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Olfaction-based anthropophily in a mosquito-specialist predator

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Evarcha culicivora **is an unusual salticid spider because it feeds indirectly on vertebrate blood by choosing blood-carrying mosquitoes as preferred prey. Its preferred mosquitoes are *Anopheles*, the genus to which all human malaria vectors belong. Here, we show that human odour, which is known to be salient to malaria vectors, is also salient to the adults and juveniles of *E. culicivora*. Test spiders spent more time in the vicinity of a source of human odour (previously worn socks) when the alternative was unworn socks.**

Keywords: mosquito; olfaction; Salticidae; spider

1. INTRODUCTION

Anopheles is well known as the mosquito genus to which all human malaria vectors belong and, among these species, *Anopheles gambiae* is well known for being anthropophilic [1]. Part of what 'anthropophily' means for *An. gambiae* is that the specific odour of their human hosts is salient [2,3]. Here, we show that human odour is also salient to *Evarcha culicivora*, an East African jumping spider (Araneae: Salticidae) that feeds indirectly on vertebrate blood by expressing an active preference for blood-carrying mosquitoes [4]. It also singles out *Anopheles* as its preferred mosquito [5]. Salticids, including *E. culicivora*, have excellent eyesight [6], and experiments have shown that *E. culicivora* can identify its preferred prey when forced to rely on vision alone. Yet, *E. culicivora* can also identify blood-carrying mosquitoes when forced to rely on olfaction alone [7] and, in general, *E. culicivora*'s proficiency at using olfaction appears to be unusual for a salticid [8–10]. *Evarcha culicivora*'s strong reliance on chemoreception, coupled with how it is often found in or near buildings occupied by people [11], was the impetus for deciding to investigate how *E. culicivora* responds to human odour.

2. MATERIAL AND METHODS

(a) *General*

Our field site and laboratory were in western Kenya at ICIPE's Thomas Odhiambo Campus (Mbita Point). All spiders were from laboratory culture (F_2 generation; standard spider-laboratory procedures adopted; see [7]). Each spider was fed to satiation three times a week on blood-carrying female mosquitoes (*An. gambiae s.s.* from laboratory culture) and 'lake flies' (*Nilodorum brevibucca*, Chironomidae; collected as needed from the field). Hunger level was standardized by subjecting each test spider to a 7-day pre-trial fast.

Test spiders were virgin adult males (body length 4.5 mm) and virgin adult females (5.5 mm) that had matured two to three weeks beforehand, as well as two size classes of 'juveniles' (3.5 and 4.5 mm). We streamline the text by referring to test spiders as 'males', 'females', 'small juveniles' and 'large juveniles', respectively. Each juvenile had last moulted 5–7 days before being tested and did not moult again in fewer than 10 days after being tested. Each individual test spider was used in a single experimental and a single control trial.

As a source of human odour, we used previously worn white socks made from 100 per cent cotton (length 300 mm). These socks were worn by the same anonymous male donor as in an earlier study on *An. gambiae* [3]. The socks had been worn continuously for the 12 h immediately preceding use in an experimental trial. Another pair of unworn, but otherwise identical, socks were used in each control trial.

(b) *Retention tests*

An olfactometer designed for 'retention testing' [9] was used, with the underlying rationale for this testing method being an expectation that spiders would remain near a more preferred odour source longer than they would remain near a control. An alternate-day design was adopted, with each test spider being presented with the human-odour source during a trial on one day and with the control on the next or the previous day (sequence determined at random).

During testing, air was pushed successively through three chambers (stimulus chamber, holding chamber and exit chamber) connected with silicone tubing (figure 1), with airflow set at 1500 ml min^{-1} (Matheson FM-1000 airflow regulator). There was no evidence of this airflow setting affecting the test spider adversely. All trials began between 08.00 and 14.00 h (laboratory photoperiod 12 L : 12 D, lights on at 07.00 h). Between trials, the olfactometer was dismantled and cleaned with 80 per cent ethanol, followed by distilled water, and then dried.

The stimulus chamber (figure 1) was an aluminium box (inner dimensions 150 × 130 × 130 mm) with a lid, and with eight inflow holes and 16 outflow holes (i.e. 16 spiders, a random combination of males, females and juveniles, were tested at any one time). The diameter of each hole was 13 mm. There was a wire rack (110 × 100 × 90 mm) inside the stimulus chamber, with one sock draped over the top of one 110 mm side and another sock over the top of the other 110 mm side. Socks were put in the stimulus chamber 30 min before testing began.

Each inflow hole was connected to a separate air pump (i.e. eight air pumps were used), and each outflow hole was connected to a separate holding chamber, which then connected to a separate exit chamber (i.e. 16 holding chambers and 16 exit chambers were used). Each holding chamber was a glass tube (length 90 mm; inner diameter 15 mm), with a rubber stopper in one end and the other end open. The open end of the holding chamber fitted snugly in a hole in a glass exit chamber (flush with the inner wall of the exit chamber). The exit chamber was a glass cube (inner dimensions 70 × 70 × 70 mm) with a glass lid. The holding chamber for each individual test spider was connected to the same hole in the stimulus chamber during the experimental and the control trial.

Each of the stoppers used for plugging the holding chambers and holes in the stimulus chamber had a hole through which a glass tube (length 45 mm, diameter 4 mm) passed. These glass tubes were connected to silicone tubing, enabling air to move throughout the olfactometer. Nylon netting covering the stopper in the holding chamber ensured that the only way the test spider could leave the holding chamber was via the opening into the exit chamber. New netting was used for each trial.

The test spider was kept in the holding chamber for 2 min before each trial. During this time, the holding chamber was not connected to the stimulus chamber or the exit chamber, and both ends of the holding chamber were plugged with hole-less stoppers. We began testing by unplugging the two sides of the holding chamber, connecting one side to the stimulus chamber and connecting the other to the exit chamber, with a prerequisite being that the test spider was in the half of the holding chamber distal to the exit chamber.

During each trial, we recorded the test spider's departure latency (accurate to nearest minute; defined as time elapsing between trial beginning and test spider entering exit chamber). Maximum time allowed was 60 min and, by default, latency was recorded as 60 min whenever the 60 min trial period ended with the test spider still in the holding chamber.

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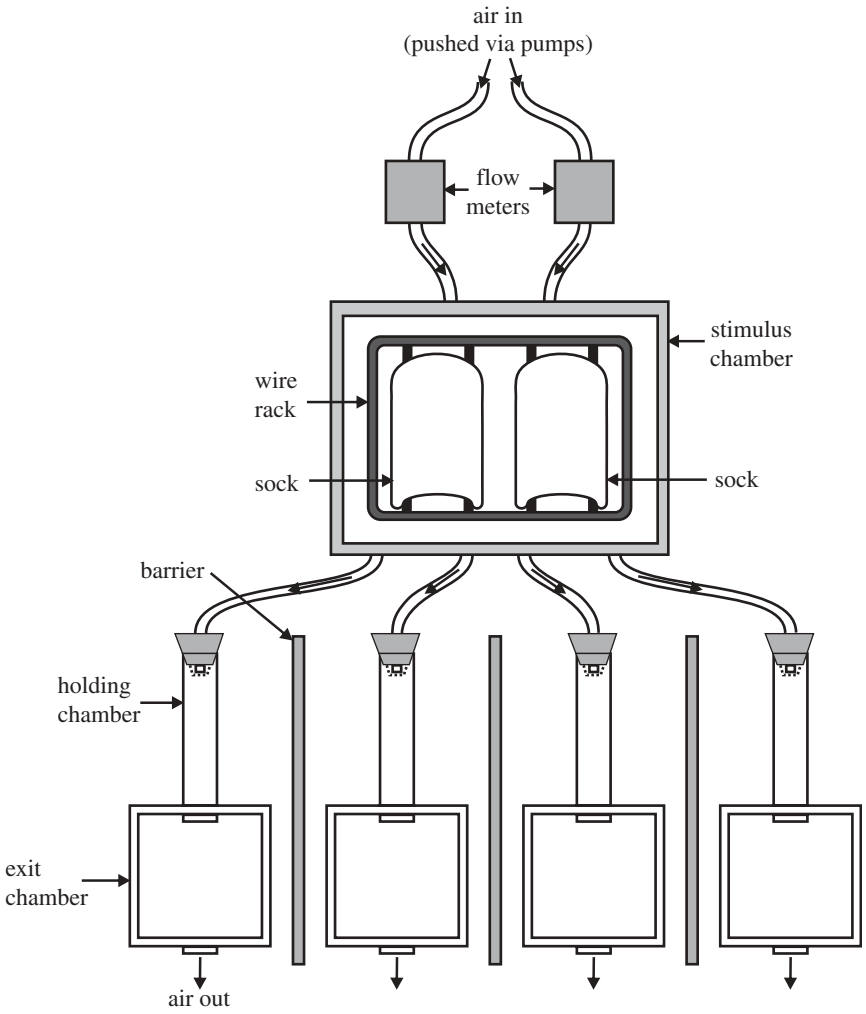

Figure 1. Olfactometer designed for retention testing (not drawn to scale). Only two of eight inputs from pumps connected to flowmeters and only four of 16 holding chambers connected to exit chambers are shown. Arrows in silicone tubes indicate direction of airflow. Two socks (previously worn for experimental; unworn for control) were draped over the wire rack inside the stimulus chamber. Start of trial: one test spider in each holding chamber at end distal to the exit chamber. During testing, the test spider can leave the holding chamber and enter the exit chamber. Barriers between holding and exit chambers ensure that test spiders in holding chambers cannot see test spiders in other holding chambers.

Table 1. Latency (min) of adults (males and females) and juveniles of *E. culicivora* to leave holding chambers when tested with or without odour from a previously worn sock. For each test spider, a 'score' was calculated by subtracting latency when tested with unworn socks from latency when tested with previously worn socks.

test spider	*n*	median score (min)	first quartile (min)	third quartile (min)	Wilcoxon result
male	27	21.0	8	32	$Z = 3.530$, $p < 0.001$
female	21	29.0	0	36	$Z = 3.340$, $p < 0.001$
small juvenile (3.5 mm)	32	15.5	0	29	$Z = 3.665$, $p < 0.001$
large juvenile (4.5 mm)	29	20.0	3	29	$Z = 3.189$, $p = 0.001$

(**c**) ***Data analysis***

As our data often did not meet the assumptions required for parametric data analyses, we used Wilcoxon tests for paired comparisons (null hypothesis: latency in experimental and control trial matched). A score was calculated for each test spider by subtracting latency when tested with unworn socks from latency when tested with previously worn socks (positive score when test spider's latency was longer in the trial with previously worn socks). Mann–Whitney *U*-tests were also used for comparing scores across groups (males, females, small juveniles and large juveniles), with the null hypothesis being that scores for one group matched scores for another group. Wilcoxon test results are in table 1 and Mann-Whitney *U*-test results are in the text. For details about statistical procedures, see Howell [12].

3. RESULTS

Male, female, small juvenile and large juvenile test spiders spent significantly more time in the holding chamber when human odour was present than when human odour was absent (i.e. scores were significantly larger than zero; table 1). Scores for males were not

significantly different from scores for females ($Z = 0.281$, $p = 0.779$), and scores for small juveniles were not significantly different from scores for large juveniles ($Z = 0.238$, $p = 0.812$). Pooled scores for adults (males plus females) were not significantly different from pooled scores for juveniles (large plus small; $Z = 1.273$, $p = 0.203$).

4. DISCUSSION

A number of human-related stimuli may alert *An. gambiae* to a host's presence, including CO_2 [13], heat and moisture [14] and a variety of more specific compounds associated with human odour [2,15,16]. However, our findings are, to our knowledge, the first experimental evidence of human odour being salient to a spider or a mosquito-specialist predator. Our methods for acquiring odour matched those previously adopted for testing *An. gambiae* [3], including having the same individual who wore the socks used when testing *An. gambiae* also wear the socks we used when testing *E. culicivora*. These findings suggest a remarkable predator–prey convergence. Our test spiders were not specifically trained to respond to human odour before the experiment and, although our experiment was not designed specifically to test this hypothesis, we propose that *E. culicivora* has an innate predisposition to respond to human odour.

For *An. gambiae* and for *E. culicivora*, detecting human odour appears to be an important step towards finding blood meals, but with an interesting difference. For *An. gambiae*, the source of the odour corresponds more directly to the source of the blood meal. For *E. culicivora*, detecting human odour may be important not so much for finding people but more for finding the mosquitoes that are carrying the blood of recently fed upon people.

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