How often are male mosquitoes attracted to humans?

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Abstract

Many mosquito species live close to humans where females feed on human blood. While male mosquitoes do not feed on blood, it has long been recognized that males of some species can be attracted to human hosts. To investigate how commonly male mosquitoes are attracted to humans, we review existing literature and performed human-baited field trials. We then undertook further laboratory and tent experiments to examine attraction to humans in males and females of three common *Aedes* species. We find that male attraction to humans is restricted to a handful of species including *Aedes aegypti* and *Ae. albopictus*. The presence of male host-seeking behaviour in some species may promote mating success and contribute to these species being globally invasive. Male and female *Ae. aegypti* show similar patterns in preferential attraction between different human subjects. We also demonstrate that mosquito repellents applied to human skin repel male mosquitoes. Our findings provide insights into mosquito evolution and behaviour and have implications for mosquito control programs, particularly those that involve monitoring of the male mosquito population.

1. Introduction

Many insect species exhibit distinct behavioural differences between sexes, often as adaptations in behaviour such as mating, foraging, territoriality and feeding that affect the relative contribution of the sexes to their offspring (1–3). For example, foraging in female pollinators is focused on gaining provisions for offspring production whereas male pollinators search resources that help with locating mates (4). Similarly, mate acquisition behaviour in male insects is typically associated with territoriality (5), lekking displays (6), and locating sites where females emerge (7). Females, on the other hand, more rarely actively search for mates but may focus on accepting males following courtship and nuptial gifts (8–10).

In blood-sucking insects like mosquitoes, males feed on nectar while most females require blood to reproduce and thus have behavioural adaptations for host-seeking and blood-feeding (11,12). The evolution of blood-feeding in insects is believed to have occurred through different routes, such as accidental biting of vertebrates by plant-sucking insects, which then developed the ability to digest and utilize protein-rich blood (13). Another possibility is that blood-feeding evolved through the close association between chewing insects and vertebrates, where insects became accustomed to recognizing and biting vertebrates (14). As blood became crucial for these insects, parallel evolution occurred between insects and their hosts, with the insect developing preference for specific hosts based on cues that optimize reproduction (15). Anthropophilic mosquitoes exhibit a strong drive to seek out human hosts for blood-feeding. They use cues such as CO_2, body-heat, and skin odour to locate their target. Skin odour specifically identifies humans as targets over other warm-blooded animals (16,17).

The study of host-seeking behaviour in mosquitoes has traditionally focused on females, as they are the primary vectors of disease transmission. However, as the utilization of *Wolbachia*-infected (18) or sterilized males (19) as a control strategy for reducing mosquito populations becomes increasingly prevalent, understanding the behaviour of male mosquitoes is of growing importance. This is because the efficacy of these methods hinges upon the ability of released males to locate and reproduce with wild females. Male mosquitoes have sophisticated auditory and olfactory systems (12) used to locate females (20,21), nectar and other sugar sources (22), and conspecific males (23). Despite their inability to blood feed, field observations report that males of *Aedes aegypti* (24–27) and *Ae. albopictus* (28) are attracted to humans, with males swarming around and landing on humans. Capture rates of males in both species also increase when traps are baited with CO_2 or human odour mimics (29–31). Amos *et al* (32) confirmed the attraction of *Ae. aegypti* to humans experimentally under semi-field conditions. In contrast, studies on other mosquito species frequently report no attraction of males to humans and traps that use human cues. For example, studies on *Ae. notoscriptus* have reported exceptionally low capture rate of males through CO_2-baited BG traps (33,34), indicating that there may be differences in male behaviour between mosquito species. These may be due to species differences in mating strategies and/or sensory abilities, although the inability to detect male attraction in some cases may be a consequence of study designs which fail to detect male attraction (32).

Species differences in male attraction to humans provide a basis for further investigations into the underlying mechanisms governing this behaviour and how they vary across mosquito species. Species differences are also of applied importance as releases of incompatible or sterile male mosquitoes start to be used to suppress mosquito species; public acceptance of this strategy may be problematic if males are attracted to humans. To investigate species differences, we conducted a comprehensive literature review of previous observations from field collections that employ human-baited methods, and we present results of our own human-baited field collections of both male and female mosquitoes from various regions in Australia. We also evaluated the attraction of male and female mosquitoes of three common *Aedes* species to human hosts in laboratory experiments. For species that exhibited human attraction by males, we determined whether preferences for specific human hosts are similar for males and females and tested the effectiveness of mosquito repellents on male mosquitoes.

2. Methods

2.1. Literature review

We compiled observations on male mosquito attraction to humans from studies on human-baited field collections. We looked for studies that presented catches of both males and females across any mosquito species. We searched the term "human landing catch mosquito male" as well as "human bait mosquito male" on the Google Scholar platform on September 16, 2022. We went through the first 600 results for each search term to identify references and then also searched for relevant references within the articles. Studies retained needed to identify mosquitoes to the species level and present numbers of caught mosquitoes for both sexes. A study must also have collected mosquitoes through Human Landing Catches (HLC), Human Baited Traps (HBT) or Human Baited Collections (HBC) under field conditions. If these criteria were met (Figure S1), we extracted relevant data from the text and tables within the article.

2.2. Human-baited field trials

We performed human-baited trials in Victoria (VIC), New South Wales (NSW), Australian Capital Territory (ACT), South Australia (SA) and Queensland (QLD) Australia. Participants ran trials for 0.5 to 1 hr duration at a private residence or public space at any time of the day but focused on dusk or dawn. Mosquitoes were collected when landing or hovering around exposed skin, using mechanical aspirators (Spider & Insect Vac, Select IP Australia Pty Ltd), electric rackets (Pestill USB Rechageable Mosqutio & Fly Swatter, Kogan Australia Pty Ltd), or tube collection. Keys from Dobrorwsky (1965) were used to morphologically identify the species and sex of collected mosquitoes. Mosquitoes that could not be confidently identified to species level were excluded from the study. We ran 115 individual trials from 2014-2022.

2.3. *Aedes* laboratory experiments

2.3.1. Mosquito strains and maintenance

Laboratory colonies were established from field collections from Cairns, Australia in 2019 (*Ae. aegypti*) and Brisbane, Australia in 2014 (*Ae. notoscriptus*) or 2020 (*Ae. vigilax*). *Aedes aegypti* and *Ae. notoscriptus* were reared at 26°C and a 12:12 cycle with a 1 hr dawn and dusk period. Adults were maintained in 30 x 30 x 30 cm BugDorm-1 cages and provided with 70% sucrose solution and females were blood fed using human volunteers (ethics approval from The University of Melbourne 0723847). We collected and partially dried eggs, before hatching them in 3 L of Reverse Osmosis (RO) water containing a total of 0.2 g baker's yeast. Mosquito larvae were reared on fish food (TetraMin Tropical Fish Food, Tetra, Melle, Germany) and pupae allowed to emerge into cages. *Aedes vigilax* were reared under identical conditions but adults were maintained in a BugDorm® M4590 Insect rearing cage (93 x 44 x 32 cm) and larvae were reared in 30% saltwater solution (API Aquarium salt, USA).

2.3.2. Male attraction to humans – *Aedes* species comparison

We conducted experiments on mosquito attraction to humans using three species: *Ae. aegypti*, *Ae. notoscriptus*, and *Ae. vigilax*. The experiments were conducted in a 3 x 3 m tent under constant light levels and at room temperature. Each trial involved releasing 100 males, aged between 1 and 2 weeks, that had previously been allowed to mate, into the tent. The males were given 30 minutes to acclimate before the experiment began. The experiments were filmed using GoPro Hero 10 cameras placed at either end of the tent, with white panels (84.1 x 118.9 cm) as a background. In each trial, one side was baited with a human subject, while the other side was left unbaited as a control. Subjects stood facing the camera with their bare feet and shins in the field of view, with this position remaining consistent across trials. Subjects did not wear any perfume. The side of the baited and unbaited treatment was alternated for each trial. The number of trials, human subjects, and number of days the experiments are summarized in Table S1. The same batch of males was used for multiple trials on the same day but replaced daily. Treatments were recorded for 30 mins using the time-lapse function immediately after the human subject assumed their position inside the tent. The number of mosquitoes in view of the camera was scored every 20 s, distinguishing between males that were in flight and males that landed on the human subject. For data analysis, we calculated the average number of male mosquitoes in each category over the entire trial period.

2.3.3. Mosquito preferences for different human subjects

In our experiments we found that *Ae. aegypti* males exhibit preferences towards certain human subjects (see Figure S2). While previous research has demonstrated differential attraction of female *Ae. aegypti* mosquitoes to different human hosts (17,35,36), this has not yet been quantitatively reported in males. We conducted additional experiments in which we used a consistent set of five human subjects (coded A-E) who stood in pairs in opposite positions in the tent setup described in 2.3.2. The subjects were filmed for 5 minutes on each side before the sides were swapped and the procedure was repeated. This was done for each pairwise combination of the five subjects (20 combinations in total), with a fresh batch of males being used for each day of four separate days. The footage was scored as described in 2.3.2. For data analysis, we calculated the average number of male mosquitoes in view (combining flight and landed) over the 5 minutes of each trial for each human subject.

We then tested all pairwise combinations of the same five human subjects for their attraction to female *Ae. aegypti* and *Ae. notoscriptus*. We used a two-port olfactometer (30 x 30 x 30 cm) similar to those used in previous studies by Ross et al (37) and Amos et al (32). The mosquitoes used in this experiment were 6-7 days post-emergence and had been allowed to mate prior to the experiment. We released approximately 50 females into the set-up and allowed them to acclimatise for approximately one minute. A box fan placed at the opposite end of the cage drew air through two traps into the cage. Pairs of subjects placed one hand each in front of one of the traps. After 5 minutes, we closed the entrance to the traps and counted the number of females in each trap and individuals remaining in the cage. The combinations of subjects and sides were randomized until all 20 pairwise comparisons between subjects and sides were completed. We repeated the experiment using the same 5 subjects for another four days using a fresh batch of females each day for a total of 10 replicates (5 per side). *Aedes vigilax* females were not assessed in this experiment due to relatively low rates of attraction to humans observed in a pilot trial using this olfactometer design.

Prior to data analysis, we calculated a preference index for each person to reflect the relative attraction of each subject by dividing the number of mosquitoes attracted to one human subject by the number of mosquitoes attracted to both subjects. We determined the average preference index for all replicates of each human subject. Statistical analyses were performed using the preference index averaged across replicates.

2.3.4. Effect of mosquito repellent on male mosquitoes that show attraction to humans

After we confirmed that male *Ae. aegypti* show attraction to humans in our tent experiments, we tested whether they are repelled by a commercial mosquito repellent (Aerogard tropical strength insect repellent, Reckitt Benckiser, NSW, Australia) containing 191 g/kg Diethyltoluamide and 40 g/kg N-Octyl Bicycloheptene Dicarboximide. We used the same tent setup as described in 2.3.2. The repellent was applied to the knees downwards to one of the two human subjects positioned on either site of the tent within 5 min before the trial began. The number of males in view was recorded every 20 s for 10 minutes. The person wearing the repellent and the sides of the treatment and control were randomized. We ran 20 trials over 5 days with a rotation of 9 human subjects, with the batch of 100 *Ae. aegypti* males replaced each day. The footage was scored as described in 2.3.2. For data analysis, we calculated the average number of male mosquitoes in view (combining flight and landed) over the 5 minutes of each trial.

2.4. Data analysis

All statistical analyses were conducted using R (v. 4.1.2) (38). Wilcoxon-signed-rank tests were used to assess differences in male attraction between three *Aedes* species. The influence of human subject on the number of male and female *Ae. aegypti* and female *Ae. notoscriptus* attracted to humans were assessed by first calculating a preference index for each person to reflect their relative attraction. This involved dividing the number of mosquitoes attracted to one human subject by the number of mosquitoes attracted to both subjects, which was then averaged across the replicates. We then performed an ANOVA, followed by Tukey's post-hoc tests using this averaged index to test for differences between human subjects. Using the preference indexes (without averaging), we applied Jonckheere-Terpstra tests to determine whether mosquito attraction to one subject was affected by the attractiveness of the other human subject used in a pairwise comparison (ranked apriori by their overall attractiveness). We also ran Mantel tests were run to compare the matrices of preferences obtained with different groups of mosquitoes to assess whether patterns of preferences differed between species and sexes. Finally, we used a t-test to determine whether the application of mosquito repellent significantly reduced the attraction of male *Ae. aegypti* to humans.

3. Results

3.1. Literature review

Our literature search identified 46 studies containing evidence of male mosquito attraction to humans across species using human-baited field collections. A further 355 studies did not meet all our inclusion criteria (Figure S1), including 179 studies that were excluded because they did not specify the sex of the collected mosquitoes.

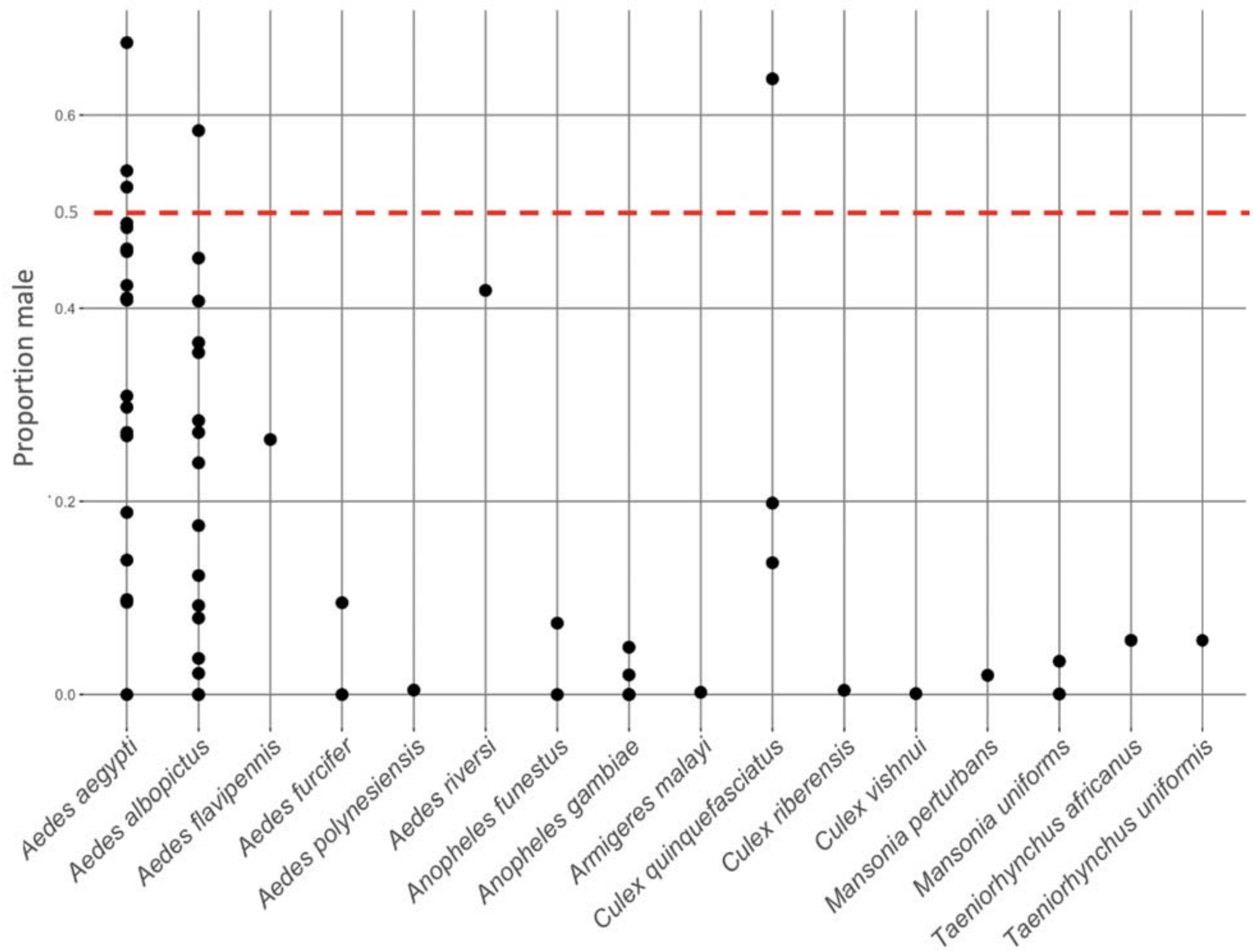

Figure 1. Proportion of males collected across mosquito species from the literature search of human-baited field collections. Dots show the proportion of males collected out of the total catch, with each dot representing a single study. The red dashed line indicates an equal ratio between male and female catches (0.5 proportion). Data are only presented for species with catches having n > 50 individuals and where males were collected. See table S4 for the complete dataset.

In the 46 studies involving 136 different mosquito species meeting the inclusion criteria, male catches were reported for 16 species. Among these, only five species (*Ae. aegypti*, *Ae. albopictus*, *Ae. flavipennis*, *Ae. riversi* and *Cx. quinquefasciatus*) reported greater than 10% male catches. The evidence for male attraction to humans by *Ae. aegypti* and *Ae. albopictus* was robust, with male catches recorded in 18 (out of 19) and 15 (out of 17) studies respectively (Figure 1, Table 1).

Table 1. Numbers of females and males collected across mosquito species from the literature search of human-baited field collections. HLC = Human landing catch, HBT = Human baited trap, HBC = Human baited collection. We only present data for species with catches n > 50 individuals. Average proportion males was calculated by determining the proportion of males out of the total catch for each study, then averaging this proportion across studies. See table S4 for the complete dataset which includes proportions for each individual study.

Spe[illegible]	[illegible] collected	[illegible] collected	[illegible] males	[illegible] method	[illegible]
Species with male catches					
Aedes aegypti	5796	11929	0.34	HLC, HBT	19
Aedes albopictus	7417	18630	0.21	HLC, HBT, HBC	17
Aedes flavipennis	14	39	0.26	HLC	1
Aedes furcifer	2	1987	0.05	HLC	2
Aedes polynesiensis	43	9225	0.005	HBC	1
Aedes riversi	90	125	0.42	HBC	1
Anopheles fuenstus	278	3703	0.025	HLC, HBT	3
Anopheles gambiae	115	15819	0.01	HLC, HBT	5
Armigeres malayi	2	869	0.002	HLC	1
Culex quinquefasciatus	711	1267	0.23	HLC, HBT	4
Culex riberensis	1	226	0.004	HBC	1
Culex vishnui	3	2983	0.001	HLC	1
Mansonia perturbans	246	12056	0.02	HLC, HBT	1
Mansonia uniforms	3	1747	0.03	HLC	2
Taeniorhychus africanus	17	185	0.06	HBT	1
Taeniorhychus uniforms	144	2418	0.06	HBT	1
Species without male catches					
Aedes africanus	0	98		HLC	1
Aedes apicoargenteus	0	33		HLC	2
Aedes poiciius	0	125		HLC	1
Aedes serratus	0	125		HLC	1
Anopheles albimanus	0	4474		HLC	1
Anopheles aquasalis	0	5175		HLC	2
Anopheles darling	0	631		HLC	1
Anopheles flavirostris	0	61		HLC	1
Anopheles implexus	0	108		HLC	1
Anopheles pharoensis	0	1803		HLC, HBT	2
Anopheles ziemanni	0	191		HLC	1
Culex annulioris	0	410		HLC	2
Culex atratus	0	465		HLC	1

Culex bastagarius	0	320	HLC	1
Culex clastrieri	0	2541	HLC	1
Culex eastor	0	1085	HLC	1
Culex pedroi	0	65	HLC	1
Culex sccettae	0	532	HBC	1
Culex taeniopus	0	335	HLC	1
Culex theobaldi	0	127	HLC	1
Culex vaxus	0	182	HLC	1
Culex vomerifer	0	2366	HLC	1
Culex ybarmis	0	248	HLC	1
Downsiomyia Ganapathi	0	199	HLC	1
Mansonia africana	0	5644	HLC, HBT	3
Mansonia fucopennata	0	2154	HLC	2
Psorophora amazonia	0	350	HLC	1
Psorophora ferox	0	59	HLC, HBT	2

3.2. Human baited field collections

We conducted human-baited field trials in Australia in both temperate and tropical regions between 2014 and 2022. Over this period, we collected 13 mosquito species as shown in Table 2.

Table 2. Summary of human-baited field collections targeting male mosquitoes in Australia. Detailed information about collections can be found in Table S5. Köppen climate-zone codes: Am = Tropical Monsoon; Cfa = Humid subtropical; Cfb = Marina west coast; Csb: Mediterranean.

Species	Males collected	Females collected	State	Köppen climate-zone
Aedes aegypti	89	140	QLD	Am
Aedes notoscriptus	0	441	NSW	Cfa
	0	1501	ACT	Cfb
	0	223	VIC	Cfb
	0	1	SA	Csb
	0	94	QLD	Am
Aedes alboannulatus	0	5	ACT	Cfb
	0	5	NSW	Cfa
	0	1	VIC	Cfb
Aedes vigilax	0	19	NSW	Cfa
	0	2	QLD	Am
Aedes vittiger	0	3	ACT	Cfb
	0	3	QLD	Am
Aedes procax	0	16	NSW	Cfa
Aedes rubrithorax	0	1	ACT	Cfb
	0	21	VIC	Cfb
Culex orbostiensis	0	2	NSW	Cfa
	0	5	QLD	Am
Culex quinquefasciatus	5	11	VIC	Cfb
Culex molestus	0	2	VIC	Cfb

	0	9	NSW	Cfa
Culex annulirostris	0	5	ACT	Cfb
	0	34	NSW	Cfa
	2	38	VIC	Cfb
	1	24	VIC	Cfb
	0	1	SA	Csb
	0	2	ACT	Cfb
Anopheles annulipes	1	1	VIC	Cfb
	0	2	ACT	Cfb
Coquillettidia lienalis	0	2	ACT	Cfb

We found evidence of male attraction of *Ae. aegypti* to humans in our field collections, with males collected in 16/22 catches. We also collected males from three other species (*Cx. quinquefasciatus, Cx. annulirostris*, and *An. annulipes*) but overall numbers were low. *Aedes notoscriptus* was by far the most prevalent mosquito captured, but no male individuals were collected despite recording thousands of females of this species.

3.3. Species-specific attraction of male *Aedes* mosquitoes to humans under laboratory conditions

In human-baited tent trials, we found no convincing evidence of attraction to humans in male *Ae. notoscriptus* or *Ae. vigilax*, with 5 or fewer observations of mosquitoes across all trials in each of the human-baited and unbaited treatments (Figure 2). Males of both species were inactive in the presence of human subjects and typically rested on the walls of the tent. However, we observed consistent attraction to humans in *Ae. aegypti* (Figure 2). The number of males observed in human-baited treatments after 30 min was significantly higher than in unbaited treatments across all tested human subjects (Wilcoxon signed-rank test: landed: $z = 1.072$, $p < 0.001$; in flight: $z = 7.755$, $p < 0.001$; total: $z = 1.056$, $p < 0.001$). Attraction was persistent, with males observed in flight around human subjects for the entire 30 minutes. Additionally, we observed an increasing number of males that had landed on the subject throughout the trials (Figure 2). While human subjects were not compared directly in this experiment, mosquito observations were much higher for some subjects, suggesting differential attraction (Figure S2).

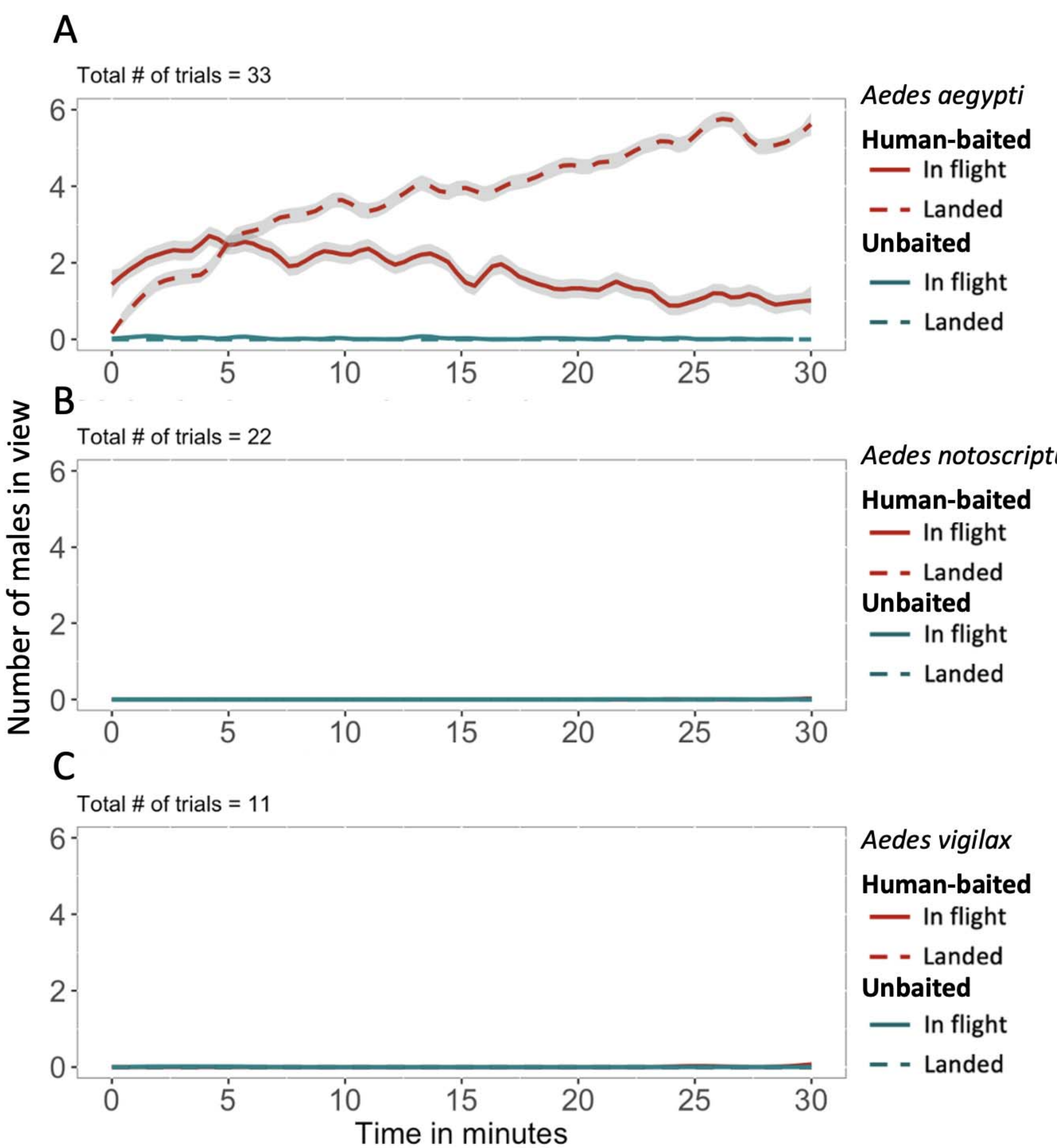

Figure 2. Comparison of male attraction to humans for three *Aedes* species in tent trials. The number of male mosquitoes of *Aedes aegypti* (A), *Aedes notoscriptus* (B) and *Aedes vigilax* (C) observed in view of a camera every 20 s. Mosquitoes that were in-flight and landed are shown

with solid and dashed lines respectively. Human-baited treatments are indicated in red, with unbaited controls shown in blue. 95% confidence intervals are shown in grey. Data were averaged across all human subjects, with data for *Ae. aegypti* males presented separately for each human volunteer in Figure S2.

3.4 **Mosquito preferences for different human subjects**

We observed significant host preferences among male and female *Ae. aegypti* and female *Ae. notoscriptus* in pairwise comparisons between five human subjects (ANOVA: *Ae. aegypti* males: $F = 5.019$, $df = 4, 15$, $p = 0.008$; *Ae. aegypti* females: $F = 5.81$, $df = 4, 15$, $p = 0.005$; *Ae notoscriptus* females: $F = 4.137$, $df = 4, 15$, $p = 0.018$). Although less pronounced, male *Ae. aegypti* showed a preference for the same human subjects as female *Ae. aegypti* (Figure 3). Tukey's posthoc tests showed that significantly more mosquitoes were attracted to certain human subjects over others (*Ae. aegypti* males: Subject A vs Subject E: $p = 0.009$; Subject B vs Subject E: $p = 0.017$; *Ae. aegypti* females: Subject A vs Subject D: $p = 0.025$; Subject A vs Subject E: $p = 0.04$; *Ae notoscriptus* females: Subject B vs Subject D: $p = 0.035$; Subject B vs. Subject E: $p = 0.03$) (Figure 3). Jonckheere-Terpstra tests comparing preference index values for the focus subject against the other subjects ranked in order of overall attractiveness showed that the attractiveness to one subject was not influenced by the other human subject present in the pairwise comparison; this lack of dependence on the other subject was found for *Ae. aegypti* females and males as well as for *Ae. notoscriptus* females (Table S3). Mantel tests on preference index matrices between *Ae. aegypti* males and females as well as *Ae. notoscriptus* females were positive but not significant (Table S2), suggesting a similar pattern of preferences for human subjects among the three groups.

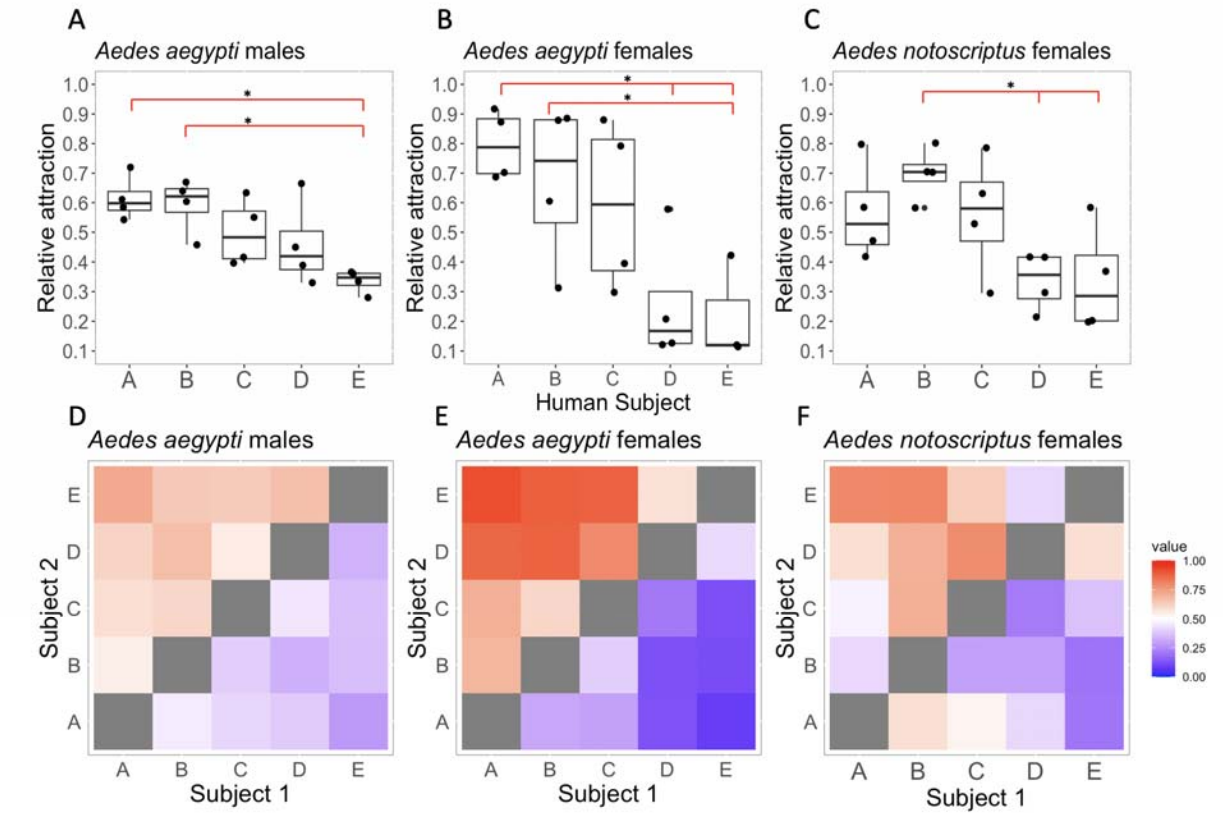

Figure 3. Relative attraction of female *Ae. notoscriptus* and male and female *Ae. aegypti* to different human subjects in pairwise comparisons. The upper row (A-C) shows boxplots of relative attraction between the five human subjects across *Ae. aegypti* males (A) and females (B) and *Ae. notoscriptus* males (C). The preference index was calculated by dividing the number of mosquitoes attracted to one human subject over the number of mosquitoes attracted to both subjects. Dots represent the mean attraction of the relevant subject to the other four subjects across 8 replicate trials. Stars above red bars indicate comparisons with significant ($P < 0.05$) pairwise differences. The lower row (D-F) presents heat maps displaying the preference index in pairwise comparisons between human subjects. Preference indices are shown on a 0-1 scale, with higher values (red) indicating stronger attraction to subject 1, lower values (blue) indicating stronger attraction to subject 2, and 0.5 (white) indicating no preferential attraction between pairs of human subjects.

3.5 Effect of mosquito repellent on male mosquitoes that show attraction to humans

Commercial mosquito repellent applied to exposed skin was effective in reducing the attraction of male *Ae. aegypti* to human subjects (Figure 4). Significantly fewer mosquitoes landed on the exposed skin of human subjects wearing repellent (t-test: $t = 6.51$, $df = 8$, $p < 0.001$). Furthermore, fewer male *Ae. aegypti* mosquitoes were observed flying in field of view of the camera towards humans wearing repellent compared to untreated subjects (t-test: $t = 8.18$, $df = 8$, $p < 0.001$).

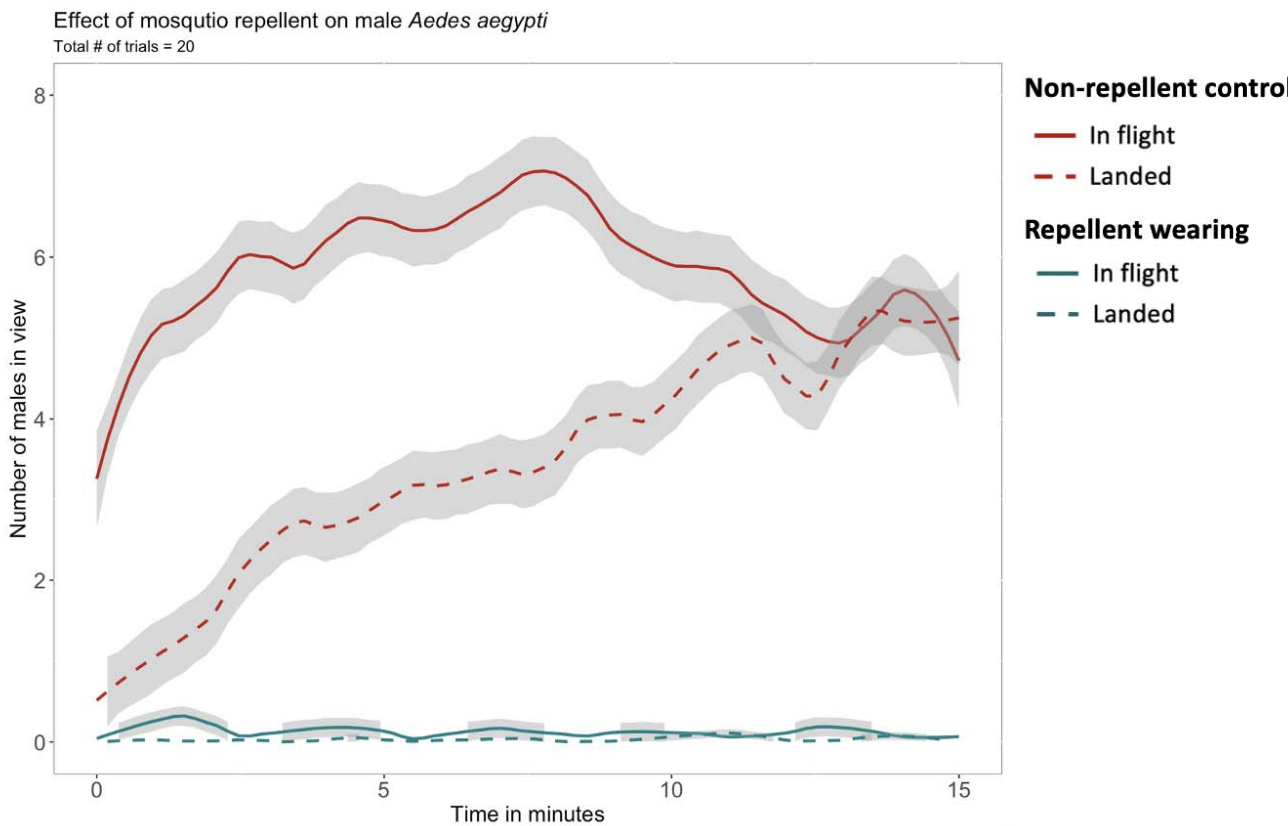

Figure 4. Effect of mosquito repellent applied to exposed skin on swarming and landing by male *Aedes aegypti*. the number of male *Ae. aegypti* in view of a camera was recorded every 20 s. Mosquitoes that were in-flight and landed are shown with solid and dashed lines respectively. Repellent-wearing treatments are indicated in red, with non-repellent controls

shown in blue. 95% confidence intervals are shown in grey. Data were pooled across all human subjects.

Discussion

We present a thorough examination of existing literature and our own field collections to determine the attraction of male mosquitoes towards human hosts. Our laboratory experiments focused on evaluating the attraction of male mosquitoes of three common *Aedes* species to a human host, as well as the preferences of males and females to different human hosts for two species, *Ae. aegypti* and *Ae. notoscriptus*. We also evaluated the effectiveness of mosquito repellents on male mosquitoes that exhibited attraction to humans. In this work, our study provides important information that can improve public support for mosquito control measures by demonstrating the effectiveness of mosquito repellent against male mosquitoes (Figure 4). Importantly, we found that repellent not only reduces mosquito landing but also decreases swarming behaviour. Even though males do not bite, they can still be regarded as a nuisance, as reported in some communities (*https://www.todayonline.com/voices/project-wolbachia-residents-are-killing-helpfulmosquitoes-which-can-be-nuisance*).

Our literature review revealed a paucity of observational data of male mosquito attraction to humans. This is largely because many field studies did not report on male catches, limiting the number of studies included in our analysis (e.g., 39–41). Additionally, human landing catches are a commonly used technique for collecting mosquitoes in the field (42). These approaches can introduce bias because, even if males are attracted to humans, they may fly around the subject without landing, resulting in a higher collection rate of females compared to males. It is worth noting that most studies screened were focused on collecting female mosquitoes or testing the attraction of female mosquitoes to different traps or hosts. Relatively few studies considered males in their design or did not report data on male catches. Thus, the findings presented in our literature review should be interpreted with caution. Despite these limitations, we found a clear distinction in male attraction to humans between species that are highly or mostly anthropophilic that are also highly invasive (e.g., *Ae. aegypti*, *Ae. albopictus*, *Cx.*

quinquefasciatus) and species with a broader range of preferred host species and lower invasiveness (Figure 1, Table 1). Our own field collections targeting males support these findings, as we only found consistent male attraction in *Ae. aegypti*, while other species presented no male attraction, or very low numbers (Table 2).

Our laboratory experiments comparing different *Aedes* species provide clear evidence that male attraction to humans is a species-specific phenomenon. Male *Ae. aegypti* persistently swarmed and landed on humans, while *Ae. notoscriptus* and *Ae. vigilax* displayed no attraction (Figure 2). Our results also indicate that male *Ae. aegypti* exhibit varying levels of attraction towards different human participants (Figure 3; Figure S2), a phenomenon well documented in female mosquitoes of different species (43,44), including *Ae. aegypti* (16,35,36,45,46). Consistent preferences for specific human subjects were found across females of *Ae. aegypti* and *Ae. notoscriptus*, indicating that these species respond to similar host-specific cues. These findings are noteworthy as *Ae. notoscriptus* feeds on a broader range of hosts (47) compared to *Ae. aegypti,* and it is important to acknowledge that blood feeding patterns may not necessarily reflect host preferences as they could also be influenced by host availability (48).

Male *Ae. aegypti* demonstrated similar individual host preferences as female *Ae. aegypti* (Figure 3). Mosquito genome studies have identified several receptor families that detect volatile chemicals (49–51). Studies investigating the *Ae. gambiae* inotropic receptor family have revealed that the expression of receptors was largely similar between the sexes, but males generally have a lower expression level of all receptors (52), suggesting that they may be responsive to the same chemical compounds as females, but at a reduced sensitivity. This view aligns with Amos et al (32) who described long-range attraction of *Ae. aegypti* males but no detectable short-range attraction, indicating that males respond to some cues but not all, or that the response to cues is less sensitive and additional circumstances need to be met (e.g., room for swarming). Recent research revealed that preferences in female *Ae. aegypti* for specific humans is influenced by their skin-derived carboxylic acid levels (36), which implies that males may also detect this odour cue since they show a similar preference to different humans in our experiments (Figure 3). While our results show a similar preference pattern between male and female *Ae. aegypti*, it is important to note that male and female attraction were measured in different ways (tent trials vs. a two-port olfactometer) which could have introduced differences in overall preference levels.

The presence of species-specific attraction to humans in male mosquitoes raises intriguing questions about the evolution of this behaviour and the underlying reasons for differences between species. Different factors could influence male attraction to humans, such as mating and feeding behaviour. Males of several species, including *Ae. albopictus* and *Ae. aegypti* have been observed aggregating near hosts in nature (25,27,28,53). Females entering these swarms were engaged by males, leading to copulation (28), which suggests that males swarm around hosts to find mates. These observations are consistent with the results of our literature review (for *Ae. aegypti* and *Ae. albopictus*) and our own field collections (*Ae. aegypti).* Both species are known to be active and bite during the day, which might reduce the distinction between host seeking and mating behaviour (53,54). Males of *Ma. uniformis* and *Ma. africana* also reportedly orient towards non-human animals in search of females to mate with (55,56). Although we focused on humans in this study, it would certainly be worthwhile broadening these investigations to other non-human hosts.

Observations in *Anopheline* and *Culicine* have shown swarming behaviour that does not require hosts (57,58). *Anopheles gambiae* forms large swarms in the absence of host animals, likely relying on visual cues (58). This species also exhibits nocturnal feeding and crepuscular mating patterns, which may factor into the lack of male attraction to hosts as feeding and mating occurs at different times. In species such as *Ae. communis* and *Ae. stimulans*, swarming is a pre-requisite for mating and has been observed in large walk-in cages, with mating pairs forming in flight (59). In previous investigations of insemination rates in mosquitoes, it was found that *Ae. polynesiensis* mates near larval habitats and exhibits higher insemination rates than *Ae. aegypti* (60). These observations may explain the lack of males collected for many of the species in our literature review and field collections.

Developing an understanding of male mating behaviour is important because successful mating with wild females is critical for mass-reared male mosquitoes released for disease control efforts (61–63). However, being able to facilitate the right circumstances for this when planning releases is a challenge without knowing the factors that influence mating behaviour. Male mosquito release programs need to consider what species-specific mating and host-seeking behaviour their target species displays. For example, releases with mosquitoes including *Ae. aegypti* should consider that the presence of humans may be important for inducing mating, while releases of *An. gambiae* should focus on other factors and areas away from humans that induce this behaviour. Finding the right species-specific swarming marker or cues will be useful for the development of efficient male trap techniques to benefit surveillance.

Mating behaviour is also important in the establishment and maintenance of laboratory colonies. For example, Watson et al (64) argued that difficulties to establish *Ae. notoscriptus* colonies in the laboratory stems from mating behaviour that cannot easily be facilitated in cages. Understanding these behaviours can help researchers to identify the best methods for maintaining colonies, such as using bigger cages with larger numbers of males to induce swarming, adding swarm markers such as plants or providing host odours if the species shows male attraction to hosts. Furthermore, understanding the mating behaviour of mosquitoes can help researchers to investigate the evolution of different mating strategies and how they influence the population dynamics of mosquitoes, as well as the underlying genetic and physiological mechanisms that drive these behaviours.

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