Acta Parasitologica (2022) 67:1463–1470
https://doi.org/10.1007/s11686-022-00621-6

REVIEW

Manipulation by *Plasmodium* Parasites of *Anopheles* Mosquito Behavior and Human Odors

Tristan Sanford[1] · **Dave Shutler**[1]

Received: 8 August 2022 / Accepted: 20 September 2022 / Published online: 19 October 2022

Abstract

Purpose The phenomenon of parasites manipulating host phenotypes is well documented; the best-known examples are manipulations of host behavior. More recently, there has been interest in whether parasites can manipulate host odor phenotypes to enhance their attractiveness to vectors. We review here evidence that *Plasmodium*-infected mosquitoes have enhanced attraction to human hosts, especially when the parasite is sufficiently developed to be transmissible. We also review evidence suggesting that malaria-infected host odors elicit greater mosquito attraction compared to uninfected controls.

Methods We reviewed and summarized the relevant literature.

Results Though evidence is mounting that supports both premises we reviewed, there are several confounds that complicate interpretation. These include differences in *Plasmodium* and mosquito species studied, stage of infection tested, age of human participants in trials, and methods used to quantify volatiles. In addition, a key requirement to support the hypothesis of manipulation by parasites is that costs of manipulation be identified, and ideally, quantified.

Conclusions Substantial progress has been made to unlock the importance of odor for enhancing transmission of *Plasmodium*. However, there needs to be more replication using similar methods to better define the odor parameters involved in this enhancement.

Keywords Malaria · Manipulation of host odor · Transmissible stages · Volatile organic compounds

Abbreviations

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
GCMS	Gas chromatography mass spectrometry
GC–EAG	Gas chromatography–electroantennography
HMBPP	(E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate
MTP	Methylthio-propane
MTPNE	(E)-1-methythio-1-propene
MTPNZ	(Z)-1-methylthio-1-propene
RBCs	Red blood cells
VOCs	Volatile organic compounds

Manipulation and *Plasmodium* Parasites

Manipulation of hosts by parasites entails alteration of the former's phenotype to enhance the latter's transmission [1]. Changes can be caused indirectly (e.g., a parasite passively excretes metabolites, such as waste products, which affect hosts) or directly (e.g., a parasite upregulates production of metabolites that are released and affect hosts). Manipulations can include affecting host habitat preferences, changing host appearance, or reducing fear of predators [1]. Less well-studied are manipulations of phenotype that affect chemosensory interactions. Nonetheless, evidence is accumulating of odor changes in parasitized animals [e.g., 2, 3]. Regardless of the phenotypic change, parasite manipulations are predicted to be related to infection or infestation intensity [sensu 4], and to occur only when parasites have reached a developmental stage that is ready for transmission. Manipulations are assumed to help increase parasite transmission rates; multiple independent evolutions in myriad host–parasite relationships are acknowledged to have made such manipulations ubiquitous [1].

✉ Dave Shutler
dave.shutler@acadiau.ca

[1] Department of Biology, Acadia University, Wolfville, NS B4P 2R6, Canada

Plasmodium spp. have complex life cycles that include transmission of asexual sporozoites between *Anopheles* (*An*.) mosquitoes and vertebrate hosts [5]. Inside vertebrate hosts, *Plasmodium* parasites invade liver cells, where they multiply before spreading to the blood to infect erythrocytes (red blood cells, RBCs). While multiplying in RBCs, some merozoites develop into presexual stages called gametocytes that differentiate into haploid sexual micro- and macrogametes when ingested by a mosquito during a blood meal. Inside midguts of mosquitoes, micro- and macro-gametes fuse to form diploid zygotes. Zygotes then burrow into mosquito epithelial cells to form asexual oocysts where further multiplication occurs. After ~ 10 days developing inside a mosquito midgut, sporozoites migrate to salivary glands where they can be delivered to a vertebrate host during a subsequent blood meal [5].

Transmission of *Plasmodium* parasites between human and mosquito hosts relies on mosquito feeding behavior [6]. Male and female mosquitoes are generally nectarivorous [7, 8], but, during maturation of eggs, females require protein-rich blood meals. To locate a suitable host, female mosquitoes rely primarily on olfactory chemoreceptors that detect volatile organic compounds (VOCs) [9]. During host location, mosquitoes are first attracted to carbon dioxide (CO_2), a gas released during vertebrate respiration [10]. Once in close range (≤ 1 m), mosquitoes may become attracted to odors (i.e., blends of VOCs) of suitable hosts [11]. Most VOCs in host odor arise from either breath or skin [12]. In general, blood-feeding by mosquitoes is a complex process that involves detection of a host, landing on a host, piercing and probing a host, ingestion of blood, and termination of a blood meal [6]. Each of these steps offers opportunities for manipulation by *Plasmodium* parasites of both mosquitoes and vertebrates to enhance transmission.

We review here evidence that *Plasmodium* parasites manipulate mosquito host-seeking behavior, and that *Plasmodium* parasites manipulate vertebrate host odors to enhance attractiveness to mosquitoes. Our objectives are to clarify the extent of *Plasmodium* parasites' manipulation of hosts, as well as identify areas in which further research is needed.

Manipulation of Mosquito Behavior

Vector manipulation is considered evolutionarily advantageous and is thought to be under intense selection [1]. Nonetheless, studies assessing effects of *Plasmodium* parasites manipulating mosquito behaviour have at times had conflicting results [13]. For instance, *An. gambiae* mosquitoes with transmissible *P. falciparum* sporozoites had increased attraction to human odors compared to uninfected controls [14], but this result was not obtained in another experiment [15]. Furthermore, increased attraction to human odors was not observed in *An. coluzzi* mosquitoes carrying *P. falciparum* sporozoites, nor were changes in host-seeking observed [15].

Tests using mosquito dual choice assays indicated that human patients with high intensities (i.e., levels detectable by microscopy) of *P. falciparum* or *P. vivax* gametocytes were twice as attractive to *An. gambiae* as were uninfected patients or patients infected with non-transmissible malarial stages [3, 17]. Infected *An. gambiae* fed on more individuals per night than did uninfected controls [18]; this enhances opportunities for *Plasmodium* parasite transmission. Similarly, *Aedes aegypti* infected with *P. gallinaceum* probed longer, took smaller blood meals per feeding, and probed more than uninfected controls [19–21]. Finally, *An. stephensi* with *P. chabaudi* and *P. yoelii nigeriensis*, respectively, took smaller blood meals and were more persistent in feeding [20, 22].

As further evidence of manipulation of mosquito hosts, *An. gambiae* with *P. falciparum* oocysts (a non-infective stage) had increased attraction to plant volatiles compared to uninfected controls. This increased attraction to plant volatiles is proposed to prevent gravid females (who normally seek blood meals to meet increased nutritional requirements) from being killed during host-feeding. This enhanced attraction to plant volatiles appears to last only until *Plasmodium* parasites develop into transmissible sporozoite stages, where interfering with host attraction would be deleterious to transmission [13, 14].

Apyrase, an ATP- and ADP-degrading enzyme, helps mosquitoes locate blood vessels during probing and thereby makes feeding faster [23, 24]. Thiévent *et al*. [25] found that apyrase activity in *An. gambiae* was negatively associated with sporozoite intensity of *P. berghei*, and they observed a correlation between the amount of blood ingested and apyrase activity. They also confirmed results of Li *et al*. [26], who had suggested that mosquitoes, such as *An. gambiae*, that have intrinsically high levels of apyrase, are most affected when apyrase levels are reduced. Because *An. gambiae* has high levels of apyrase, it is thought to play a more important role in its feeding compared to mosquitoes with lower levels. By reducing production of apyrases in the salivary glands, mosquitoes would probe longer because of difficulties locating blood vessels, and have more incomplete blood meals. This could lead to more blood meals and higher chances of transmission.

Manipulation of Human Odors

Recently, manipulation of human VOCs by *Plasmodium* spp. parasites has been hypothesized [27]. Gas chromatography mass spectrometry (GCMS), gas chromatography–electroantennography (GC–EAG), and dual choice assays have

been used to test this hypothesis [e.g., 9, 12, 17, 28, 29]. *An. gambiae* mosquitoes have enhanced attraction to humans infected with *P. falciparum* [17] attributed, in part, to odor emissions [30]. Because mosquitoes locate hosts primarily by detecting VOCs, and show non-random host selection [31, 32], Lacroix *et al*. [27] hypothesized that malaria may alter levels of VOCs in either skin or breath of humans to increase transmission. Accordingly, Robinson *et al*. [28] found changes in VOCs released from the feet of children infected with *P. falciparum*. Notably, increases in aldehydes such as heptanal, octanal, nonanal, (E)-2-octenal, and (E)-2-decenal were found (Table 1). Aldehydes are VOCs of human skin and are reportedly used by hematophagous arthropods during host location [28]. The ketone 2-octanone was associated with submicroscopic gametocyte infections (i.e., infections at intensities too low to be detected by microscopy). Because submicroscopic infections can still transmit *Plasmodium* parasites, and because they can pose health risks for infants and children, methods for detecting them in the field are highly desirable [33]. In addition, all VOCs described by Robinson *et al*. [28] were also present in samples from healthy children, but at much lower levels than in infected children. These findings support the deceptive signaling hypothesis that predicts upregulation of natural host-seeking cues, tricking vectors, regardless of potential negative fitness consequences to them, into being attracted to infected hosts [12, 34, 35].

VOCs identified by Robinson *et al*. [28] were then tested in dual choice assays to evaluate their attractiveness to *An. coluzzii*. GC–EAG was used to test for neurological stimulation of mosquitoes. Because antennae of mosquitoes are highly selective, GC–EAG ensures that VOCs are associated with a neurological response. VOCs from parasite-free patients were similarly tested for their attractiveness to mosquitoes. Ten µL of heptanal at a concentration of 10^{-8} g/mL increased attraction, but 10 µL at a concentration of 10^{-7} g/mL produced no effect, i.e., there was dose-dependent attraction. Adding octanal, nonanal, (E)-2-octenal, (E)-2-decenal, or 2-octanone by themselves to uninfected control odors (in this case, socks from patients) produced no change in attraction levels. Heptanal, the only compound increasing attraction when added to controls on its own, was then added to the Mbita synthetic mosquito lure, which is composed of ammonia, L-(+)-lactic acid, tetradecanoic acid, 3-methyl-1-butanol, and butan-1-amine, without changes in attraction [29]. These results indicate that synergism among specific doses of chemicals may be responsible for increased mosquito attraction [28].

To control for differences in intrinsic human attraction, the same patients' odors were again collected 21 days after administration of antimalarial drugs [28]. In addition, these patients were determined to be parasite-free via both microscopy and 18S RNA (ssrDNA) qualitative polymerase chain reaction (PCR). Interestingly, post-infection, these individuals were less attractive even when compared to uninfected patients, or patients carrying only asexual stages. These results suggest that gametocytes could play a key role in increased attraction of mosquitoes.

De Moraes *et al*. [36] collected VOC samples from the arms and feet of > 400 children from across Kenya. Samples were separated into uninfected, asymptomatic infected, and symptomatic infected treatments. De Moraes *et al*. [36] did not separate samples based on species of *Plasmodium*, and the majority of samples were from mixed infections (*P. falciparum*, *P. vivax*, and *P. malariae*), with *P. falciparum* most prevalent. Using machine-learning algorithms and GCMS, VOCs were tested to detect signatures indicative of infection. Foot VOCs significantly associated with infection status were 4-hydroxy-4-methylpentan-2-one, nonanal, and toluene, among others (Table 1). 2-ethylhexan-1-ol, an underarm volatile, also significantly predicted infection status. Of compounds identified as being produced by *Plasmodium* parasites, toluene and hexanal had been reported [32, 37]. Predictive models obtained from machine-learning algorithms were comparable to PCR in detecting submicroscopic infections [36]. Because PCR is costly, machine-learning algorithms paired with portable eNose technology could provide a more affordable and accurate field-based diagnostic test [38].

In VOCs collected by De Moraes *et al*. [36], decreases in hexanal were significant for identification of both symptomatic and asymptomatic malaria infections. Hexanal can elicit neurological responses in mosquitoes, and is produced by *Plasmodium* parasites *in vitro* [32, 37]. Hexanal also occurs in VOCs produced by cancer patients and appears to be associated with cell lysis [39].

Breath VOCs Associated with Infection

Changes in breath VOCs are reported for a growing number of infectious diseases [40–43]. During an infection, a disease's unique pathology [44] causes characteristic physiological changes that can alter VOCs exhaled from lungs [29]. Lacroix *et al*. [27] suggested that diseases spread by mosquito vectors could benefit from manipulating host breath VOCs to increase attraction. Berna *et al*. [45] collected exhaled VOCs from clinically induced blood stage *P. falciparum* and *P. vivax* infections in humans. Four thioethers, allyl methyl sulfide, 1-methylthio-propane (MTP), (Z)-1-methylthio-1-propene (MTPNZ), and (E)-1-methythio-1-propene (MTPNE), were most indicative of infection (Table 1). Levels of these thioethers differed significantly between infected and uninfected controls with a 27-fold increase in MTPNZ, and similar increases in MTP and MTPNE in patients with *P. falciparum* infections [46].

Table 1 Volatile organic compounds (VOCS) of breath, skin, or microbiota samples associated with *Plasmodium* spp. infection

VOC	Location	Change in VOCs	Age	*Plasmodium* species	Reference(s)
(E)-1-Methythio-1-propene	Breath	Increased	Adults	*falciparum*	[52]
(E)-2-Decenal	Foot	Increased	Children	*falciparum*	[14]
(E)-2-Octenal	Foot	Increased	Children	*falciparum*	[14]
(R)- or (S)- 2-Methylbutanal	Foot	Increased	Adult	*falciparum*	[64]
(R)- or (S)- 3-Hydroxy-2-butanone	Foot	Increased	Adult	*falciparum*	[64]
(R)- or (S)- 3-Methylbutanal	Foot	Increased	Adult	*falciparum*	[64]
(Z)-1-Methylthio-1-propene	Breath	Increased	Adults	*falciparum*	[52]
1-Dodecene	Foot	Increased	Adult	*falciparum*	[64]
1-Methylthio-propane	Breath	Increased	Adults	*falciparum*	[52]
2-Ethylhexan-1-ol	Foot/arm	Increased/Decreased	Children	*falciparum*, *vivax*, *malariae*	[43, 65]
2-Octanone	Foot	Increased	Children	*falciparum*	[14]
2-Pentadecanone	Skin	Decreased[b]	Adults	*falciparum*	[46]
3-Carene	Breath	Increased	Children	*falciparum*	[15]
4-Hydroxy-4-methylpentan-2-one	Foot/arm	Increased/decreased	Children	*falciparum*, *vivax*, *malariae*	[43, 65]
6-Methyl-5-hepten-2-one	Foot	Decreased	Adult	*falciparum*	[64]
Acetone	Breath	Varied	Adults	*falciparum*	[52]
Allyl methyl sulfide	Breath	Increased	Adults	*falciparum*	[52]
Benzene	Breath	Varied	Adults	*falciparum*	[52]
Butyl 2-methylbutanoate	Skin	Increased[a]	Adults	*falciparum*	[46]
Butyl acetate	Skin	Increased[a]	Adults	*falciparum*	[46]
Butyl butyrate	Skin	Increased[a]	Adults	*falciparum*	[46]
Butyl isobutyrate	Skin	Increased[a]	Adults	*falciparum*	[46]
Carbon dioxide	Breath	Increased	Adults	*falciparum*	[52]
Cyclohexanone	Breath	Varied	Adults	*falciparum*	[52]
Decane	Foot/arm	Decreased	Children	*falciparum*	[65]
Dimethyl decane	Breath	Increased	Children	*falciparum*	[15]
Dimethyl sulfide	Skin	Increased[b]	Adults	*falciparum*	[46]
Dodecanal	Foot	Increased	Adult	*falciparum*	[64]
Ethylbenzene	Foot	Increased	Children	*falciparum*, *vivax*, *malariae*	[43]
Ethylcyclohexane[c]	Foot/arm	Increased/decreased	Children	*falciparum*, *vivax*, *malariae*	[43, 65]
Heptanal	Foot	Increased	Children	*falciparum*	[14]
Hexanal	Foot/arm	Decreased/increased	Children	*falciparum*, *vivax*, *malariae*	[43, 65]
Isoprene	Breath	Varied/ decreased	Adults and Children	*falciparum*, *vivax*	[14, 52]
Limonene	Breath	Increased	Adults	*falciparum*, *vivax*	[53]
M-cymene	Breath	Increased	Adults	*falciparum*, *vivax*	[53]
Methyl dodecanoate	Foot	Increased	Adult	*falciparum*	[64]
Methyl undecane	Breath	Decreased	Children	*falciparum*	[15]
Nonanal	Breath/foot/arm	Increased/decreased	Children	*falciparum*	[3, 14, 15]
O-xylene	Foot/arm	Decreased	Children	*falciparum*	[65]
Octanal	Foot	Increased	Children	*falciparum*	[14]
Octane	Foot/arm	Decreased	Children	*falciparum*	[65]
Octonal	Foot/arm	Decreased	Children	*falciparum*	[65]
Propylcyclohexane	Foot	Increased	Children	*falciparum*, *vivax*, *malariae*	[43]
Terpinolene	Breath	Increased	Adults	*falciparum*, *vivax*	[53]
Toluene	Foot	Increased	Children	*falciparum*, *vivax*, *malariae*	[43]
Tridecane	Breath	Decreased	Children	*falciparum*	[15]
Trimethyl hexane	Breath	Decreased	Children	*falciparum*	[15]
α-Pinine	Breath	Increased	Children	*falciparum*	[15]
α-Terpinene	Breath	Increased	Adults	*falciparum*, *vivax*	[53]

[a]Indicates dose-dependent chemicals whereas

[b]Indicates dose-independent chemicals

[c]Indicates chemicals that have been used to distinguish children that are symptomatic but are not infected with malaria. Table data and sources adapted from [3]

Infection with *P. vivax* was not associated with significantly higher thioether levels. Interestingly, levels of thioethers cycled every 24 h, whereas cycles for *P. falciparum* gametocytes were 48 h; however, no direct relationships between the two cycles were found. These results were contrary to Berna *et al*.'s [46] subsequent study which suggested that cycles of thioethers were directly correlated with gametocyte cycles. Importantly, because induced blood-stage infections were used, infections were treated before gametocyte stages would occur (~ 1 week) [29]. Because gametocytes affect VOC production, their absence could have negatively affected results [28]. MTP, MTPNZ, and MTPNE were 98% accurate in predicting infection status of human adults carrying *P. falciparum* [46]. However, this only occurred when the time of collection was controlled, because VOCs exhibit diurnal variation.

Thioethers were not detected when the study was replicated using breath from patients with *P. vivax*, indicating that thioethers released may be specific to *P. falciparum* [46]. Comparing breath VOCs between infections with *P. vivax* and *P. falciparum* revealed four terpenes (α-terpinene, m-cymene, limonene, and terpinolene) that rose and fell similarly during infection/treatment (Table 1). Interestingly, when comparing VOCs between infected and uninfected RBCs, high levels of terpenes in headspace (gas found at the top of sealed samples so that direct sampling of substrate is not needed) were associated with infection [32].

Schaber *et al*. [29] collected breath VOCs of febrile children with or without *P. falciparum*. Six VOCs (methyl undecane, dimethyl decane, trimethyl hexane, nonanal, isoprene, and tridecane) were most useful in determining infection status (Table 1). Levels of these VOCs were not associated with patient age, sex, or nutrition, and all six were also found in healthy breath samples but at lower concentrations. In terms of detected VOCs, isoprene is a known endogenous compound, while the other five are believed to be produced by oxidative stress-induced lipid peroxidation, which is characteristic of *Plasmodium* infection. Average intensity of parasitemia was over one hundred thousand times the average reported by Berna *et al*. [46], which could explain differences in VOCs detected between studies.

Terpenes, in general, are a class of hydrocarbons typically associated with plants [32]. Terpenes found by both Berna *et al*. [45] and Schaber *et al*. [29] are of particular interest, because many are released from mosquito's preferred, nectar-producing plants. In humans, terpenes in healthy breath are believed to be derived from diet [47]. A-pinene and 3-carene were of particular importance, because they have been found in the headspace of infected RBCs, and terpenes such as pinene and limonene are mosquito attractants [9, 32]. Production of these terpenes is thought to be caused by *Plasmodium* parasites synthesizing isoprenoids (important for growth, membrane components, and hormones) via the methylerythritol phosphate pathway. Enammi *et al*. [48] showed that by adding (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) (a precursor isoprene molecule produced by *Plasmodium* spp. parasites) to samples of uninfected RBCs, terpenes (such as α-pinene) were produced. These terpenes were also released by mosquito-preferred nectar-producing plants [32]. This mechanism could account for the increase in host attraction as well as offer a plausible route for parasite manipulation. However, Miller and Odom John [49] failed to reproduce these results. In sum, tests on exhalates from patients with malaria have produced diverse and conflicting results.

Manipulation of Microbiomes

Human gut microbiota are integral to maintaining homeostasis [50]. Changes to host microbiota composition may, therefore, produce profound effects on health and microbial interspecific interactions. Malaria has wide-ranging effects on hosts and new research suggests that the gut microbiome not only influences malaria severity, but also may play a role in transmission via production of an antibody that binds to antigens on both *E. coli* and *Plasmodium* species [51].

Unsurprisingly, human body odor differs among individuals [30, 35], and some variation in odor is associated with differences in skin microbiota [30]. Differences in odor arise from skin microorganisms breaking down odorless eccrine sweat, producing VOCs attractive to mosquitoes after 1–2 days. Skin bacteria cultured on blood agar plates were more attractive to mosquitoes than uncultured blood agar plates [52, 53]. Using GCMS, VOCs produced from skin bacteria were tested for their attractiveness to *An. gambiae*. *Corynebacterium minutissimum*, a foot microbe, produced the most attractive VOCs, with *Bacillus subtilis* and *Staphylococcus epidermidis* also being attractive [54]. *Pseudomonas aeruginosa* produced VOCs that were repellent to mosquitoes. 2-pentadecanone, butyl 2-methylbutanoate, butyl isobutyrate, dimethyl disulfide, butyl butyrate, and butyl acetate were the most significant attractants (Table 1). When added to a mosquito lure, dimethyl disulfide increased attraction, a result not observed for other compounds.

Infection, therefore, could induce changes in the density or diversity of microbiomes and affect mosquito attraction. Currently, however, it is unclear to what extent changes in diet, bed rest, and fever/chills caused by malaria contribute to microbial changes. In addition, mosquito attraction is only beneficial while the parasite is transmissible [55]. Further research into manipulation of human microbiomes by *Plasmodium* species should test if changes in species composition or diversity are associated with the presence or absence of transmissible stages.

Costs of Manipulation

Brown [56] and others [57, 58] have argued that "true" manipulation by parasites must entail costs and not be a simple by-product of parasitism. To illustrate, several bacterial infections cause human patients to have distinct, recognizable odors [59], but these manipulations do not enhance transmission and may be of no adaptive value (i.e., they are "spandrels", sensu [60]). Whereas manipulation is presumed to increase net fitness, benefits of manipulating mosquito behavior or human odor are infrequently quantified, and costs can vary widely based on how a parasite manipulates its host [58]. In addition, costs can be difficult to quantify due to complex interactions between parasites and hosts, with interspecific parasite interactions adding further complexity.

Interpreting the Literature, and Future Work

The literature suggests enhanced mosquito attraction to humans carrying transmissible stages of *Plasmodium* parasites relative to uninfected individuals or those carrying non-transmissible stages [3]. Changes in infected mosquito behaviors are consistent with manipulation of both mosquitoes and humans. However, differences in *Plasmodium* species studied, stage of infection tested, intensities of infection, age of participants, and experimental procedures make straightforward interpretation of these results difficult. Moreover, there are studies that failed to reproduce earlier, suggestive results [15, 16]. Some differences can be ascribed to experimental design [see 29, 45], and some to variation among species of mosquito/*Plasmodium* studied (Table 1). A wide array of tests that sampled various aspects of human, mosquito, and *Plasmodium* relationships have produced inconsistent results. Though some VOCs were associated with attraction and accurately predicted infection status, the compounds themselves were rarely similar among studies (Table 1). Curiously, VOCs associated with attraction (e.g., heptanal, nonanal, and octanal) were also found at significantly higher levels among individuals categorized as less attractive to *Aedes aegypti* mosquitoes [61].

Another issue is a lack of standardization in VOC collection methods that makes it difficult to compare results among studies [62, 63]. Collection methods influence the diversity and concentration of VOCs detected [63]. Whereas flexibility and customization of GCMS appeals to researchers seeking to tailor experimental designs, variation in GCMS methods paired with differences in study design (e.g., species of *Plasmodium*, age of patients, infection status) make comparisons challenging and may be responsible for inconsistencies among studies in reported significant VOCs. This deficit of replication hampers synthesis. Through the practice of careful replication, researchers can take a more focused and empirical approach to solving the enigma of *Plasmodium* parasite odor manipulation.

Current research highlights the importance of understanding interactions between microbiota and human health [50], and presently there is still much we do not know about how parasites interact with human microbiomes. Future research on *Plasmodium*–host microbiome interactions and their subsequent effects on microbial diversity in key areas such as guts, lungs, and skin may help determine if manipulation is indeed occurring.

To date, mechanisms underlying manipulation of both mosquito behavior and human odors are poorly understood. This means we can only speculate about costs that *Plasmodium* parasites may incur from manipulation. Further research into the metabolic pathways of *Plasmodium* may shed light on hidden costs borne by these parasites and help determine if true manipulation is occurring. Although the literature provides several examples suggesting that *Plasmodium* parasites manipulate host chemosensory interactions, we should not blindly jump onto the bandwagon and conclude that true manipulation is occurring, particularly given the counter-examples and inconsistencies we cite herein.

Acknowledgements We thanks two reviewers for their helpful comments that improved the manuscript.

Author contributions TS and DS: conceived and designed the review. TS: collected the data. TS and DS: analyzed and interpreted the literature. TS: drafted the initial manuscript. TS and DS: did iterative revisions. Both authors reviewed the results and approved the final version of the review.

Funding TS was supported by an Undergraduate Summer Research Award from the Natural Sciences and Engineering Research Council (NSERC) of Canada, and DS was supported by a Discovery Grant from NSERC.

Data availability Data supporting conclusions in this article are cited or included within.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have none.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

1. Moore J (2002) Parasites and the behavior of animals. Oxford University Press
2. Hudson PJ, Dobson AP, Newborn D (1992) Do parasites make prey vulnerable to predation? Red grouse and parasites. J Anim Ecol 61:681–692. https://doi.org/10.2307/5623
3. Busula AO, Bousema T, Mweresa CK, Masiga D, Logan JG, Sauerwein RW et al (2017) Gametocytemia and attractiveness of *Plasmodium falciparum*–infected Kenyan children to *Anopheles gambiae* mosquitoes. J Infect Dis 216:216–291. https://doi.org/10.1093/infdis/jix214
4. Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575–583. https://doi.org/10.2307/3284227
5. Roberts LS, Janovy J Jr (2005) Foundations of parasitology, 7th edn. McGraw-Hill, New York, NY
6. Friend WG, Smith JJ (1977) Factors affecting feeding by blood-sucking insects. Annu Rev Entomol 22:309–331. https://doi.org/10.1146/annurev.en.22.010177.001521
7. Foster WA (1995) Mosquito sugar feeding and reproductive energetics. Annu Rev Entomol 40:443–474. https://doi.org/10.1146/annurev.en.40.010195.002303
8. Gu W, Müller G, Schlein Y, Novak RJ, Beier JC (2011) Natural plant sugar sources of *Anopheles* mosquitoes strongly impact malaria transmission potential. PLoS One 6:e15996. https://doi.org/10.1371/journal.pone.0015996
9. Nyasembe VO, Teal PEA, Mukabana WR, Tumlinson JH, Torto B (2012) Behavioural response of the malaria vector *Anopheles gambiae* to host plant volatiles and synthetic blends. Parasit Vectors 5:1–11. https://doi.org/10.1186/1756-3305-5-234
10. Costantini C, Gibson G, Sagnon N, Della Torre A, Brady J, Coluzzi M (1996) Mosquito responses to carbon dioxide in a west African Sudan savanna village. Med Vet Entomol 10(3):220–227. https://doi.org/10.1111/j.1365-2915.1996.tb00734.x
11. Kennedy JS (1940) The visual responses of flying mosquitoes. Proc Zool Soc Lond 109:221–242
12. Busula AO, Verhulst NO, Bousema T, Takken W, de Boer JG (2017) Mechanisms of *Plasmodium*-enhanced attraction of mosquito vectors. Trends Parasitol 33(12):961–973. https://doi.org/10.1016/j.pt.2017.08.010
13. Cator LJ, Lynch PA, Read AF, Thomas MB (2012) Do malaria parasites manipulate mosquitoes? Trends Parasitol 28(11):466–470. https://doi.org/10.1016/j.pt.2012.08.004
14. Smallegange RC, van Gemert G-J, van de Vegte-Bolmer M, Gezan S, Takken W et al (2013) Malaria infected mosquitoes express enhanced attraction to human odor. PLoS One 8(5):e63602. https://doi.org/10.1371/journal.pone.0063602
15. Nguyen PL, Vantaux A, Hien DF, Dabiré KR, Yameogo BK et al (2017) No evidence for manipulation of *Anopheles gambiae*, *An. coluzzii* and *An. arabiensis* host preference by Plasmodium falciparum. Sci Rep 7(1):9415. https://doi.org/10.1038/s41598-017-09821-x
16. Vantaux A, de Sales Hien DF, Yameogo B, Dabiré KR, Lefèvre T (2015) Host-seeking behaviors of mosquitoes experimentally infected with sympatric field isolates of the human malaria parasite *Plasmodium falciparum*: no evidence for host manipulation. Front Ecol Evol 3:1–12. https://doi.org/10.3389/fevo.2015.00086
17. Batista EP, Costa EF, Silva AA (2014) *Anopheles darlingi* (Diptera: Culicidae) displays increased attractiveness to infected individuals with *Plasmodium vivax* gametocytes. Parasit Vectors 7:1–4. https://doi.org/10.1186/1756-3305-7-251
18. Koella JC, Sørensen FL, Anderson RA (1998) The malaria parasite, *Plasmodium falciparum*, increases the frequency of multiple feeding of its mosquito vector, *Anopheles gambiae*. Proc Roy Soc Lond B: Biol Sci 265(1398):763–768. https://doi.org/10.1098/rspb.1998.0358
19. Rossignol PA, Ribeiro JM, Spielman A (1984) Increased intradermal probing time in sporozoite-infected mosquitoes. Am J Trop Med Hyg 33(1):17–20. https://doi.org/10.4269/ajtmh.1984.33.17
20. Rossignol PA, Ribeiro JM, Spielman A (1986) Increased biting rate and reduced fertility in sporozoite-infected mosquitoes. Am J Trop Med Hyg 35(2):277–279. https://doi.org/10.4269/ajtmh.1986.35.277
21. Koella JC, Rieu L, Paul REL (2002) Stage-specific manipulation of a mosquito's host-seeking behavior by the malaria parasite *Plasmodium gallinaceum*. Behav Ecol 13(6):816–820. https://doi.org/10.1093/beheco/13.6.816
22. Anderson RA, Koella JC, Hurd H (1999) The effect of *Plasmodium yoelii nigeriensis* infection on the feeding persistence of *Anopheles stephensi* Liston throughout the sporogonic cycle. Proc Roy Soc Lond B Biol Sci 266(1430):1729–1733. https://doi.org/10.1098/rspb.1999.0839
23. Ribeiro JM, Rossignol PA, Spielman A (1984) Role of mosquito saliva in blood vessel location. J Exp Biol 108:1–7. https://doi.org/10.1242/jeb.108.1.1
24. Lehane MJ (2005) The biology of blood-sucking in insects. Cambridge Univ Press, Cambridge
25. Thiévent K, Zilio G, Hauser G, Koella JC (2019) Malaria load affects the activity of mosquito salivary apyrase. J Insect Physiol 116:10–16. https://doi.org/10.1016/j.jinsphys.2019.04.003
26. Li X, Sina B, Rossignol PA (1992) Probing behaviour and sporozoite delivery by *Anopheles stephensi* infected with *Plasmodium berghei*. Med Vet Entomol 6(1):57–61. https://doi.org/10.1111/j.1365-2915.1992.tb00036.x
27. Lacroix R, Mukabana W, Gouagna LC, Koella J (2005) Malaria infection increases attractiveness of humans to mosquitoes. PLoS Biol 3(9):1590–1593. https://doi.org/10.1371/journal.pbio.0030298
28. Robinson A, Busula AO, Voets MA, Beshir KB, Caulfield JC et al (2018) *Plasmodium*-associated changes in human odor attract mosquitoes. Proc Natl Acad Sci USA 115(18):E4209–E4218. https://doi.org/10.1073/pnas.1721610115
29. Schaber CL, Katta N, Bollinger LB, Mwale M, Mlotha-Mitole R et al (2018) Breathprinting reveals malaria-associated biomarkers and mosquito attractants. J Infect Dis 217(10):1553–1560. https://doi.org/10.1093/infdis/jiy072
30. Pombi M, Jacobs F, Verhulst NO, Caputo B, della Torre A, Takken W (2014) Field evaluation of a novel synthetic odour blend and of the synergistic role of carbon dioxide for sampling host-seeking *Aedes albopictus* adults in Rome, Italy. Parasit Vectors 7(1):1–5. https://doi.org/10.1186/s13071-014-0580-9
31. Kingsolver JG (1987) Mosquito host choice and the epidemiology of malaria. Am Nat 130(6):811–827. https://doi.org/10.1086/284749
32. Kelly M, Su C-Y, Schaber C, Hsu FF, Carlson JR, Odom AR (2015) Malaria parasites produce volatile mosquito attractants. MBio 6(2):e00235-e315. https://doi.org/10.1128/mbio.00235-15
33. Kasstan B, Hampshire K, Guest C, Logan J, Pinder M et al (2019) Sniff and tell: the feasibility of using bio-detection dogs as a mobile diagnostic intervention for asymptomatic malaria in sub-Saharan Africa. J Biosoc Sci 51(3):436–443. https://doi.org/10.1017/s0021932018000408
34. Mauck KE, De Moraes CM, Mescher MC (2010) Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. Proc Natl Acad Sci USA 107(8):3600–3605. https://doi.org/10.1073/pnas.0907191107
35. Verhulst NO, Qiu YT, Beijleveld H, Maliepaard C, Knights D et al (2011) Composition of human skin microbiota affects attractiveness to malaria mosquitoes. PLoS One 6(12):e28991. https://doi.org/10.1371/journal.pone.0028991

36. De Moraes CM, Wanjiku C, Stanczyk NM, Pulido H, Sims J et al (2018) Volatile biomarkers of symptomatic and asymptomatic malaria infection in humans. Proc Natl Acad Sci USA 115(22):5780–5785. https://doi.org/10.1073/pnas.1801512115
37. Correa R, Coronado LM, Garrido AC, Durant-Archibold AA, Spadafora C (2017) Volatile organic compounds associated with *Plasmodium falciparum* infection in vitro. Parasit Vectors 10(1):1–8. https://doi.org/10.1186/s13071-017-2157-x
38. Stanczyk N, De Moraes C, Mescher M (2018) Can we use human odors to diagnose malaria? Future Microbiol 14(1):5–9. https://doi.org/10.2217/fmb-2018-0312
39. Fuchs P, Loeseken C, Schubert JK, Miekisch W (2010) Breath gas aldehydes as biomarkers of lung cancer. Int J Cancer 126(11):2663–2670. https://doi.org/10.1002/ijc.24970
40. Peng G, Tisch U, Adams O, Hakim M, Shehada N et al (2009) Diagnosing lung cancer in exhaled breath using gold nanoparticles. Nat Nanotechnol 4(10):669–673. https://doi.org/10.1038/nnano.2009.235
41. Phillips M, Basa-Dalay V, Bothamley G, Cataneo RN, Lam PK et al (2010) Breath biomarkers of active pulmonary tuberculosis. Tuberculosis 90(2):145–151. https://doi.org/10.1016/j.tube.2010.01.003
42. Koo S, Thomas HR, Daniels SD, Lynch RC, Fortier SM et al (2014) A breath fungal secondary metabolite signature to diagnose invasive aspergillosis. Clin Infect Dis 59(12):1733–1740. https://doi.org/10.1093/cid/ciu725
43. Nakhleh MK, Amal H, Jeries R, Broza YY, Aboud M et al (2017) (2017) Diagnosis and classification of 17 diseases from 1404 subjects via pattern analysis of exhaled molecules. ACS Nano 11(1):112–125. https://doi.org/10.1021/acsnano.6b04930.s001
44. Peterson JW (2011) Bacterial pathogenesis. In: Baron S (ed) Medical microbiology. University of Texas Medical Branch at Galveston, Galveston
45. Berna AZ, McCarthy JS, Wang RX, Saliba KJ, Bravo FG et al (2015) Analysis of breath specimens for biomarkers of *Plasmodium falciparum* infection. J Infect Dis 212(7):1120–1128. https://doi.org/10.1093/infdis/jiv176
46. Berna AZ, McCarthy JS, Wang XR, Michie M, Bravo FG et al (2018) Diurnal variation in expired breath volatiles in malaria-infected and healthy volunteers. J Breath Res 12(4):046014. https://doi.org/10.1088/1752-7163/aadbbb
47. de Lacy CB, Amann A, Al-Kateb H, Flynn C, Filipiak W et al (2014) A review of the volatiles from the healthy human body. J Breath Res 8(1):p014001. https://doi.org/10.1093/infdis/jiv176
48. Emami SN, Lindberg BG, Hua S, Hill SR, Mozuraitis R et al (2017) A key malaria metabolite modulates vector blood seeking, feeding, and susceptibility to infection. Science 355(6329):1076–1080. https://doi.org/10.1126/science.aah4563
49. Miller JJ, Odom John AR (2020) The malaria metabolite HMBPP does not trigger erythrocyte terpene release. ACS Infect Dis 6(10):2567–2572. https://doi.org/10.1021/acsinfecdis.0c00548
50. Fan Y, Pedersen O (2020) Gut microbiota in human metabolic health and disease. Nat Rev Microbiol 19:55–71. https://doi.org/10.1038/s41579-020-0433-9
51. Mutoni d'A J, Coutelier JP, Rujeni N, Mutesa L, Cani PD (2022) Possible interactions between malaria, helminthiases and the gut microbiota: a short review. Microorganisms 10:721. https://doi.org/10.3390/microorganisms10040721
52. Day JF, Ebert KM, Edman JD (1983) Feeding patterns of mosquitoes (Diptera: Culicidae) simultaneously exposed to malarious and healthy mice, including a method for separating blood meals from conspecific hosts. J Med Entomol 20(2):120–127. https://doi.org/10.1093/jmedent/20.2.120
53. Verhulst NO, Beijleveld H, Knols BG, Takken W, Schraa G et al (2009) Cultured skin microbiota attracts malaria mosquitoes. Malar J 8:1–12. https://doi.org/10.1186/1475-2875-8-302
54. Verhulst NO, Andriessen R, Groenhagen U, Bukovinszkiné Kiss G, Schulz S et al (2010) Differential attraction of malaria mosquitoes to volatile blends produced by human skin bacteria. PLoS One 5(12):e15829. https://doi.org/10.1371/journal.pone.0015829
55. Hurd H (2003) Manipulation of medically important insect vectors by their parasites. Annu Rev Entmol 48:141–161. https://doi.org/10.1146/annurev.ento.48.091801.112722
56. Brown S (1999) Cooperation and conflict in host-manipulating parasites. Proc Roy Soc Lond B Biol Sci 266:1899–1904. https://doi.org/10.1098/rspb.1999.0864
57. Thomas F, Adamo S, Moore J (2005) Parasitic manipulation: where are we and where should we go? Behav Processes 68(3):185–199. https://doi.org/10.1016/j.beproc.2004.06.010
58. Poulin R, Fredensborg B, Hansen E, Leung T (2005) The true cost of host manipulation by parasites. Behav Processes 68:241–244. https://doi.org/10.1016/j.beproc.2004.07.011
59. Shirasu M, Touhara K (2011) The scent of disease: volatile organic compounds of the human body related to disease and disorder. J Biochem 150(3):257–266. https://doi.org/10.1093/jb/mvr090
60. Gould S, Lewontin R (1979) The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. Proc Roy Soc Lond B: Biol Sci 205(1161):581–598. https://doi.org/10.1098/rspb.1979.0086
61. Logan JG, Birkett MA, Clark SJ, Powers S, Seal NJ et al (2008) Identification of human-derived volatile chemicals that interfere with attraction of *Aedes aegypti* mosquitoes. J Chem Ecol 34(3):308–322. https://doi.org/10.1007/s10886-008-9436-0
62. Berna AZ, Schaber CL, Bollinger LB, Mwale M, Mlotha-Mitole R et al (2019) Comparison of breath sampling methods: a post hoc analysis from observational cohort studies. Analyst 144(6):2026–2033. https://doi.org/10.1039/c8an01823e
63. Berna AZ, DeBosch B, Stoll J, Odom John AR (2019) Breath collection from children for disease biomarker discovery. J Vis Exp 144:e59217. https://doi.org/10.3791/59217
64. De Boer JG, Robinson A, Powers SJ, Burgers SL, Caulfield JC et al (2017) Odours of *Plasmodium falciparum*-infected participants influence mosquito-host interactions. Sci Rep 7(1):1–9. https://doi.org/10.1038/s41598-017-08978-9
65. Pulido H, Stanczyk NM, De Moraes CM, Mescher MC (2021) A unique volatile signature distinguishes malaria infection from other conditions that cause similar symptoms. Sci Rep 11(1):1–9. https://doi.org/10.1038/s41598-021-92962-x