






RESEARCH ARTICLE

Influence of the host sex on the duration of protection by topical repellents against mosquitoes and ticks

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Abstract

Vector-borne diseases pose significant public health challenges globally, necessitating effective personal protection strategies against insect bites. Topical repellents have been widely used to mitigate these risks, yet their efficacy is affected by several factors, including the biological characteristics of users. Typically, studies assessing the efficacy of topical repellents require a balanced representation of participants, with a sample size of at least 10 individuals, evenly divided between females and males, to simulate the population. This study aims to evaluate the correlation between the sex of volunteers and the protection duration provided by DEET and Icaridin against four mosquito species (*Culex pipiens*, *Anopheles gambiae*, *Aedes albopictus*, *Culex quinquefasciatus*), and the tick species *Ixodes ricinus*. Fourteen separate trials were conducted, and no significant differences in complete protection time (CPT) were observed between male and female participants. These findings suggest that the human host sex may not influence the efficacy of topical repellents significantly. Future research should focus on a more diverse range of demographic and biological characteristics to help optimise usage guidelines, contributing to better protection against vector-borne diseases in different populations and settings.

Keywords

personal protection – VBD – complete protection time – arm in cage

1 Introduction

Mosquitoes and ticks are among the most important vectors of pathogens worldwide, being responsible for the transmission of infectious agents such as viruses, bacteria, nematodes, and protozoa in humans (Bezerra-Santos *et al.*, 2024). Consequently, protection against these arthropods is very important, as mosquitoes alone

account for an annual human death toll of more than 700,000 (WHO, 2009).

The struggle against these insects can be addressed either through preventive measures that hinder or reduce their development, or through curative methods, such as insecticides, which eliminate larvae and adults. Another strategy focuses on keeping mosquitoes away from us by using repellents, rather than kill them.

Repellents can either be distributed in the environment, (spatial and ambient repellents like coils, mats, candles, sprays, electric devices with refills, etc.), or they can be applied directly on the skin, like topical repellents. Topical repellents, which are widely used and available in several formats such as lotions, sprays, creams, gels, roll-ons, or towels, are an important category of products. Interestingly, not only humans use topical repellents, as capuchin monkeys also rub arthropods and plants on their hair to repel mosquitoes (Weldon *et al.*, 2003).

Although the mode of action of mosquito repellents is controversial (Dickens and Bohbot, 2013), it is clear that host odour plays a fundamental role on the person's attractiveness. Consequently, the many factors which can affect the odour of a person may potentially modify that person's number of bites. This variability in attractiveness is commonly observed, with different individuals exhibit varying levels of attractiveness to mosquitoes, a finding that has been confirmed by different studies (De Obaldia *et al.*, 2022; Logan *et al.*, 2008; Qiu *et al.*, 2006). Interestingly, attractiveness can also fluctuate within a single individual, as highlighted by Moreno *et al.* (2025), who found that these variations in attractiveness were comparable to those observed among distinct individuals.

Other factors, such as infection status, also influence mosquito attraction. In 2022, Zhang *et al.* showed that when mice and humans are infected with dengue and Zika viruses, their skin smell change increases mosquito-seeking behaviour. This finding aligns with earlier work by Lacroix *et al.* (2005) who found that children infected by malaria are almost twice as attractive when harbouring gametocytes than during the non-infective stage or when not infected at all. Similarly, pregnancy is also a factor affecting the odour of the skin, which makes pregnant women twice as attractive to *Anopheles (Cellia) gambiae* Giles, 1902 than non-pregnant ones, as found by Lindsay *et al.* (2000).

Beyond infection status, diet also may play a role in modulating attractiveness, although the role played is still controversial (Ellwanger *et al.*, 2021). For example, while banana consumption increases our attractiveness, eating grapes does not have any effect, as proved by Paskewitz *et al.* in 2018. In the same way, Rajan *et al.* (2005) found that the consumption of garlic did not influence attractiveness to *Aedes (Stegomyia) aegypti* (Linnaeus, 1762), and Ives and Paskewitz (2005) showed that providing additional vitamin B to the test subjects also had no effect in terms of attraction. In addition, if it has been proven that several factors affect the

attractiveness of the subjects toward mosquitoes, there are no studies which demonstrate that different attractiveness affect the duration of the protection provided by the topical repellents. One example is represented by Smith *et al.* (1963) which found no correlation between attractiveness to *Ae. aegypti* and the relative protection provided by dimethyl phthalate repellents.

The efficacy of topical repellents is usually evaluated using a protocol defined as 'arm in cage'. Although there are several guidelines available with different parameters, the basic concept is to introduce a treated arm into a cage where starved mosquitoes have been released. The arm is introduced at time intervals to establish the length of protection. The parameters of the test which may change are species of mosquito, strain, cage size, number of mosquitoes, age of mosquitoes, time and frequency of counting. All of them can potentially affect the biting pressure and the efficacy of repellents (Barnard *et al.*, 1998).

The protocol used to determine the endpoint, known as Complete Protection Time (CPT), may also influence the results related to protection duration. This parameter varies depending on the guidelines selected, such as those from the Environmental Protection Agency EPA (EPA, 2008), the World Health Organization WHO (WHO, 2009), or European Chemical Agency ECHA (ECHA Guidance on the Biocidal Products Regulation, 2018, 2021, 2022, 2023). WHO defines the CPT as the number of minutes elapsed between the time of repellent application and the first mosquito landing and/or probing. This is interpreted as: if the first bite is after 4 hours, the CPT is 4 hours (WHO, 2009). In 2010, the EPA (EPA, 2010) defined the CPT as 'the time from application of a repellent until efficacy failure', which sounds identical to WHO's definition, and therefore efficacy failure corresponds with the CPT, with the difference that EPA considers a failure event not just the first bite but the confirmed bite (a bite confirmed by a second bite within the same or the next assessment). The ECHA guideline up to version 3.0 defines CPT as the time between repellent application and the first confirmed bite, and in this sense, it is identical to the EPA guidelines. The next version, published in December 2021 (4.0) as well as the following 4.1, 5.0 and 6.0, defines the CPT as 'the time from the application of a repellent until the last effective observation, before efficacy failure by a confirmed event'. The CPT to be specified corresponds to the time interval before the confirmed event. This new definition reduces the CPT to the previous assessment, shortening the claim of 30 or 60 minutes, depending on the interval between assessments.

Different organisations (ECHA, EPA, WHO) printed their own guidelines indicating to use study participants of both sexes. Although the WHO guidelines do not specifically require the inclusion of both sexes in repellent efficacy studies, the EPA guidelines for topical repellents (EPA, 2010) and the ECHA guidelines recommend including adults of both sexes, preferably at a 50:50 ratio. However, is there a true difference in attractiveness between males and females? And does the sex of the persons influence the protection duration of a topical repellent?

The potential influence of the subjects' sex on the outcomes of efficacy evaluation tests is a significant consideration, as it could impact both participant selection and the conclusions drawn about the effectiveness of a formula. The few studies exploring the effect of human sex attractiveness to mosquitoes have so far provided inconsistent results, emphasising the need for further research.

Most of these studies found no significant differences in attractiveness between male and female subjects to *Anopheles gambiae* (Carnevale *et al.*, 1978; Qiu *et al.*, 2006) or *Anopheles (Cellia) stephensi* Liston, 1901 (Golenda *et al.*, 1999). However, a study by Gilbert *et al.* (1966) reported that female subjects were less attractive to *Ae. aegypti* than males. While attractiveness may influence mosquito-host interactions, it is important to note that differences in attractiveness between host sexes do not necessarily correlate with differences in repellent protection time. For example, Gilbert *et al.* (1996) observed that females were less attractive to mosquitoes and experienced significantly longer protection times than males. In contrast, Golenda *et al.* (1999) found that women experienced significantly shorter protection times when using DEET-based topical repellents than men, despite showing no significant differences in mosquito attractiveness. The inconsistent findings show that further research is required to better understand how the sex of the person might affect repellent efficacy, particularly considering the complex interplay of biological and environmental factors.

Given the limited information available, it remains unclear whether a balanced male-to-female ratio (i.e. 50:50) should continue to be the best approach in participant selection for repellent studies, or whether other factors are more relevant than the sex of participants themselves. To address this knowledge gap, this study aims to investigate the impact of people's sex on the protection provided by different topical repellents – including those containing Icaridin and

DEET – at varying concentrations, against four mosquito species and one tick species.

2 Materials and methods

The efficacy tests of this study were conducted at Entostudio's facilities between 2018 and 2021 on four mosquito species, including *Aedes (Stegomyia) albopictus* (Skuse, 1894), *Anopheles gambiae*, *Culex (Culex) pipiens* (Linnaeus, 1758) and *Culex (Culex) quinquefasciatus* (Say, 1823), as well as on nymphs of the tick species *Ixodes ricinus* (Linnaeus 1758).

The data analysed were not gathered to examine the effect of the subjects' gender on the CPT, but to evaluate the efficacy of commercial topical repellents. These tests were performed within the process of Research and Development of new products to collect the necessary efficacy data for their registration as biocides, according to the European Biocide Product Regulation BPR, or for national registrations. Consequently, some of the data include parameters that are not constant across all treatments, such as the species evaluated, active substance (AS), concentration of AS, or dosage. Other parameters were adjusted to meet specific guideline requirements, like the length of assessments (3 or 5 min) or to account for variations in mosquito activity (with mosquito numbers ranging from 50 to 100). Additionally, information about co-formulants is missing, as it was not provided by the customers. The data set includes two of the most common active ingredients available on the market: Icaridin and N,N-Diethyl-meta-toluamide (DEET). The 6 different formulas used are specified in Table 1. A total of 14 treatments were conducted.

The efficacy of topical repellents against mosquitoes was evaluated using the 'Arm in Cage' method, as described in version 3.0 of the ECHA guidelines. All tests involved 10 participants, with a 50:50 ratio of females to males.

Species used to test the efficacy of the topical repellents *Mosquitoes*

The test includes data from *Ae. albopictus*, *An. gambiae*, *Cx. pipiens* and *Cx. quinquefasciatus*. The procedure to test a product starts by moving a varying number of adult females depending on the species, ranging from 50 for *Ae. albopictus* to 100 for the other species. All mosquitoes are between 5 to 10 days old and exhibit trophic activity. They are moved from the breeding cages to the test cage 24 hours before each replicate. A source of water is provided in the test cage.

TABLE 1 Tested formulas and corresponding formulation description specifying target species, length of assessment, active substance (AS), active substance percentage in the formula (AS%), product format and dose applied (mg/cm²).

Formula	Target species	Length of assessment	Formula description			
			AS	AS%	Product format	Dose (mg/cm ²)
1	<i>Aedes albopictus</i> <i>Anopheles gambiae</i> <i>Culex quinquefasciatus</i> <i>Ixodes ricinus</i>	3	Icaridin	10.0%	Lotion	0.80
2	<i>Ae. albopictus</i> <i>An. gambiae</i> <i>Cx. quinquefasciatus</i>	5	Icaridin	7.5%	Aerosol	1.41
3	<i>Ae. albopictus</i>	5	DEET	20.0%	Lotion	0.83
4	<i>Ae. albopictus</i> <i>An. gambiae</i> <i>Cx. quinquefasciatus</i> <i>I. ricinus</i>	5	DEET	20.0%	Lotion	0.83
5	<i>Cx. quinquefasciatus</i>	5	Icaridin	25.0%	Aerosol	1.66
6	<i>Cx. pipiens</i>	5	Icaridin	12.0%	Aerosol	1.66

The test cage is cube shaped and made of a 1 mm mesh steel net with an aluminium or PVC frame measuring 40 cm on each side. The cage floor and front are made of plexiglass, and the front panel has a 25 cm diameter hole closed by a tulle sleeve, through which the forearm is introduced.

Before starting each replicate, the mosquito propensity to bite is assessed by inserting an untreated forearm into the cage. The replicate can begin if at least 10 probing occur in 30 s (for *Aedes* species) or in 60 s for *Culex* and *Anopheles* species (ECHA guidelines).

The term 'probing' indicates a mosquito's attempt to bite. Probing is counted when the insect has alighted or 'landed' on the forearm, when it assumes the posture to bite (the act of penetrating the human skin by the mouthparts of a mosquito without blood ingestion). At this point, the insect is shooed away by moving the arm or blowing.

Having assessed the mosquitoes are sufficiently active, the product is applied to the other forearm (treated forearm) of the person performing the test, called 'volunteer', pausing for 5 min to allow the repellent to dry. The treated forearm is then inserted into the cage for 3 or 5 min. The assessments are performed every hour. The activity of the mosquitoes is evaluated

for each assessment, introducing the control forearm into the cage for 30 s (or until the minimum number of probing is recorded). If the threshold is reached, the test can proceed, and the treated forearm is introduced.

Ticks

The study was conducted with nymphs of the tick species *I. ricinus*. Regarding the procedures to evaluate the efficacy of topical repellent against ticks, the indication from EPA and the ECHA guidelines 3.0, 4.0, 5.0 and 6.0 are quite similar. WHO guidelines on this topic are not available.

Three lines are drawn on the volunteers' forearms: a release line 2 cm above the wrist indicating the line where the ticks are released; a crossing line 3 cm above the release line, indicating the beginning of the treated area (crossing zone) and one 3 cm above the crossing line, indicating the end of the crossing zone (Figure 1).

The first assessment is performed after a few minutes from product application, and then assessments are repeated every hour, until the CPT is reached, and in any case for no longer than 8 h.

The control arm is used to pre-screen the ticks for sufficient crawling activity (control test): ticks are released on the release line using a brush, and the ones capable

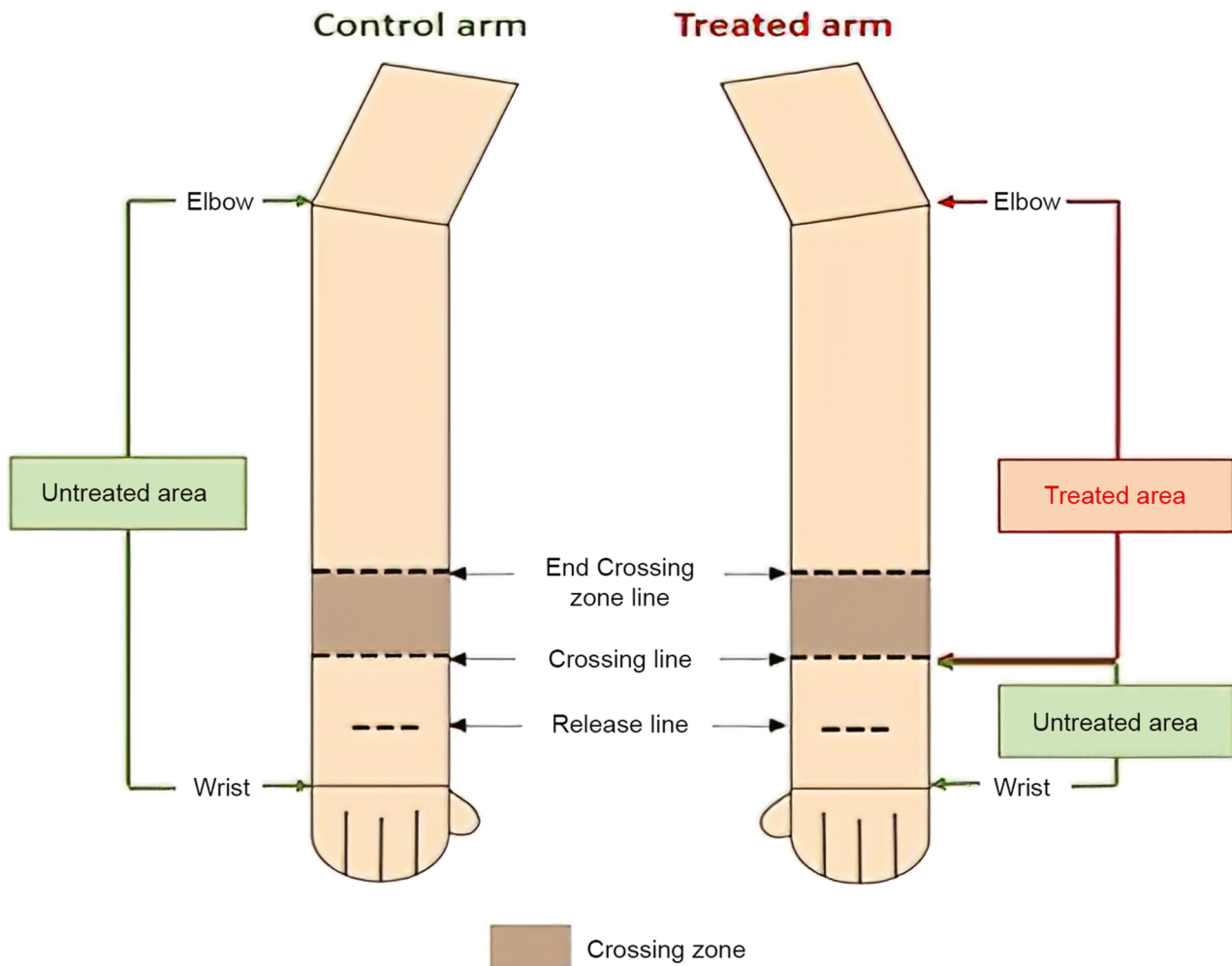


FIGURE 1 Procedure used to test topical repellents against ticks.

to cross the crossing zone are considered sufficiently locomotive and immediately used for the test on the treated forearm.

A tick is considered not repelled when, within the 3 mins' observation time, it either crawls onto the treated area and remains there for 1 min, or enters the treated area and crawls further across the 3 cm crossing zone. Five ticks/hour are used, while the number of replicates is 10 per test, as for mosquitoes. Each tick is only used once to evaluate the repellent, and then it is discharged.

Study participants

The study was undertaken in accordance with the applicable national ethical regulations on mixed-sex volunteers, on the basis of their informed written consent (written in their own language). All test subjects (volunteers) freely participated to the test. The volunteers were

fully informed of the nature and purposes of the test and of any reasonably foreseeable physical and mental health consequences. Volunteers were not identified by name in data collection forms or study reports. The volunteers were allowed to stop the procedure at any time without penalty. Volunteers were suitable to participate in the test if they did not use fragrances, repellent products and/or alcohol within 12 h before and during testing. Volunteers were not tobacco users, or at least refrained from tobacco use for 12 h prior to and during testing. Pregnant or nursing women and people under 18 or over 55 years were not used as volunteers for the study.

Product formulations

The test products came in two formats, lotion and aerosol. Lotion formulations were applied on the volunteer's arm adding small quantities and weighing the product container until the desired reduction was recorded,

ranging from 0.5 to of 1 g per 600 cm². Within the same formula, the dosage was kept consistent, although it could vary between different formulas. The product was then smeared on the forearm by an assistant.

To apply the exact amount of aerosol, the product was first discharged into a cup and then collected using a pipette. It was then applied on the volunteer's arm and smeared by an assistant.

Endpoints

Complete protection failure makes the replicate stop and defines the CPT. As we already described, there are several methods to calculate the CPT. For the purpose of this test, the following procedures were indicated in version 3.0 of the ECHA guideline in use at the time of the study.

Statistical analysis

This study assessed differences in CPT between males and females across different formulas and arthropod species. CPT median values and corresponding 95% confidence intervals (CI) were estimated using the Kaplan-Meier statistical test following the WHO methodology for data with right-censorship (WHO, 2009). This indicates that median CPT is estimated as the

shortest time at which $\geq 50\%$ of the participants failed complete protection.

To compare CPTs between subjects of the two sexes, generalised linear models (GLMs) were employed, utilising Poisson error distribution with a log link function, or quasi-Poisson when the dataset was over dispersed. In these models, CPT was designated as the response variable, while the sex was considered a fixed factor. GLMs were conducted for each of the 14 treatments (i.e. different formulas tested against each arthropod species) separately. Analyses were performed under R version 4.1.3 and an alpha level of 0.05 was employed (R Core Team, 2022).

3 Results

No significant differences in CPT between the two sexes were detected in any of the treatments. Median CPT values and 95% CI are shown in Table 2. These values ranged from 0 to 8 both in females and males depending on the applied formula and target species.

For formula 1 against *An. gambiae*, the median CPT was 0.00±NA hours for both female and male groups, as over 50% of participants in each group had lost protection within the first hour of testing.

TABLE 2 Median CPT and 95% CI values from each sex in any formula and species tested. The 'CPT comparison' column shows the results of the GLM analysis between females and males.

Target specie	Formula ¹	Median ± CI95% (hours)		CPT comparison			
		Females	Males	estimate	SE	z-ratio	P-value
<i>Aedes albopictus</i>	1	4.00±1.56	2.00±0.88	-0.60	0.62	-0.96 ²	0.36
	2	8.00±NA	8.00±NA	0.00	0.22	0.00	1.00
	3	8.00±0.24	7.00±0.88	-0.11	0.24	-0.48	0.63
	4	8.00±0.41	7.00±0.29	-0.05	0.23	-0.23	0.82
<i>Anopheles gambiae</i>	1	0.00±NA	0.00±NA	0.29	1.00	0.29 ²	0.78
	2	8.00±0.41	6.00±0.58	-0.21	0.25	-0.85	0.39
	4	5.00±0.88	5.00±1.07	0.08	0.28	0.28	0.78
<i>Culex quinquefasciatus</i>	1	1.00±1.34	2.00±0.88	0.22	0.65	0.34 ²	0.74
	2	8.00±NA	8.00±NA	0.00	0.22	0.00	1.00
	5	8.00±NA	8.00±0.41	-0.05	0.23	-0.23	0.82
	4	8.00±0.35	7.00±0.17	-0.09	0.24	-0.36	0.72
<i>Culex pipiens</i>	6	6.00±4.29	5.00±1.07	-0.07	0.26	-0.26	0.79
<i>Ixodes ricinus</i>	1	8.00±NA	8.00±0.35	-0.11	0.23	-0.47	0.64
	4	6.00±1.34	6.00±1.07	0.03	0.26	0.13	0.90

¹ Formula description is specified in Table 1.

² Comparisons were conducted using a GLM with a quasi-Poisson error distribution, with the resulting values corresponding to t-ratios.

Treatments that achieved CPT $8.00 \pm NA$ provided protection that exceeded the maximum observation time of 8 h, leading to right-censorship in our data. CPT $8.00 \pm NA$ values were observed in the same treatments for both females and males (i.e. Formula 2 against *Ae. albopictus* and *Cx. quinquefasciatus*), while others were observed only in females, with corresponding median CPT values of 8.00 in males (i.e. Formula 5 against *Cx. quinquefasciatus* and formula 1 against *I. ricinus*).

4 Discussion

This study explored the efficacy of topical mosquito repellents, focusing in particular on how the sex of the study participants might influence the protection provided by topical repellents. Our findings indicate that the protection time was not significantly affected by the sex of the participants across the 14 different treatments conducted. These treatments involved two AS, DEET and Icaridin, at different concentrations and doses, tested against four species of mosquitoes, *Ae. albopictus*, *An. gambiae*, *Cx. pipiens* and *Cx. quinquefasciatus* and one tick species, *I. ricinus*.

The relationship between sex of the human subject and mosquito attraction remains inconsistent in the literature. Gilbert *et al.* (1966) conducted a study with 100 subjects, 50 for each sex, to assess attractiveness to *Ae. aegypti*. Using an olfactometer, they found that women were significantly less attractive than men, with the difference being highly significant. Additionally, they observed that the average protection time provided by DEET was significantly longer for women than men. The study also explored the correlation between attractiveness and protection time, finding that attractiveness influenced repellent efficacy in men. However, this correlation was not observed when the data were analysed for women. In contrast, Qiu *et al.* (2006) and Carnevale *et al.* (1978) found no significant sex effects in the attractiveness to *An. gambiae*, suggesting that it did not play a crucial role in determining the relative attractiveness of male and female subjects to these mosquitoes. Golenda *et al.* (1999), in line with these results, tested the efficacy of DEET on 60 subjects of each sex against *An. stephensi* and found no difference in attractiveness between males and females. However, they did report that women experienced significantly less protection over time than men. These findings contrast sharply with those of Gilbert *et al.* (1966). The differences may be attributed to the use of different mosquito species in the experiments. While Gilbert *et al.* (1966) studied *Ae. aegypti*, a diurnal species

with relatively consistent biting patterns throughout the day (Egid *et al.*, 2021), Golenda *et al.* (1999) used *Anopheles*, which typically do not feed during midday hours (Korgaonkar *et al.*, 2012). Other variables, such as the number of mosquitoes used in the tests, may also have influenced the outcomes. Gilbert *et al.* (1966) used between 115 and 1,500 mosquitoes for their attractiveness and protection tests, respectively, while Golenda *et al.* (1999) employed only 15 mosquitoes per cage. Our study aimed to compare results using a similar methodology, with slight variations in mosquito and tick numbers, in accordance with established guidelines. Although we did not evaluate differences in terms of attractiveness, our results showed no significant differences between sexes in terms of protection time and were consistent across all treatments.

To further complicate the relationship between the sex of individuals and mosquito attraction, recent research by Das *et al.* (2017) investigating blood-feeding behaviour in *An. (Cellia) funestus* Giles, 1900 and *An. gambiae* found no bias in human host sex preference in field conditions. These findings further reinforce the idea that human sex may not impact significantly mosquito attraction in natural settings.

In addition to sex-based differences in mosquito attraction, there is growing interest in how other factors, such as skin microbiome, influence human odour and mosquito attraction. Several studies have also explored the effects of physiological and behavioural differences between males and females. It is well-known that certain areas of the human body have a unique odour, produced partially by microbial action. Overall body odour is mainly the result of chemicals emanated by the skin, with at least 400 compounds detected by coupled gas chromatography / mass spectrometry (GC/MS), of which only 135 have been properly identified (Sastry *et al.*, 1980). Li *et al.* (2019) demonstrated that while males and females have comparable axillary bacterial loads, the composition of corynebacteria differs between the sex. This difference may result in distinct odour profiles, which could influence mosquito attraction.

Other studies have demonstrated that changes in the volatile compounds on the skin can influence a host's attractiveness to mosquitoes. These studies suggest that higher levels of certain compounds may contribute to increased attractiveness, while others may reduce it. For example, various volatile organic compounds, (VOCs) such as 3-methyl-1-butanol, have been identified as potential attractants for mosquitoes, while compounds like 6-methyl-5-hepten-2-one, octanal, nonanal, decanal, and geranylacetone have been linked to repellence

(Logan *et al.*, 2008). Human feet are another notable source of strong body odour, often described as 'cheesy'. Male and female differences in footwear also play a significant role in the feet's microenvironment, and have been linked to repellence (Marples, 1982), as male footwear tends to be more enclosed than the typically more open footwear worn by females.

Hormonal factors have also been implicated in altering odour profiles or skin temperature, further complicating the understanding of sex-based variations in attractiveness (Lindsay *et al.*, 2000; Rossler, 1963). As Ellwanger *et al.* (2021) emphasise, individual susceptibility to mosquito bites arises from a complex interplay of various human-related factors, environmental conditions, and the inherent characteristics of mosquitoes themselves.

As regards ticks, although they play a crucial role in disease transmission, the factors influencing variation in human attractiveness to ticks have been largely overlooked (Josek *et al.*, 2019). Some studies have shown that VOCs play a crucial role in tick attraction and repellence (Bezerra-Santos *et al.*, 2024). For example, the attraction of some volatiles (such as methyl salicylate, benzoic acid, ammonium hydroxide, salicylic acid) have been tested in nymphs of *Amblyomma sculptum* Berlese, 1888 ticks (Ferreira *et al.*, 2020). The same tick species has also demonstrated to be repelled by benzaldehyde and isobutyric acid, which, in turn, are compounds previously detected in tick-resistant mammalian hosts (Borges *et al.*, 2015; Ferreira *et al.*, 2020). Moreover, in another study performed on donkeys, the (E)-2-octenal was shown to act as a repellent for this tick species (Ferreira *et al.*, 2019).

Regarding sex-based differences, previous research on *Amblyomma americanum* (Linnaeus, 1758) demonstrated these differences in host attraction under controlled conditions (Josek *et al.*, 2019). Specifically, a binary choice bioassay revealed that female human breath attracted a higher proportion of ticks than male breath (Josek *et al.*, 2019). Ixodid ticks are capable of detecting chemicals such as carbon dioxide and ammonia (Carr *et al.*, 2013), two major components of human breath, as well as thousands of other volatile organic compounds emitted in small quantities (Ogimoto *et al.*, 2015; Popov, 2011). Since the chemical composition of male and female breath differs significantly (Doty *et al.*, 1982), these differences may partially explain the observed variation in host attraction. Notably, this finding pertains to host attraction rather than protection. In our study with *I. ricinus*, we did not investigate attraction or repellence. However, in line with results obtained

from studies on mosquitoes, we found no differences in protection provided by repellent formulations between men and women participants. This suggests further research on sex-specific cues in tick-host interaction is needed.

Despite these complexities, our study found no significant differences in protection time between males and females, supporting the idea that this factor may play a limited role in influencing the protection time provided by topical repellents. These findings suggest that future research on repellent efficacy could benefit from reconsidering the necessity of strict sex representation in experimental designs. There may be a potential shift toward a more streamlined approach to testing, where the inclusion of an equal 50:50 male-to-female ratio is not critical for assessing repellent effectiveness. However, until further research clarifies the need for sex-specific analyses, it remains advisable to include both male and female participants.

References

- Barnard, D.R., Posey, K.H., Smith, D. and Schreck, C.E., 1998. Mosquito density, biting rate and cage size effects on repellent tests. *Medical and Veterinary Entomology* 12: 39–45.
- Bezerra-Santos, M.A., Benelli, G., Germinara, G.S., Volf, P. and Otranto, D., 2024. Smelly interactions: host-borne volatile organic compounds triggering behavioural responses in mosquitoes, sand flies, and ticks. *Parasites and Vectors* 17(1): 227. <https://doi.org/10.1186/s13071-024-06299-1>.
- Borges, L.M., Oliveira, J.G.F., Ferreira, L.L., Louly, C.C., Pickett, J.A. and Birkett, M.A., 2015. Identification of non-host semiochemicals for the brown dog tick, *Rhipicephalus sanguineus sensu lato* (Acari Ixodidae), from tick-resistant beagles, *Canis lupus familiaris*. *Ticks and Tick-Borne Diseases* 6(5): 676682. <https://doi.org/10.1016/j.ttbdis.2015.05.014>.
- Carnevale, P., Frezil, J.L., Bosseno, M.F., Le Pont, F. and Lancien, J., 1978. Etude de l'agressivité d'*Anopheles gambiae* en fonction de l'âge et du sexe des sujets humains. *Bulletin of the World Health Organization*. <https://iris.who.int/handle/10665/65741>.
- Carr, A.L., Roe, R.M., Arellano, C., Sonenshine, D.E., Schal, C. and Apperson, C.S., 2013. Responses of *Amblyomma americanum* and *Dermacentor variabilis* to odorants that attract haematophagous insects. *Medical and Veterinary Entomology* 27: 86–95. <https://doi.org/10.1111/j.1365-2915.2012.01024.x>.
- Das, S., Muleba, M., Stevenson, J.C., Pringle, J.C. and Norris, D.E., 2017. Beyond the entomological inoculation rate: characterizing multiple blood feeding behavior and

- Plasmodium falciparum* multiplicity of infection in *Anopheles* mosquitoes in northern Zambia. *Parasites and Vectors* 10: 45. <https://doi.org/10.1186/s13071-017-1993-z>.
- De Obaldia, M.E., Morita, T., Dedmon, L.C., Boehmler, D.J., Jiang, C.S., Zeledon, E.V., Cross, J.R. and Vosshall, L.B., 2022. Differential mosquito attraction to humans is associated with skin-derived carboxylic acid levels. *Cell* 185(22): 4099–4116.e13. <https://doi.org/10.1016/j.cell.2022.09.034>.
- Dickens, J.C. and Bohbot, J.D., 2013. Mini review: Mode of action of mosquito repellents. *Pesticide Biochemistry and Physiology* 106(3): 149–155.
- Doty, R.L., Green, P.A., Ram, C. and Yankell, S.L., 1982. Communication of gender from human breath odors: relationship to perceived intensity and pleasantness. *Hormones and Behavior* 16: 13–22. [https://doi.org/10.1016/0018-506X\(82\)90002-2](https://doi.org/10.1016/0018-506X(82)90002-2).
- European Chemicals Agency (ECHA), 2018. Guidance on the biocidal products regulation – volume ii Efficacy – assessment and evaluation (parts b+c), version 3.0.
- European Chemicals Agency (ECHA), 2021. Guidance on the biocidal products regulation – volume ii Efficacy – assessment and evaluation (parts b+c), version 4.0.
- European Chemicals Agency (ECHA), 2022a. Guidance on the biocidal products regulation – volume ii Efficacy – assessment and evaluation (parts b+c), version 4.1.
- European Chemicals Agency (ECHA), 2022b. Guidance on the biocidal products regulation – volume ii Efficacy – assessment and evaluation (parts b+c), version 5.0.
- European Chemicals Agency (ECHA), 2023. Guidance on the biocidal products regulation – volume ii Efficacy – assessment and evaluation (parts b+c), version 6.0.
- Egid, B.R., Coulibaly, M., Dadzie, S.K., Kamgang, B., McCall, P.J., Sedda, L., Toe, K.H. and Wilson, A.L., 2021. Review of the ecology and behaviour of *Aedes aegypti* and *Aedes albopictus* in Western Africa and implications for vector control. *Current Research in Parasitology and Vector-Borne Diseases* 2: 100074. <https://doi.org/10.1016/j.crvpbd.2021.100074>.
- Ellwanger, J.H., Da Cruz Cardoso, J. and Bogo Chies, J.A., 2021. Variability in human attractiveness to mosquitoes. *Current Research in Parasitology and Vector-Borne Diseases* 1: 100058. <https://doi.org/10.1016/j.crvpbd.2021.100058>.
- Environmental Protection Agency (EPA), 2008. EPA product performance test guidelines. Insect repellents to be applied to human skin. Version 23. Environmental Protection Agency, Washington, DC, USA.
- Ferreira, L.L., Sarria, A. L.F., De Oliveira Filho, J.G., De Silva, F.D.O., Powers, S.J., Caulfield, J.C., Pickett, J.A., Birkett, M.A. and Borges, L.M.F., 2019. Identification of a non-host semiochemical from tick-resistant donkeys (*Equus asinus*) against *Amblyomma sculptum* ticks. *Ticks and Tick-borne Diseases* 10(3): 621–627. <https://doi.org/10.1016/j.ttbdis.2019.02.006>.
- Ferreira, L.L., De Oliveira Filho, J.G., De Oliveira Silva, F., Lacerda Ferraz, A.L. and Mascarin, G.M., 2020. Attract or repel *Amblyomma sculptum* ticks: Screening of semiochemicals. *Veterinary Parasitology* 278: 109036. <https://doi.org/10.1016/j.vetpar.2020.109036>.
- Gilbert, I.H., Gouck, H.K. and Smith, N., 1966. Attractiveness of men and women to *Aedes aegypti* and relative protection time obtained with deet. *Florida Entomologist* 49(1): 53–66.
- Golenda, C.F., Solberg, V.B., Burge, R., Gambel, J.M. and Wirtz, R.A., 1999. Gender-related efficacy difference to an extended duration formulation of topical N,N-diethyl-m-toluamide (DEET). *American Journal of Tropical Medicine and Hygiene* 60(4): 654–657. <https://doi.org/10.4269/ajtmh.1999.60.654>.
- Ives, A.R. and Paskewitz, S.M., 2005. Testing vitamin B as a home remedy against mosquitoes. *Journal of American Mosquito Control Association* 21: 213–217. [https://doi.org/10.2987/8756-971X\(2005\)21\[213:TVBAAH\]2.0.CO;2](https://doi.org/10.2987/8756-971X(2005)21[213:TVBAAH]2.0.CO;2).
- Josek, T., Gardner, A.M., Hedlund, T.J., Parker, A.T., Allmann Updyke, E. and Allan, B.F., 2019. Fatal attraction: Lone star ticks (*Amblyomma americanum*) exhibit preference for human female breath over male breath. *Experimental and Applied Acarology* 77(1): 59–64. <https://doi.org/10.1007/s10493-018-00338-0>.
- Korgaonkar, N.S., Kumar, A., Yadav, R.S., Kabadi, D. and Dash, A.P., 2012. Mosquito biting activity on humans and detection of *Plasmodium falciparum* infection in *Anopheles stephensi* in Goa, India. *Indian Journal of Medical Research* 135(1): 120–126. <http://doi.org/10.4103/0971-5916.93434>.
- Lacroix, R., Mukabana, W.R., Gouagna, L.C. and Koella, J.C., 2005. Malaria infection increases attractiveness of humans to mosquitoes. *PLoS Biology* 3(9): e298. <https://doi.org/10.1371/journal.pbio.0030298>.
- Li, M., Budding, A.E., Van der Lugt-Degen, M., Du-Thumm, L., Vandeven, M. and Fan, A., 2019. The influence of age, gender and race/ethnicity on the composition of the human axillary microbiome. *International Journal of Cosmetic Science* 41: 371–377. <https://doi.org/10.1111/ics.12549>.
- Lindsay, S., Ansell, J., Selman, C., Cox, V., Hamilton, K. and Walraven, G., 2000. Effect of pregnancy on exposure to malaria mosquitoes. *The Lancet* 355: 1972. [https://doi.org/10.1016/S0140-6736\(00\)02334-5](https://doi.org/10.1016/S0140-6736(00)02334-5).
- Logan, J.G., Birkett, M.A., Clark, S.J., Powers, S., Seal, N.J., Wadhams, L.J., Mordue Luntz, A.J. and Pickett, J.A., 2008. Identification of human-derived volatile chemicals that interfere with attraction of *Aedes aegypti* mosquitoes. *Journal of Chemical Ecology* 34: 308–322. <https://doi.org/10.1007/s10886-008-9436-0>.

- Marples, R.R., 1982. Sex, constancy, and skin bacteria. *Archives of Dermatology Research* 272: 317–320.
- Moreno-Gómez, M., Monsonís-Güell, E., Abril-Melendez, S., Manzanares-Sierra, A. and Mayol-Pérez, J., 2025. Robustness of insect repellent efficacy research: exploring the impacts of sampling design and participant heterogeneity. *Journal of the European Mosquito Control Association*. <http://doi.org/10.52004/2054930x-20251017>.
- Ogimoto, Y., Selyanchyn, R., Takahara, N., Wakamatsu, S. and Lee, S.W., 2015. Detection of ammonia in human breath using quartz crystal microbalance sensors with functionalized mesoporous SiO₂ nanoparticle films. *Sens Actuators B Chemical* 215: 428–436. <http://doi.org/10.1016/j.snb.2015.03.103>.
- Paskewitz, S., Irwin, P., Konwinski, N. and Larson, S., 2018. Impact of consumption of bananas on attraction of *Anopheles stephensi* to humans. *Insects* 9: 129. <http://doi.org/10.3390/insects9040129>.
- Popov, T.A., 2011. Human exhaled breath analysis. *Annals of Allergy, Asthma and Immunology* 106: 451–456. <http://doi.org/10.1016/j.anai.2011.02.016>.
- Qiu, T., Smallegange, R.C., Van Loon, J.J.A., Ter Braak, C.J.F. and Takken, W., 2006. Interindividual variation in the attractiveness of human odours to the malaria mosquito *Anopheles gambiae* s.s. *Medical and Veterinary Entomology* 20(3): 280–287. <http://doi.org/10.1111/j.1365-2915.2006.00627.x>.
- Rajan, T.V., Hein, M. and Wikel, S., 2005. A double-blinded, placebo-controlled trial of garlic as a mosquito repellent: a preliminary study. *Medical and Veterinary Entomology* 19(1): 84–89. <http://doi.org/10.1111/j.0269-283X.2005.00544.x>.
- R Core Team, 2022. R: A language and environment for statistical computing. Vienna, Austria.
- Roessler, P., 1963. The attractiveness of steroid and amino acids to female *Aedes aegypti*. In: *Proceedings of the Fifth Annual Meeting of the New Jersey Mosquito Extermination Association and the Nineteenth Annual Meeting of the American Mosquito Control Association*, pp. 250–255.
- Sastry, S.D., Buck, K.T., Janák, J., Dressler, M. and Preti, G., 1980. Volatiles emitted by humans. In: Waller, G.R. and Dermer, O.C. (eds.). *Biochemical Applications of Mass Spectrometry*. John Wiley & Sons, New York, NY, USA, pp. 1085–1129.
- Smith, C.N., Gilbert, I.H., Gouck, H.K., Bowman, M.C., Acree Jr, F. and Schnmidt, C.H., 1963. Factors affecting the protection period of mosquito repellents. *Technical Bulletin 1285*. U.S. Dept of Agriculture.
- Weldon, P.J., Aldrich, J.R., Klun, J.A., Oliver, J.E. and Debboun, M., 2003. Benzoquinones from millipedes deter mosquitoes and elicit self-anointing in capuchin monkeys (*Cebus spp.*). *Naturwissenschaften* 90: 301–304. <https://doi.org/10.1007/s00114-003-0427-2>.
- World Health Organization (WHO), 2009. Guidelines for efficacy testing of mosquito repellents for human skin. WHO/HTM/NTD/WHOPES/2009.4. World Health Organization, Geneva, Switzerland.
- Zhang, H., Zhu, Y., Liu, Z., Peng, Y., Peng, W., Tong, L., Wang, J., Liu, Q., Wang, P. and Cheng, G., 2022. A volatile from the skin microbiota of flavivirus-infected hosts promotes mosquito attractiveness. *Cell* 185(14): 2510–2522.e16. <https://doi.org/10.1016/j.cell.2022.05.016>.