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Human Odour Attracts *Anopheles* and *Culex* Mosquitoes only Upon Sweating

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Abstract—Mosquitoes transmit many pathogens, like malaria (by *Anopheles*), lymphatic filariasis (by *Culex*), Zika virus and Dengue virus (by *Aedes*). Female mosquitoes have robust preferences for human odours over animal odours. A recent study showed that some olfactory glomerulus within the *Aedes aegypti* was significantly activated by human odours but weakly activated by animal odours. Among the odour chemicals, decanal and undecanal played dominant roles. However, most previous studies adopted lab-reared mosquitoes who were investigated in 1-way or 2-way enclosed tunnels. Here we studied wild *Anopheles* and *Culex* mosquitoes in an open environment and found that no mosquito could be caught without either CO_2 or heat, indicating that both CO_2 and heat gate the responses to other odorant stimuli. Only wet clothes soaked with human sweat (but not human-scented dry clothes) attracted 20–50% more mosquitoes at 37°C and showed no effect at 25°C. Thus for *Anopheles* and *Culex*, human odour may only play a significant role in the host-seeking behavior only upon sweating. And neither decanal nor undecanal attracted mosquitoes in our field study, indicating inefficacy of any single volatile substance.

Keywords: human odour, *Anopheles*, *Culex*, heat, CO_2

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INTRODUCTION

Mosquitoes transmit many pathogens, like malaria (by *Anopheles*), lymphatic filariasis (by *Culex*), Zika virus and Dengue virus (by *Aedes*) (Dieme et al., 2015; Braack et al., 2018; Singh et al., 2019; Bamou et al., 2021; Killeen et al., 2021). Although many species of mosquitoes prefer to attack animals, such as birds, rather than humans, the synanthropic ("domestic") form of the *Aedes aegypti* mosquitoes prefer humans (McBride et al., 2014). They can discriminate host species by recognizing body odours and have robust preferences for human odours over animal odours (McBride et al., 2014). Recently, Zhao et al. (2022) showed that animal odours and human odours evoked different olfactory glomeruli within antennal lobes of *Aedes aegypti*. One glomerulus (the H glomerulus) was significantly activated by human odours but weakly or insignificantly activated by animal odours. These human-odour-sensitive glomeruli were selectively activated by the long-chain aldehydes undecanal and decanal, which are usually enriched in human odours and may originate from human skin lipids specifically (Zhao et al., 2022). Either Undecana, Decanal or the combo treatment (decanal + undecanal) evoked significant and prolonged activities in the H glomeruli (Zhao et al., 2022).

However, previous studies suggested that besides human odour, CO_2, heat, and the black color also play key roles in mosquito trapping (Hallem and Carlson, 2006; Turner et al., 2011; van Breugel et al., 2015; Vinauger et al., 2018; Alonso San Alberto et al., 2022; Sumner and Cardé, 2022). The study of Zhao et al. (2022) did not investigated their relationships. Indeed, female mosquitoes showed only a weak attraction to human-scented clothes in the absence of CO_2, and this response was three-fold promoted when human-scented clothes were presented accompanying with CO_2 (DeGennaro et al., 2013). One the other hand, most previous studies adopted lab-reared mosquitoes who were investigated in 1-way or 2-way enclosed tunnels (Hallem and Carlson, 2006; Turner et al., 2011; DeGennaro et al., 2013; van Breugel et al., 2015; Vinauger et al., 2018; Alonso San Alberto et al., 2022; Sumner and Cardé, 2022; Zhao et al., 2022). There are much more distractions, if the mosquitoes are in a natural open environment enclosed with complex

Fig. 1. The trapping device without (a) or with a human scented shirt (b).

attractions. And human odour scatters much more rapidly in an open space than in a wind tunnel. Furthermore, most human odour should be usually absorbed into the clothes (the skin that tends to sweat is usually covered with clothes). Human odour thus may not play an important role in mosquito trapping in a real environment. Here we studied wild *Anopheles* and *Culex* mosquitoes in an open environment and used a sticky pad/trap catches with or without CO_2, heat source, black color and human odour (a dry sports shirt worn for a whole day or a wet shirt soaked with sweat).

MATERIALS AND METHODS

Animals

The behavior of wild mosquitoes (*Culex quinquefasciatus* and *Anopheles sinensis*) was investigated. *Aedes* mosquitoes only appears after late summer and disappears after mid autumn, and thus were not counted. All experiments were carried out from June 1st to September 5th in 2022, when the mosquitoes were most active.

Field Trapping

The field experiments were carried out at the Sichuan Agricultural University—Chengdu Campus (103°49′ E 30°41′ N with an altitude of 556 m). The mosquito trap was placed in a hallway adjacent to a teaching building (about 1.2 m to the wall of the building; 1.2 m to the bushes near a drainage channel) from 16:30 to 19:30 of each rainless day (The trapping was paused during the rainy days). The illumination intensity during the trapping experiments ranged from approximate 50 to 200 μmol photons m^{-2} s^{-1} and the ambient temperature ranged from 18.5 to 25.5°C.

The Trapping Device

A metal block was heated to 25, 37 or 50°C, a petri dish of chemical solution (see below) was placed directly above the block, and then a pale-yellow 15 × 15 cm stickyplate with four 0.8 cm diameter holes was placed above. A simple CO_2 generator with $NaHCO_3$ and sodium citrate was placed near to the sticky plate (the hose outlet faced the sticky plate) with a gas flow of approximate 500 mmol h^{-1}. A black plastic bag (90 × 60 cm) was placed near to the sticky plate (Fig. 1). To test the effect of black or white color on mosquito landing, the black plastic bag was replaced by a white paper (90 × 60 cm). Four independent traps about 9.8 m apart were placed simultaneously on the same day (Zhou et al., 2018).

Chemical Treatments

Attractive odour compounds 1-octen-3-ol, decanal and undecanal were tested. All chemicals were purchased from Sigma-Aldrich Comp. (St. Louis, MO, USA) and dissolved to 10^{-1} or 10^{-2} in paraffin oil (Zhou et al., 2018). 50 mL chemical solution was supplied to the petri dish of each trap (Zhou et al., 2018).

Human Odour Treatments

Five participants (three males and two females of 23–42 years old) were enrolled. Their dry sports shirts worn for a whole day or the wet shirts soaked with sweat (immediately after a vigorous exercise) were replaced under the trapping device (Fig. 1).

Temperature Measurement

The ambient temperature was 22.4°C during the measurement. Temperatures at distances of 0–30 cm away from the sticky plate were detected by a digital

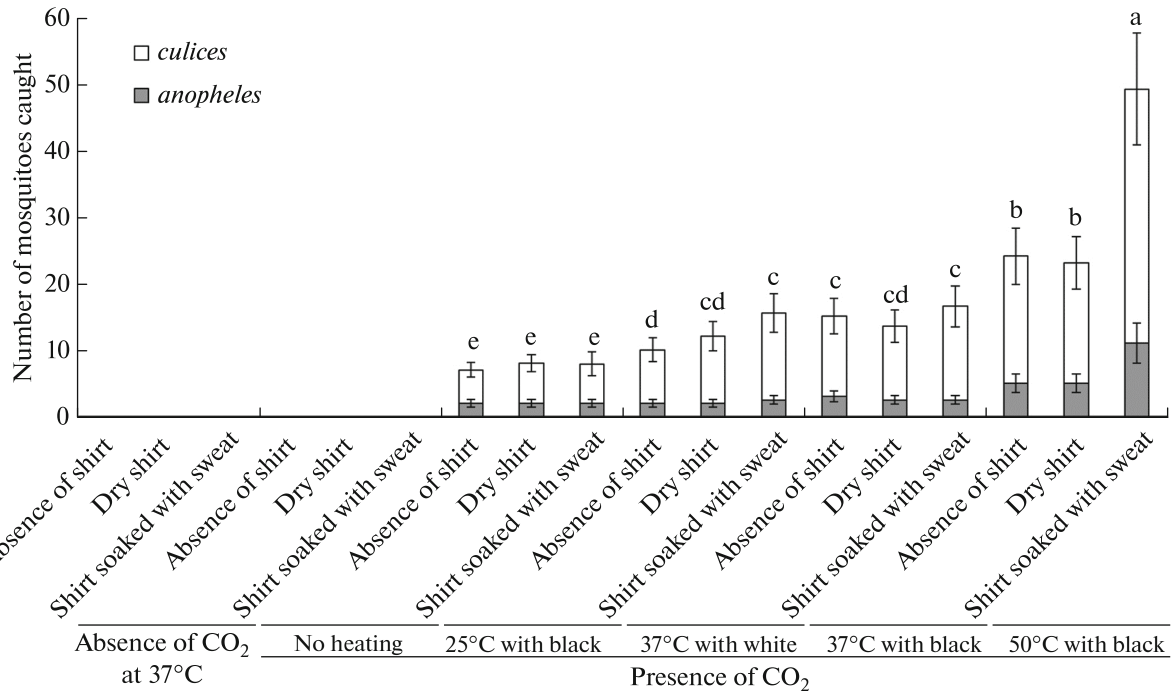

Fig. 2. Mosquitoes are attracted to human odour in the presence of both CO_2 and heat. A metal block was heated to 25, 37 or 50°C, a petri dish of water was placed directly above the blocks, and then a faint-yellow 15 × 15 cm sticky plate with four 1 cm diameter holes was placed above. A simple carbon-dioxide generator was placed on the left side of the sticky plate with a gas flow of about 500 mmol h^{-1}. A black polyethylene bag (90 × 60 cm) or a white paper (90 × 60 cm) was placed adjacent to the sticky plate. A dry sports shirt worn for a whole day or the wet shirt soaked with sweat was replaced under the trapping device. The mosquito traps were placed in a ventilated corridor from 16:30 to 19:30 of each rainless day. Error bars show standard deviations (n = 5). Significant differences are indicated by different lowercase letters.

thermometer (van Breugel et al., 2015; Zhou et al., 2018).

The Stability Test and Statistical Analysis

A stability test to the basic trap was performed (37°C with a black polyethylene bag but without any chemical/adour treatment). According to 18 independent replicates in 18 different sunny or cloudy days (from June 1st to July 10st in 2022), the basic trap should catch 1–5 *Anopheles* and 6–18 *Culex* per evening (16 : 30 to 19 : 30) in the open environment. Therefore for the following experiments, four independent traps about 9.8 m apart, including one basic trap, were placed simultaneously on the same day. If the basic trap caught more or fewer mosquitoes than the normal values, the data of that day was ruled out because of strong wind, heavy rain, or other disturbances (Zhou et al., 2018).

Significant differences among different treatments or different temperatures were determined by the Duncan's multiple range test ($p < 0.05$). ANOVA was carried out with the software package SPSS v22.0.

RESULTS AND DISCUSSION

Synergistic Effects of CO_2, Heat, Black Color and Human Odour on Mosquito Trapping

Consistent with our previous study (Zhou et al., 2018), no mosquitoes were caught at 37°C but without CO_2 (Fig. 2). Though black color attracted more mosquitoes than the white color, the temperature of the trap had a greater effect on mosquito trapping, and no mosquito was caught without a heat (Fig. 2). A dry shirt worn for a day barely affected the capture rate; however in the presence of a wet shirt soaked with sweat, more mosquitoes were caught (no increase at 25°C; 20–50% increase at 37°C; doubled at 50°C). *Anopheles* and *Culex* showed a similar variation trend that their ratios were not significantly changed by the different treatments. These results imply that the people who just sweat should not go to bushes or other places where mosquitoes are crowded to avoid mosquito bites.

Interestingly, no mosquito was caught in the presence of a human-scented shirt, but in the absence of CO_2 or heat (Fig. 2), indicating that both CO_2 and heat gate the responses to other sensory stimuli.

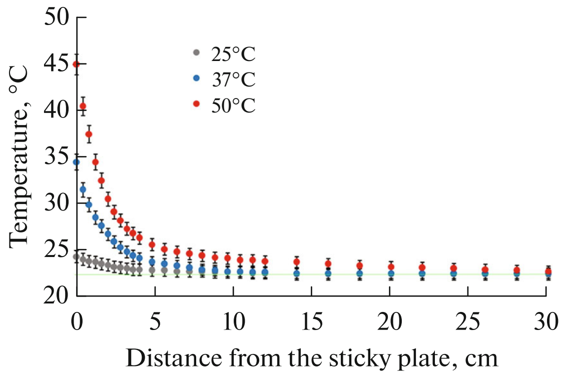

Fig. 3. Thermal signatures of the heated sticky plate. Temperatures away from the sticky plate (above the heated metal blocks) were measured at an ambient temperature of 22.4°C. The metal blocks were heated to 25, 37 or 50°C respectively. Error bars show standard deviations ($n = 3$).

Skin microbiota plays a key role in the production of human body odours. It has been hypothesized that host attractiveness and selection of *Anopheles* may be affected by the species constitution, density, and metabolic rate of the skin microbiota (Verhulst et al., 2009). Recently, Showering et al. (2022) examined how skin microbiome composition of women differs in relation to level of attractiveness to *Anopheles coluzzii* mosquitoes, and found that volatiles produced by the skin microbiota were associated with individuals attractive to mosquitoes. It is possible that the attractive effect of wet clothes, described in this study, is associated precisely with the activity of such microorganisms.

Human Odour Stimulus Works Only at Short Distances

We firstly found that sweating attracts mosquitoes, especially at a high temperature of 50°C. Nevertheless at the normal human body temperature of 36–37°C, human odour only played a minor role in the host-seeking behavior, even upon sweating. Given that the shirt was replaced under the trapping device, its volatilization rate should be constant, no matter that the metal block (inside the trapping device) was heated to 25, 37 or 50°C. The doubled capture rate at 50°C upon sweating may be attributed to the longer distance (than those at 25 and 37°C) at which the mosquitoes might detect a heat signature. A mosquito that approaches all the objects from just above the floor, nevertheless, shows a preference for the warm objects from as far away as 20 cm. At a distance of 10–15 cm, the difference between ambient temperature and the heat source falls below 0.2°C, the detective threshold for *Aedes* (Davis and Sokolove, 1975). Though a mosquito only sees with an angular resolution of 4–8°C (Lacey et al., 2014), this will still allow it to detect a human arm at 30–50 cm, 3 times the distance at which it may find a heat source (van Breugel et al., 2015). The distance for the difference between ambient temperature and the heat source <0.2°C increased to about 30 cm when the heat source was heated to 50°C (Fig. 3). Therefore, the result that more mosquitoes were caught at higher temperatures may be explained. The result also suggests that the human odour stimulus works only at short distances (maybe less than 1 m).

Inefficacy of Individual Odorant Chemicals on Mosquito Trapping

The impacts of individual volatile chemicals on mosquito-trapping were further investigated. Previous studies confirmed that 1-octen-3-ol generates a positive impact on CO_2-sensing neurons (Carey et al., 2010; Tauxe et al., 2013; McMeniman et al., 2014), and however showed a weakly superimposed induction to mosquito trapping (Fig. 4). On the contrary, both decanal, undecanal and decanal : undecanal (1 : 1) combination (either in 1 or 10%) showed negative effects on mosquito trapping (Fig. 4). *Anopheles* and *Culex* showed a similar variation trend that their ratios were not significantly changed by the different treatments. However, these results do not contradict the fact that real human odorants attract mosquitoes (Zhao et al., 2022). Zhao et al. (2022) mentioned that most people secrete odorant chemicals in sweat, but the odorant chemicals essential for host discrimination are possibly derived from sebum, the oily substance secreted at the hair follicle base. The sebum components, especially the long-chain aldehyde contents vary among individual people (Green et al., 1984), although at smaller scales than the differences between animals and humans (Zhao et al., 2022). Among the participants in their study, those with long-chain aldehyde contents close to the human mean level were more likely to attract *Aedes aegypti* than those with higher or lower levels (Zhao et al., 2022). This implies an intriguing possibility that the evolution of preference to real human odour mixture over individual odorant chemicals to affect the choice a mosquito makes when targeting a real people.

Evolutionary Cues That Mosquitoes Seek Humans

A "domestic" variant of *Aedes aegypti* has evolved to bite humans specifically and is the main global vector of many viruses (McBride et al., 2014). The domestic variant coexists with the ancestors, the "forest" variants that prefer to bite non-human animals and are found in the coast of Kenya (McBride et al., 2014). So far, most human adour preferences were studied with *Aedes*. We could not rule out a possibility that, compared with *Anopheles* and *Culex*, *Aedes* maybe more prefer to humans, which requires further investigations.

Origin of mosquito species occurred 100 million years ago (Poinar, G. and Poinar, R., 2005). However,

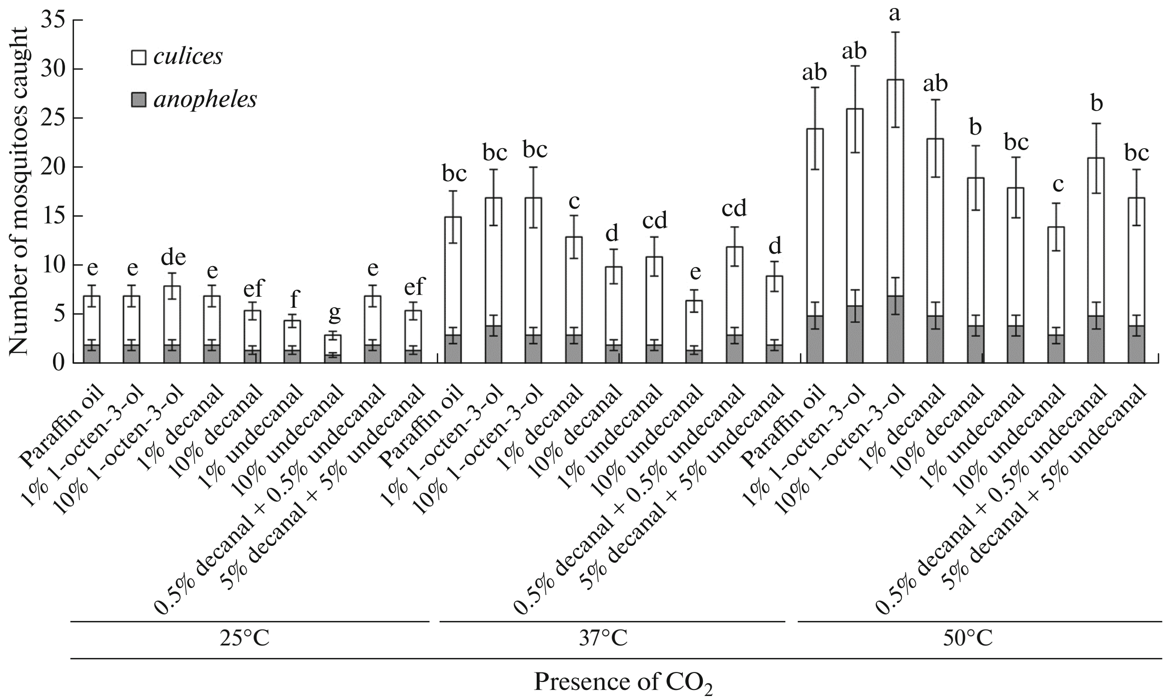

Fig. 4. Neither decanal/undecanal nor their combination attracted mosquitoes. A metal block was heated to 25, 37 or 50°C, a petri dish of paraffin oil (the control treatment), 1 or 10% decanal/undecanal, 0.5% decanal + 0.5% undecanal, or 5% decanal + 5% undecanal was placed directly above the blocks, and then the sticky plate with holes was placed above. A simple carbon-dioxide generator and a black polyethylene bag were placed adjacent to the sticky plate. The mosquito traps were placed in a ventilated corridor from 16:30 to 19:30 of each rainless day. Error bars show standard deviations ($n = 5$). Significant differences are indicated by different lowercase letters.

the earliest human remains ever discovered are merely about over 0.2 million years old (Stringer, 2016). Thus, mosquitoes may seek hosts mainly dependent on CO_2 and visual cues at a distance of 5–15 m (Bidlingmayer and Hem, 1980; Lacey and Cardé, 2011; Lacey et al., 2014), and they detect a heat source at a distance of 30–50 cm (Lacey et al., 2014; van Breugel et al., 2015), both of which may evolve 100 million years ago. And human odour only functions as an auxiliary short-range cue, which may evolve within recent 0.2 million years.

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COMPLIANCE AND ETHICAL STANDARDS

Conflicts of interest. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. The animal study was reviewed and approved by the Animal Ethics Committee of Sichuan Agricultural University, China, on May 30, 2022.

AUTHOR CONTRIBUTIONS

Xin-Yue Yang, Lin-Bei Xie, and Ting Liu contributed equally to this work.

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