

Innate Preference for Host-Odor Blends Modulates Degree of Anthropophagy of *Anopheles gambiae* sensu lato (Diptera: Culicidae)

TEUN DEKKER,¹ WILLEM TAKKEN, AND MARIETA A. H. BRAKS²

Laboratory of Entomology, Wageningen University, P.O. Box 8031, 6700 EH Wageningen, The Netherlands

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ABSTRACT In field studies, *Anopheles gambiae* Giles sensu stricto obtains most blood meals from humans, whereas *Anopheles quadriannulatus* Theobald bites predominantly bovinds. We investigated whether host odors modulate the host preference of these mosquito species. In a dual-choice olfactometer, mosquitoes were given a choice between clean air and putative host-specific odor blends. *An. gambiae* chose 'human odor' over clean air and clean air over 'cow odor.' Although *Anopheles quadriannulatus* did not choose cow odor over clean air, it chose clean air over human odor. Cheese odor, which attracted *An. gambiae*, did not result in higher trap catch of *An. quadriannulatus*. We conclude that the degree of anthropophagy of *An. gambiae* s.l. has an innate olfactory basis.

KEY WORDS *Anopheles gambiae*, *Anopheles quadriannulatus*, behavior, host choice, odors

THE HUMAN-BITING INDEX, which is the proportion of blood meals taken from a human host, has a large impact on a mosquito species' vectorial capacity for malaria. This is illustrated by the fact that the world's major malaria vectors all feed predominantly on humans (Garrett-Jones 1964). Species within the *Anopheles gambiae* Giles complex differ highly in their vectorial capacity for malaria. Because the sibling species appear equally susceptible to *Plasmodium falciparum* Welch infection (Takken et al. 1999), differences in the human biting index of the sibling species mostly determine their status as malaria vectors. *Anopheles gambiae* sensu stricto (henceforth termed *An. gambiae*) is the most important vector of malaria in Africa and is highly anthropophagic, whereas its nonvector sibling, *Anopheles quadriannulatus* Theobald, feeds for >95% on bovinds (White 1974, Sharp et al. 1984, Gillies and Coetzee 1987). Although odors are evidently used in host finding by *An. gambiae* s.l. (Takken and Knols 1999), their role in host choice has been disputed (e.g., Diatta et al. 1998). We investigated whether host choice by *An. gambiae* and *An. quadriannulatus* is modulated by an innate preference for host-odor blends from their 'preferred' hosts.

Materials and Methods

Mosquitoes. *An. gambiae* originating from Suakoko, Liberia was established in the laboratory in 1987 (courtesy of M. Coluzzi, Rome, Italy). *An. quadriannulatus* originating from Skukuza, South Africa (SKU-

QUA) was tested in the 12–18th generation after introduction in the laboratory. Mosquitoes were reared at 80% RH and a photoperiod of 12:12 (L:D) h without artificial dusk period. Larvae were reared on Baby Fish Food (Tetramin, Melle, Germany). Adults were kept in 30 by 30 by 30-cm gauze cages (*An. gambiae*) or 50 cm high by 30-cm diameter buckets with a mosquito gauze lid (*An. quadriannulatus*) and provided with a 6% glucose solution. Females of both species were blood fed on human arm only.

Bioassay and Procedures. We tested unfed, mated female mosquitoes of 5–12 d old, which had not had prior exposure to odors in a bioassay setup. Mosquitoes were transferred to release cages 15 h before testing. Each release cage contained 50 mosquitoes, 25 of each species. *An. gambiae* was dusted with a green fluorescent pigment (Day-Glo Color Co., Cleveland, OH) to be able to distinguish it from the morphologically identical *An. quadriannulatus*. A water-soaked piece of cotton was placed on top of the cage to prevent desiccation. We used a dual-choice olfactometer comprised of a transparent Lexan flight chamber of 160 long by 60 wide by 60 cm high (Braks and Takken 1999). Mosquitoes released at one end of the flight chamber had a choice of two airstreams of 20 cm/s emanating from two ports at the opposite end. Flying upwind in these ports resulted in capture in a 'no-exit' device. After 25 min, mosquitoes remaining in the flight chamber were removed. The number of each species in treatment trap (T), control trap (C) and release cage (nonresponders) were counted. The release cage and trapping devices were replaced with clean ones, and a new test could start.

Odors. We tested CO₂ at 4.5%, the concentration expired by humans and cows, and blends of 'human odor,' 'cow odor,' and cheese odor. Clean air and CO₂

¹ Current addresses: Department of Entomology, University of California, Riverside, CA 92521 (e-mail: tdekker@ucr.ucr.edu).

² Florida Medical Entomology Research Laboratory, 200 9th Street SE, Vero Beach, FL 32962.

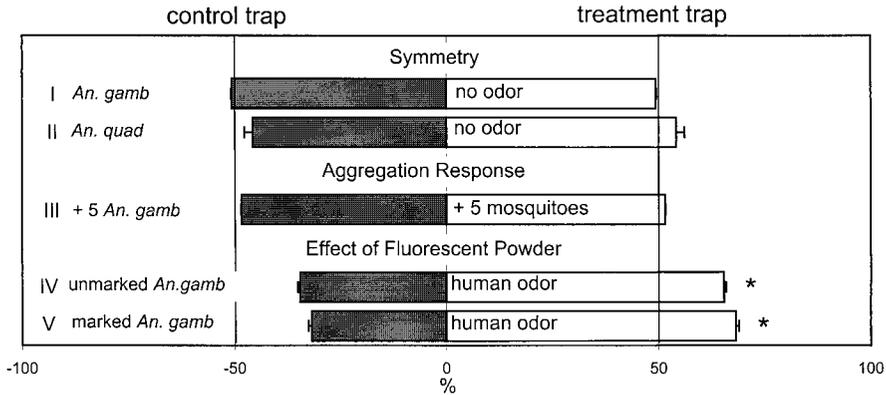


Fig. 1. Distribution of catches over control and treatment trap. Each bar represents 100%. I and II - trap catches of *An. gambiae* (*An. gamb*) and *An. quadriannulatus* (*An. quad*) with both traps unbaited. III - trap catch of *An. gambiae* with five mosquitoes in the treatment trap. IV and V - trap catch of unmarked (IV) and fluorescent-powder marked (V) *An. gambiae* with one trap baited with human odor the other unbaited. *, $P < 0.05$.

(medical air and 100% CO₂, respectively, Hoek-Loos, The Netherlands) were mixed in a 100-liter Teflon gas-sampling bag (Tedlar). We added 10 ml of distilled water to achieve a near saturation moisture level. Air from the gasbag was pumped through Teflon tubing (5 mm wide) via a flow meter (Sho-rate, Brooks Instrumental B.V., The Netherlands), into the traps at 230 ml/min. To obtain a blend of human odor a male Caucasian (28 yr old) wore a nylon sock on his foot for eight consecutive hours. The socks were stored in an airtight glass jar at room temperature for 12 h before testing. To obtain a cow-odor blend a nylon sock was wrapped around the upper hind leg of a cow. After 12 h the sock was removed and stored for 2 h in an airtight glass jar. Socks were put in the trap directly before the experiments and used singly. We used extracts of Hüttenberger Mainzer Cheese (100 g in 1 liter ethyl alcohol, courtesy Bayer AG, Leverkusen). One hundred microliters of the extract was applied on a sandblasted glass slide of 2 by 5 cm. When the alcohol was evaporated, the slide was inserted into the no-exit device and tested against clean air.

Treatments and Analysis. Baited traps were tested against clean-air controls. Control and treatment sides were alternated between experiments. Each treatment was repeated eight times. In a separate series we tested the symmetry of the setup and whether fluorescent dust on *An. gambiae* affected choice between human odor and clean air. We also tested for a possible aggregative response by scoring the choice of *An. gambiae* between two traps, one of which contained five females.

Two parameters were used to evaluate the results of the tests: the proportion of each species in the test trap calculated as $T/(T+C)$, and the total proportion caught, calculated as $(T+C)/(25 - \text{nonresponders})$. Data were arcsine transformed and differences between treatment and control were analyzed with a one-sample *t*-test (two-tailed) with $\mu = 0.5$ and $\alpha = 0.05$.

Results and Discussion

Figure 1 shows that the olfactometer is symmetric and that mosquitoes, which were already present in one of the traps at the start of the experiment, did not affect symmetry. It also shows that using fluorescent powder on *An. gambiae* did not change their response to human odor, which enabled simultaneous testing of *An. gambiae* and the morphologically identical *An. quadriannulatus*.

The total proportion *An. quadriannulatus* that entered the traps was at most 0.28, whereas irrespective of treatment this proportion was around 0.70 for *An. gambiae*. We observed *An. quadriannulatus* hovering in front of the trap opening, without entering. Other endophagic species tested, including *Aedes aegypti* (L.) (Dekker et al. 2001) and *Culex quinquefasciatus* Say (T.D. and W.T., unpublished data), quickly entered baited traps. Probably lack of innate endophagy impaired trap entry by *An. quadriannulatus* in response to host odor.

Trap catches with host odors partially reflected host choice in the field (Fig. 2). *An. gambiae* chose human odor over clean air ($P = 0.02$), whereas *An. quadriannulatus* chose clean air over human odor ($P = 0.002$). 'Cow odor,' however, caught fewer *An. gambiae* than the clean-air control ($P = 0.005$). Unexpectedly, *An. quadriannulatus* did not choose cow odor over clean air, which was possibly caused by incompleteness of the cow odor blend. CO₂, an important host stimulus for *An. quadriannulatus* (Dekker and Takken 1998), may be necessary to increase trap catch of *An. quadriannulatus* with cow odor in a similar fashion as CO₂ increases trap catches with 1-octen-3-ol for several mosquito species (Takken and Kline 1989). A recent study with cow odor and CO₂ supports this hypothesis (H. V. Pates, Wageningen University, the Netherlands, personal communication).

The reduced trap catch of *An. gambiae* and *An. quadriannulatus* with cow odor and human odor, respectively, seems to suggest that odor of 'nonpre-

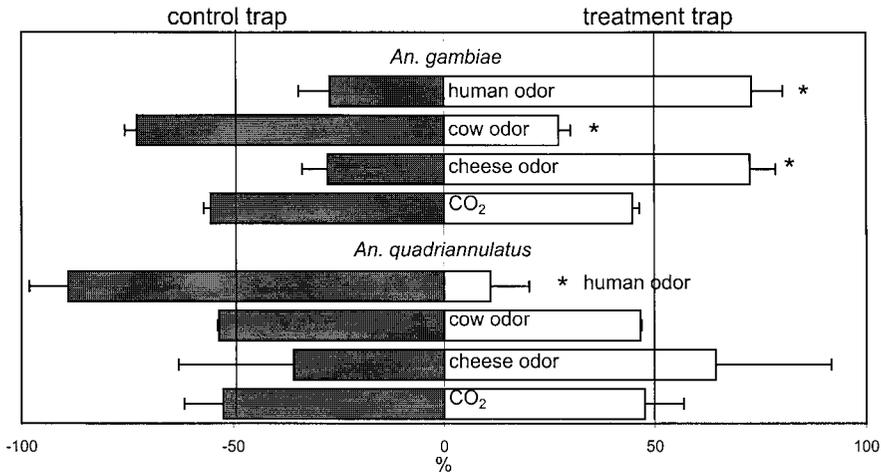


Fig. 2. Distribution of the trap catches of *An. gambiae* and *An. quadriannulatus* with odors in the treatment trap and no odor in the control trap. Each bar represents 100%. *, $P < 0.05$.

ferred' hosts are repellent to these species. Vale (1979) found that nonhost (human) odor repel tsetse, *Glossina morsitans morsitans* Westwood. Similarly, field data indicate that nonhost odor repel *An. gambiae* (Costantini et al. 1998) and *An. quadriannulatus* (Dekker and Takken 1998). In our setup, however, choice could also have been influenced by exposure of the mosquitoes to the experimenter's odor at release, or by encounters of odor filaments from previous tests still present in the flight chamber. However, although cow odor clearly contained ammonia, an attractant for *An. gambiae* at a wide range of concentrations (Braks et al. 2001), trap catches of *An. gambiae* were lower with cow odor than with clean air. It is concluded that some factor in the cow odor blend reduced the attractiveness of ammonia and possibly other attractants to *An. gambiae*.

Cheese odor, previously shown to attract *An. gambiae* (Knols and De Jong 1996), was not attractive for *An. quadriannulatus* ($P = 0.08$ versus $P = 0.01$ for *An. gambiae*; Fig. 2). This was likely caused by the high standard deviation due to the low trap-entry rates of *An. quadriannulatus*. Otherwise, the results could imply that the cheese-odor blend resembles human odor more than cow odor.

Carbon dioxide did not result in higher trap catch than the control, despite the fact that CO₂ induces trap catches of seemingly all mosquito species in the field (Mboera and Takken 1997). Field studies also have shown a differential attraction of species of the *An. gambiae* complex to CO₂ (e.g., Snow 1970, Costantini et al. 1996, Dekker and Takken 1998). This corroborates findings of Dekker et al. (2001). They used electric nets placed in front of the trap entry to demonstrate that *An. gambiae* s.s. was attracted to CO₂, but did not enter CO₂-baited traps at a higher rate than the control trap. We conclude that our setup is not well suited for testing responses of mosquitoes to CO₂ and recommend further studies on the orientation

behavior of mosquitoes to odor plumes of different constituents.

One might argue that feeding of our *An. quadriannulatus* strain on human arm may have caused a shift to more anthropophilic behavior, similar to the change in host preference of *An. atroparvus* from human to rabbit (Laarman 1958). Three factors, however, indicate that such a selection had not occurred in our *An. quadriannulatus* strain. First, blood feeding of *An. gambiae* and *An. quadriannulatus* for colony maintenance showed a marked contrast between both species. *An. gambiae* engorged within 3 min, whereas blood feeding of *An. quadriannulatus* on human arm took up to 30 min and only by frequently breathing into the cage were females induced to bite. Second, our results show that *An. quadriannulatus* and *An. gambiae* responded very differently to host odors. Finally, Pates et al. (2001) compared host-odor choice of a membrane-fed strain *An. quadriannulatus* with our human-fed strain and found no differences between the two strains.

Factors that influence the human biting index of a mosquito species include the relative availability of host species (Garrett-Jones et al. 1980, and references therein) and the innate degree of endophily (Trpis and Hauserman 1978, Coluzzi et al. 1979, Diatta et al. 1998). Our results strongly suggest that innate preferences for putative host-specific odor blends also modulate the degree of anthropophagy of *An. gambiae* s.l. Further studies on role of olfaction in host choice by mosquitoes may prove valuable for development of sampling methods that specifically lure anthropophilic species.

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