M, 1984. Heterogeneities of the malaria vectorial system in tropical Africa and their significance in malaria epidemiology and control. *Bull Wld Hlth Org* 63: 107–113.
53. White GB, Magayuka SA, Boreham PFL, 1972. Comparative studies on sibling species of the *Anopheles gambiae* Giles complex (Dipt. Culicidae): bionomics and vectorial activity of species A and species B at Segera, Tanzania. *Bull Entomol Res* 62: 295–317.
54. Githeko AK, Service MW, Mbogo CM, 1994. Origin of blood meals in indoor and outdoor resting malaria vectors in western Kenya. *Acta Trop* 58: 307–316.
55. Konate L, Faye O, Gaye O, Sy N, Diop A, Diouf M, Trape JF, Molez JF, 1999. Zoophagie et hôtes alternatifs des vecteurs du paludisme au Sénégal. *Parasite* 6: 259–267.
56. Edman JD, 1989. Are mosquitoes gourmet or gourmand? *J Am Mosq Control Assoc* 4: 487–497.
57. Beier J, Odago WO, Onyango FK, Asiago CM, Koech DK, Roberts CR, 1990. Relative abundance and blood feeding behavior of nocturnally active culicine mosquitoes in western Kenya. *J Am Mosq Control Assoc* 6: 207–212.
58. Muturi EJ, Muriu S, Shililu J, Mwangangi JM, Jacob BG, Mbogo C, Githure J, Novak RJ, 2008. Blood-feeding patterns of *Culex quinquefasciatus* and other culicines and implications for disease transmission in Mwea rice scheme, Kenya. *Parasitol Res* 102: 1329–1335.
59. Mboera LEG, Takken W, 1999. Odour mediated host preference of *Culex quinquefasciatus* in Tanzania. *Entomol Exp Appl* 92: 83–88.
60. Edman JD, Taylor DJ, 1968. *Culex nigripalpus*: seasonal shift in the bird-mammal feeding ratio in a mosquito vector of human encephalitis. *Science* 161: 67–68.