Feasibility of sand fly control based on knowledge of sensory ecology

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Abstract

Phlebotomine sand flies are vectors of multiple human pathogens but are well known for enabling transmission of *Leishmania* parasites which cause *leishmaniasis*; the visceral form constituting a serious public health disease and a second parasitic killer in the world after malaria. Sensory ecology shapes sand fly behavior including host seeking for a blood meal, nectar foraging, oviposition and reproduction which directly impact on disease transmission. As such, knowledge of sand fly sensory ecology including olfactory and physical (visual, tactile, thermal and acoustic) cues is essential to enable their exploitation in the development of novel tools for sand fly surveillance and control. A previous review discussed the chemical ecology of sand flies with a focus on plant feeding (nectar foraging) behavior. Here, we contribute to the existing literature by providing an analysis of the feasibility of using knowledge gained from studies on sand fly sensory ecology for control of the vector.

Key words: Phlebotomine sand flies, leishmaniasis, chemical ecology, *Leishmania* parasites, disease surveillance

Introduction

Phlebotomine sand flies are vectors of various species of parasitic protozoa of the genus *Leishmania* that cause leishmaniasis─ a group of neglected tropical diseases afflicting people in 99 countries globally with at least 700, 000 new cases reported annually [1]. Three clinical forms of the disease are recognized: cutaneous, mucocutaneous and visceral [2]. The latter represents the lethal form, causing death in more than 90% of cases if left untreated [1]. More than twenty Leishmania species cause disease in humans transmitted by over 90 sand fly species, creating a complex epidemiologic landscape in different WHO regions of the world.

Currently, there are no vaccines or universal approved medicines against the different forms of the disease, [1] making it impossible to use a single treatment regime. Severe toxic side effects also present another challenge to leishmaniasis treatment and management. Therefore, development and deployment of effective vector control tools to manage the disease is deemed pertinent and urgent.

Sensory ecology including chemical (olfactory-based semiochemicals) and physical cues (visual, tactile, thermal, acoustic) (Fig 1) drive sand fly behavior comprised of host seeking for food (blood meal or nectar), reproduction and oviposition which directly impact disease transmission. Like for most disease vectors, current control strategies developed from sand fly sensory ecology mainly target two sensory systems- visual and olfactory stimuli [3-5]. Here, we discuss the feasibility of sand-fly control based on knowledge of both chemical and physical cues.

Figure 1: Chemical and physical cues influencing sand fly interactions.

Chemical cues

Chemical cues of olfactory nature mediate the behavior of different sand fly developmental stages throughout their life history. Adults respond to olfactory cues to inform them about habitat attributes such as the presence of food (blood-meal, nectar) oviposition and to locate a mate for reproduction. Likewise, olfactory cues contribute to maintaining aggregations in juveniles (larvae) and adults [5,6]. Predators may also detect sand fly olfactory cues to increase their fitness in prey location. Alternatively, sand flies may detect predator olfactory cues to help them to modify their behavior, which is important for their survival like reported for many insects [7,8]. Understanding these olfactory-mediated interactions could contribute to developing sand fly control strategies.

Olfactory semiochemicals mediating host seeking for a blood meal. Olfaction plays an important role in the sand fly female host seeking process for a blood meal for egg development which is important for survival of the species [2,6]. Molecular studies based on DNA barcoding has revealed that female sand fly selectively obtains a blood meal from a wide variety of vertebrate hosts including humans, canids, livestock, rodents, birds, reptiles, and amphibians [9-14]. Breath odors dominated by CO_2 from these vertebrate hosts is a long-range attractant for females [15] which may combine with skin-derived odorants to attract sand flies, as demonstrated for *Lutzomyia intermedia* [16]. Of the three skin-derived compounds phenylacetaldehyde, 6-methyl-5-hepten-2-one (sulcatone) and nonadecane that activated sand fly behavior, phenylacetaldehyde and sulcatone attracted sand flies [16]. Other compounds important in sand fly host seeking behavior are described in [3,16-18]. Previous research has shown that skin-derived odorants from less-preferred-vertebrate hosts can serve as allomones for tsetse flies and mosquitoes [19]. As such, detailed ecological and molecular studies are needed to understand host selection and feeding patterns in different sand fly species, to understand their preferred and less preferred blood meal sources, and potentially species-specific feeding patterns. These studies could aid in the identification of additional kairomones and allomones for sand fly control via bait and ‘push (repellent)-pull’ (attractant) technologies to target specific species.

Olfactory semiochemicals mediating host seeking for a nectar source. Both sexes of sand flies feed on nectar for a sugar meal which may be obtained from fruits, floral and extrafloral nectar, honeydew and plant juices [20]. Plant sugars provide energy for survival, mating, and dispersal in both sexes of sand flies, and host seeking for a blood meal in females [21]. Like most insects, sand flies use multimodal cues comprised of olfactory and visual stimuli to locate sugar sources. DNA barcoding studies have revealed sugar sources for sand flies [21,22]. For example, in *Phlebotomus* and *Sergentomyia spp.,* most of their sugar sources were identified from the plant family Fabaceae, of which acacia species were the most dominant [21,22]. Laboratory studies identified benzyl alcohol, (*Z*)-linalool oxide, (*E*)-β-ocimene, *p*-cymene, *p*-cresol, and *m*-cresol as attractants for both sexes of *Ph. duboscqi* from the volatiles of acacia [21,22]. Additional field tests of synthetic chemicals of these attractants either individually or blends are needed to validate their importance as plant volatiles in sand fly olfactory nectar foraging behavior. Such tests would facilitate development and deployment of their formulations for use in attractive targeted sugar baits (ATSBs) in an attract and kill strategy to target both sand fly sexes. However, olfactory-mediated attraction to both sexes may involve other plant volatiles which would require additional molecular research to identify the food plants from the habitats of sand flies [23]. Since males are exclusively sugar feeders, a potent plant volatile attractant identified for males can be used to monitor the establishment and dispersal of males in a natural population in a sterile insect technique (SIT).

In addition to identifying nectar sources from plants, studies that highlight the impact of plant feeding on sand fly bionomics such as vector fecundity and life span could reveal secondary metabolites that are potential toxicants for use in ATSBs [24]. Since parasite infection has been shown to influence mosquito plant feeding [25] a similar study on sand flies could provide insights into the role of plant feeding on vectorial capacity and parasite transmission to inform disease mitigation effort [26]. It is hypothesized that plant feeding could indirectly benefit parasite-infected sand flies as they may reduce parasite load [27,28].

Olfactory semiochemicals mediating oviposition site selection and juvenile behavior. Sand flies inhabit different habitats to breed. For instance, whereas the VL vector *Ph. martini* has a micro-ecological preference for termite mounds, the related species *Ph. orientalis* prefers cracked black-cotton soils as a breeding site [29]. Organic matter present in these breeding sites emits olfactory cues that elicit oviposition responses from gravid sand fly females [30]. Examples of volatile organic compounds identified from odors released from these sites include 1-octen-3-ol, 6-methyl-5-hepten-2-one, α-pinene, benzyl alcohol, *m*-cresol, *p*-cresol and decanal. Of these compounds, only the *R*- and *S*- forms of 1-octen-3-ol attracted different species of sand flies into baited traps [31]. Noteworthy, these compounds are associated with microbial degradation of organic matter and may influence sand fly oviposition decisions in a concentration-dependent manner or ratios of individual attractants in blends. For some species like *Ph. papatasi*, females are known to distribute their eggs across multiple sites in the absence of such sites to maximize the ratio of reward to risk [32]. Knowledge of these multiple sites with fitness-related benefits to sand flies could reveal novel kairomones for sand fly control.

Sites with previously laid eggs and larvae releasing dodecanoic acid (DA) and isovaleric acid have been found to attract some species of sand flies such as gravid females of *Ph. papatasi scopoli* [33,34]. Notably, DA serves as an oviposition pheromone for the species *Lu. longipalpis* [35]. It is unknown how larvae of these sand flies use these chemicals in their behavior and whether they contribute to social integration, attract or repel predators. Knowledge of roles these chemicals play could facilitate their deployment as baits to aggregate larvae, and possibly to increase predation of juveniles at these sites. In some sand fly species (e.g. *Ph. papatasi*), social interaction among females can influence egg laying [32]. Likewise, density-dependent egg hatching olfactory/tactile stimuli can inhibit females from laying aggregated eggs compared to when dispersed [32,36]. The olfactory cues mediating these behaviors are yet to be identified.

Sex-aggregation pheromones. Sex-aggregation pheromones are well-known in certain sand fly species for reproduction [37-39]. Soon after emergence, adult male-producing volatile sex-aggregation pheromones attract both conspecific females and males for mating at resting sites or on vertebrate hosts [40,41]. For example, the terpenes (*S*)-9-methylgermagrene-B, 3-methyl-α-himachalene and sobralene are sex-aggregation pheromones of *Lu. longipalpis s.l,* while germacrene-B acetate is the sex pheromone of *Lu. cruciate* [40,42]. Of these semiochemicals, (*S*)-9-methylgermagrene-B has been synthesized and tested in field trials for potential use in vector control [43]. Of concern is the fact that the composition and amount of the pheromone might vary with geographical location and between species, thus, calling for a good understanding of the sensory architecture of sand flies before ultimate deployment in disease control. Also important is the need for additional studies highlighting chemical identity of the sex/aggregation pheromones in other species of sand flies.

Physical cues

Sand flies live in animal shelters, human dwellings, rocks and caves [30]. This suggests that sand flies must adapt to the physical (visual, thermal, tactile and acoustic) cues of these environments to survive. Although olfactory-mediated mating often takes place on a vertebrate host, the physical cues associated with the vertebrate host (texture, vibrational/acoustic) could influence sand fly successful mating [44]. Understanding these physical-mediated interactions represent avenues to develop control strategies against sand flies.

Visual cues. Sand flies are attracted to light traps, confirming the importance of visual-mediated cues in their behavior. Visual cues occur in the form of shape, color, intensity and duration, and all these may influence sand fly successful behavior in finding resting and breeding sites [45]. Sand fly species in the New World are highly attracted to the light-emitting diode (LED) colors, blue and green [46]. Different sand fly species may vary in the visual cues that they detect, in particular, wavelengths, but knowledge of this in many species is still unknown to provide the photosensory basis for modifying existing light traps for sand fly control.

Thermal cues. Temperature and moisture are usually inter-linked in insect behavior. As such, changes in temperature and humidity of an oviposition, resting or mating site of a sand fly will depend on the material composition of the site and humidity. Although sand flies are known to prefer cool environments to breed [2] a detailed understanding of thermal influence on specific behaviors could provide novel thermal-based approaches for sand fly control.

Tactile and acoustic cues. Besides olfactory cues, gravid females may rely on tactile and auditory cues to select suitable oviposition sites. Acoustic communication in the form of courtship and mating songs and their contribution to mating success are well studied for some species of sand flies [47,48]. Tactile and auditory cues may aid in foraging efficiency for a blood or sugar meal from a host. They may also provide risk assessment of the environment of the presence of a predator. Since social interaction in some sand fly species may stimulate or inhibit females from laying aggregated eggs [32,36], there is a good chance that tactile cues alone or combined with olfactory cues may modulate female behavior. It is unknown whether emerged larvae from aggregated eggs use tactile cues to remain aggregated or disperse. Nonetheless, knowledge of these mechanisms could contribute to developing emergence traps for sand flies.

Microbial-sand fly interactions

Microbes contribute to the behavior and ecology of sand flies through emission of olfactory cues that help them discriminate among suitable foraging resources and resting and oviposition sites. The microbes could include host pathogens vectored by sand flies (e.g. *Leishmania* parasites) or microbiota communities associated with environmental resources. In the transmission biology of sandfly-borne leishmaniasis, *Leishmania* infection alters host odors and attractiveness to host seeking adult sandflies.

This phenotype, which could imply enhanced transmission has been demonstrated in the rodent and dog model systems [49]. For example, hamsters or mice infected with *Leishmania* parasites are more attractive to female sandfly species *Lu. longipalpis* and *Nyssomyia neivai* in bioassays [50,51]. This parasite-induced effect is often considered a manipulative influence on sandfly behavior to promote host infection.

Chemical analysis of hair samples from *Leishmania*-infected and non-infected dogs implicated six volatile organic compounds (VOCs) as potential biomarkers of infection: β-hydroxyethylphenyl ether, nonanal, heptadecane, 2-ethylhexyl-salicylate, decanal, and octanal [52]. Dogs are important reservoir hosts of the parasite *Leishmania infantum* that causes zoonotic visceral leishmaniasis in humans in South America [51]. Further behavioral activity of these olfactory cues [53] could pave the way for developing odor-baited trapping technologies that incorporate potent chemical attractants, but also find potential application in non-invasive disease diagnosis. Overall, these findings indicate the importance of parasite-induced volatile cues in leishmaniasis transmission ecology [49-51].

Sand flies have microhabitat differences affecting their distribution in the environment. Olfactory cues contributed by microbes from such sites commonly used for resting or oviposition could play a role in such structuring of sandfly community or composition. Hassaballa et al., [30] showed that sand fly abundance and diversity was greater at livestock sites represented by fecal matter (organic matter) than termite mounds and houses. Clearly, microbial composition should contribute to the differential preference of these sites. Exploring the nature and identity of the microbes and specific cues could help define productive and non-productive habitats for selective targeting in sand fly control.
Plant nectar and vertebrate skin harbor diverse microbiota that also contributes to chemical cues that attract insects like mosquitoes. Microbiota associated volatiles that attract sandflies is surprisingly scarce, and likely to vary with different vertebrate and plant species that they exploit [54]. In-depth studies could reveal plants as sources of beneficial microbes (and underlying cues) which they ingest through nectar feeding, that can block pathogen development through the vector. A recent study demonstrated the natural acquisition of bacteria of the genus *Asaia* from plants [55] known for transmission blocking effect of malaria parasites in *Anopheles* vectors [56]. Understanding such association with certain plants may help explain the effect of plant feeding on vectorial capacity of disease vectors in nature. Identifying such plants could be exploited in microbial and paratransgenetic control of malaria and leishmaniasis.

Conclusions and future directions

The use of semiochemicals in sand fly surveillance and control is yet to be fully explored. Most investigations on sand fly sensory ecology focus on pheromonal communication mediating aggregation and mating which has been carried out on a few sand fly species in the New World [57]. Demonstrated impact of pheromones on reducing sand fly abundance and leishmaniasis burden [57] offers opportunity to combine tools from other olfactory signals, such as kairomones in sand fly-borne disease management in an ‘attract and kill’ strategy when combined with a toxicant. Advancement in understanding the molecular logic underpinning olfaction in sandflies is at its infancy. Genome sequencing and analysis could provide insights into the evolution of traits that characterise the vectorial capacity of different sand fly vectors. Such studies could help to identify olfactory receptors that detect key volatiles associated with the different sand fly behaviors.

Sequencing of the genomes of *Lu. longipalpis* and *Ph. papatasi* and sequence annotation identified chemoreceptors involved in pheromone detection, and evolution in wild *Lu. longipalpis* populations [42].

Through this, insight into molecular correlates of sand fly diversity can be gleaned. Also, chemostimuli identified from functional characterization of candidate genes can be evaluated for behavioral impact for use in odor-baited traps as attractants.

Finally, given the fact that physical cues also play an important role in sand fly sensory ecology, future studies in this area could help reveal additional tools to modify existing trapping technologies for more effective control of sand flies.

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References

1. World Health Organization (WHO). **Leishmaniasis fact sheet**. 2023, Accessed 7th March, 2024. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis
2. Cecílio P, Cordeiro-da-Silva A, Oliveira F: **Sand flies: Basic information on the vectors of Leishmaniasis and their interactions with *Leishmania* parasites.** Commun Biol 2022, **5**: 305
3. Pinto MC, Elnaiem DA, Machado VE, da Rocha Silva FB: **"Chapter 11: Host-seeking behaviour and its application for surveillance and control of sand flies". In Sensory ecology of disease vectors.** Leiden, The Netherlands: Wageningen Academic 2022
4. Warburg A, Faiman R. **Research priorities for the control of phlebotomine sand flies**. JVector Ecol 2011, **36**:S10-6.
5. Bray DP, Carter V, Alves GB, Brazil RP, Bandi KK, Hamilton JG. **Synthetic sex pheromone in a longlasting lure attracts the visceral leishmaniasis vector, *Lutzomyia longipalpis*, for up to 12 weeks in Brazil.** PLoS Negl Trop Dis 2014, **8**(3):e2723.
6. Bray DP, Yaman K, Underhilll BA, Mitchell F, Carter V, Hamilton JG. **Multi-modal analysis of courtship behaviour in the old world leishmaniasis vector *Phlebotomus argentipes***. PLoS Negl Trop Dis 2014, **8**(12):e3316.
7. Kempraj V, Park SJ, Taylor PW. **Forewarned is forearmed: Queensland fruit flies detect olfactory cues from predators and respond with predator-specific behaviour.** Sci Rep 2020, **10**, 7297.
8. Kannan K, Galizia CG, Nouvian M. **Olfactory strategies in the defensive behaviour of insects.** Insects 2022, **13**(5):470.
9. Posada-López L, Velez-Mira A, Cantillo O, Castillo-Castañeda A, Ramírez JD, Galati EAB, Galvis-Ovallos F. **Ecological interactions of sand flies, hosts, and *Leishmania panamensis* in an endemic area of cutaneous leishmaniasis in Colombia.** PLoS Negl Trop Dis 2023, **17**(5): e0011316.
10. de Souza DK, Addo SO, Desewu K, Nzelu CO, Mosore MT, Nimo-Paintsil S, Attram N, Appawu M, Wilson M, Boakye DA. **Investigating potential sand fly vectors after the first reported outbreak of cutaneous leishmaniasis in Ghana.** Parasit Vectors 2023, **16**:154
11. Owino BO, Matoke-Muhia D, Alraey Y, Mwangi JM, Ingonga JM, Ngumbi PM, Casas-Sanchez A, Acosta-Serrano A, Masiga DK. **Association of *Phlebotomus guggisbergi* with *Leishmania major* and *Leishmania tropica* in a complex transmission setting for cutaneous leishmaniasis in Gilgil, Nakuru county, Kenya.** PLoS Negl Trop Dis 2019, **18**:13(10):e0007712.

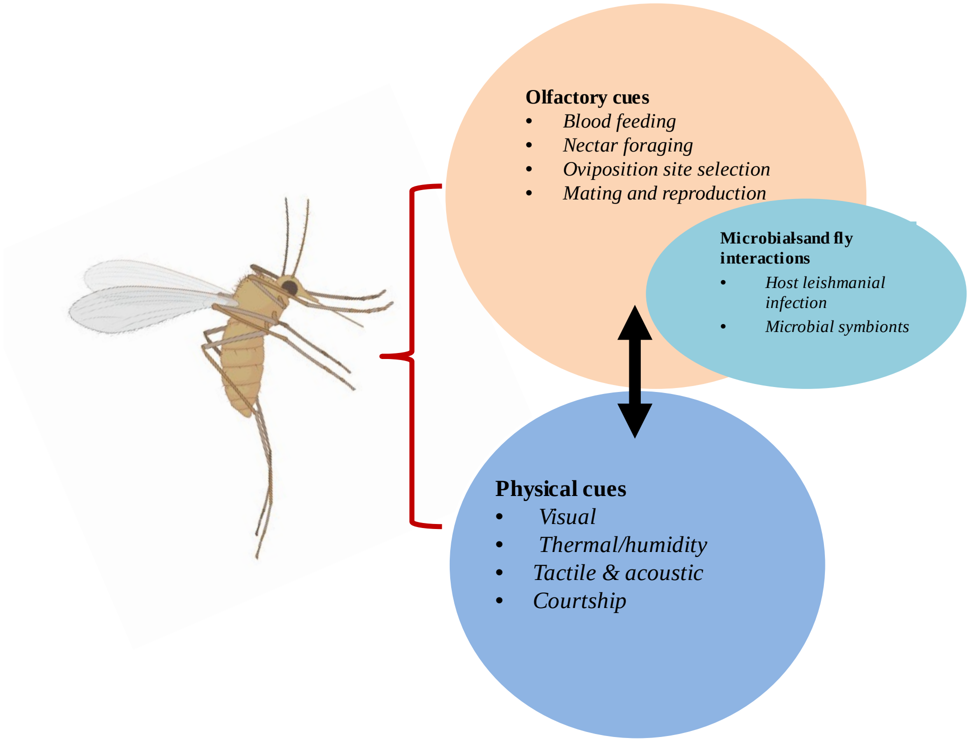
12. Abbate JM, Maia C, Pereira A, Arfuso F, Gaglio G, Rizzo M, et al. **Identification of trypanosomatids and blood feeding preferences of phlebotomine sand fly species common in Sicily, Southern Italy.** PLoS ONE 2020, **15**(3): e0229536.
13. Azmi K, Schonian G, Abdeen Z. **Specification of blood meals ingested by female sand flies caught in Palestinian foci and identification of their concomitant leishmanial infections**. PLoS Negl Trop Dis. 2020, **14**(10):e0008748.
14. Yuko E, Sang R, Owino EA, Ingonga J, Matoke-Muhia D, Hassaballa IB, Junglen S, Tchouassi DP. **Sandfly blood-feeding habits and competence in transmitting Ntepes virus, a recently discovered member of the genus *Phlebovirus*.** Biomed Res Int 2022, Article ID 4231978.
15. Benante JP, Fox J, Lawrence K, Fansiri T, Pongsiri A, Ponlawat A, Chaskopoulou A. **A comparative study of mosquito and sand fly (Diptera: Psychodidae: Phlebotominae) sampling using dry ice and chemically generated carbon dioxide from three different prototype CO_2 generators.** J. Econ. Entomol 2019, **112** (1): 494–498
16. Tavares DS, Salgado VR, Miranda JC, Mesquita PRR, Rodrigues FM, Barral-Netto M, et al. **Attraction of phlebotomine sandflies to volatiles from skin odors of individuals residing in an endemic area of tegumentary leishmaniasis.** PLoS ONE 2018, **13**(9): e0203989.
    - **The study identifies volatile organic compounds from human skin odors that attract sandflies in laboratory behavioral assays.**
17. Machado VE, da Rocha Silva FB, Goulart TM, Pinto MC. **Behavioral responses of sand fly *Nyssomyia neivai* (Psychodidae: Phlebotominae) to 1-hexanol and light**, Acta Tropica, 2022, 236, 106680
18. Bezerra-Santos MA, Benelli G, Germinara GS, Volf P, Otranto D. **Smelly interactions: host-borne volatile organic compounds triggering behavioural responses in mosquitoes, sand flies, and ticks**. Parasit Vectors 2024, **17**, 227.
19. Olaide OY, Tchouassi DP, Yusuf AA, Pirk CWW, Masiga DK, Saini RK, Torto B. **Zebra skin odor repels the savannah tsetse fly, Glossina pallidipes (Diptera: Glossinidae).** PLoS Negl Trop Dis 2019, **13**(6):e0007460. PMID: 31181060; PMCID: PMC6586361
20. Cameron MM, Pessoa FA, Vasconcelos AW, Ward RD. **Sugar meal sources for the phlebotomine sandfly *Lutzomyia longipalpis* in Ceará State, Brazil.** Med Vet Entomol 1995, **9**(3):263-72. PMID: 7548943.
21. Hassaballa IB, Sole CL, Cheseto X, Torto B, Tchouassi DP. **Afrotropical sand fly-host plant relationships in a leishmaniasis endemic area, Kenya.** PLoS Negl Trop Dis 2021, **15**(2): e0009041.
22. Hassaballa IB, Matoke-Muhia D, Masiga DK, Sole CL, Torto B, Tchouassi DP. **Behavioural responses of *Phlebotomus duboscqi* to plant-derived volatile organic compounds**. Med Vet Entomol 2021, **35**(4): 625-632
    - **The study provides evidence of attractive potential of plant-derived volatile organic compounds in a sandfly vector paving way for field validation and odor-bait development.**
23. Qualls WA, Müller GC, Khallaayoune K, Revay EE, Zhioua E, Kravchenko VD, Arheart KL, Xue RD, Schlein Y, Hausmann A, Kline DL. **Control of sand flies with attractive toxic sugar baits (ATSB) and potential impact on non-target organisms in Morocco**. Parasit Vectors. 2015,**8**:87.
24. Milugo TK, Tchouassi DP, Kavishe RA, Dinglasan RR., Torto B. **Root exudate chemical cues of an invasive plant modulate oviposition behavior and survivorship of a malaria mosquito vector**. Sci Rep 2021, **11**: 14785
25. Hien DF, Dabiré KR, Roche B, Diabaté A, Yerbanga RS, Cohuet A, Yameogo BK, Gouagna LC, Hopkins RJ, Ouedraogo GA, ET AL, **Plant-mediated Effects on mosquito capacity to transmit human malaria.** PLoS Pathog 2016, **12**(8):e1005773.
26. Torto B. **Innovative approaches to exploit host plant metabolites in malaria control.** Pest Manag Sci 2019, **75**:2341–5.
27. Palmer-Young EC, Schwarz RS, Chen Y, Evans JD. **Can floral nectars reduce transmission of *Leishmania*?** PLoS Negl Trop Dis. 2022, **16**(5):e0010373.

28. Torto B, Hassaballa IB, Tchouassi DP. **Chemical ecology of sand fly plant-feeding behaviour.** *In Sensory ecology of disease vectors*, (Eds. R. Ignell, C.R. Lazzari, M.G. Lorenzo and S.R. Hill); Wageningen Academic Publishers, Wageningen, The Netherlands ; 2022, pp 235-252.
29. Alvar J, den Boer M, Dagne DA. **Towards the elimination of visceral leishmaniasis as a public health problem in east Africa: reflections on an enhanced control strategy and a call for action.** Lancet Glob Health 2021, **9**(12):e1763-9.
30. Hassaballa IB, Torto B, Sole CL, Tchouassi DP. **Exploring the influence of different habitats and their volatile chemistry in modulating sand fly population structure in a leishmaniasis endemic foci, Kenya**. PLoS Negl Trop Dis 2021, **15**(2):e0009062.
31. Tchouassi DP, Jacob JW, Cheseto X, Chepkemoi LS, Hassaballa IB, Torto B. **Enzyme-catalyzed kinetic resolution of racemic 1-octen-3-ol and field evaluation of its enantiomeric isomers as attractants of sandflies.** Front. Trop. Dis 2024, **4**:1327349
32. McLaughlin LG, Wasserberg G. **Spatial bet hedging in sand fly oviposition: factors affecting skip oviposition in *Phlebotomus papatasi* sand flies.** Vector Borne Zoonotic Dis 2021, **21**(4):280-288.
33. Faw LR, Raymann K, Romo BN, Wasserberg G. **Larval conditioning and aging of sand fly rearing medium affect oviposition site selection in *Phlebotomus papatasi* (Diptera: Psychodidae) sand flies.** J Med Entomol 2021, **16**:58(4):1931-1935.
34. Kowacich D, Hatano E, Schal C, Ponnusamy L, Apperson CS, Shymanovich T, Wasserberg G. **The egg and larval pheromone dodecanoic acid mediates density-dependent oviposition of *Phlebotomus papatasi*.** Parasit Vectors 2020, **13**(1):280.
35. Dougherty, M., Hamilton, G. **Dodecanoic acid is the oviposition pheromone of *Lutzomyia longipalpis***. J Chem Ecol 1997, **23**:2657–2671
36. Nguyen HM, Kowacich DJ, Wasserberg G. **Temporal bet-hedging in sand fly oviposition: pharate *Phlebotomus papatasi* sand fly neonates regulate hatching time in response to organic matter and proximity to conspecific eggs**. Vector Borne Zoonotic Dis 2020, **21**(4):275-279.
37. Chelbi I, Zhioua E, Hamilton JG. **Behavioral evidence for the presence of a sex pheromone in male *Phlebotomus papatasi* scopoli (Diptera: Psychodidae).** J. Med. Entomol 2011, **48**,518–525.
38. Serrano AK, Rojas JC, Cruz-López LC, Malo EA, Mikery OF, Castillo A. **Presence of putative male-produced sex pheromone in *Lutzomyia cruciata* (Diptera: Psychodidae), vector of *Leishmania mexicana***. J Med Entomol 2016, **53**(6): 1261–1267.
39. Brazil RP, Hamilton JG. **Isolation and identification of 9-methylgermacrene-B as the putative sex pheromone of *Lutzomyia cruzi* (Mangabeira, 1938) (Diptera: Psychodidae).** Memorias do Instituto Oswaldo Cruz 2002, **97**(3):435–436.
40. Hamilton JGC. **Sand fly sex/aggregation pheromones.** *In*: Sensory Ecology of Disease Vectors. Ignell R, Lazzari CR, Lorenzo MG, Hill SR, editors; Wageningen Academic Publishers Wageningen, The Netherlands 2022.
41. Spiegel CN, Batista-Pereira LG, Bretas JA, Eiras AE, Hooper AM, Peixoto AA, Soares MJ. **Pheromone gland development and pheromone production in *lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae).** J Med Entomol 2011, **48**(3):489-95.
42. Hickner PV, Timoshevskaya N, Nowling RJ, Labbé F, Nguyen AD, McDowell MA, Spiegel CN, Syed Z. **Molecular signatures of sexual communication in the phlebotomine sand flies.** PLoS Negl Trop Dis 2020, **14**(12):e0008967.
    - **The authors identify chemoreceptors involved in sexual communication of the sandfly species *Lutzomyia longipalpis* and *Phlebotomus papatasi* based on analysis of their genome sequences and highlights genomic correlates of sandfly diversity.**
43. Gonçalves R, de Souza CF, Rontani RB, Pereira A, Farnes KB, Gorsich EE, Silva RA, Brazil RP, Hamilton JGC, Courtenay O. **Community deployment of a synthetic pheromone of the sand fly *Lutzomyia longipalpis* co-located with insecticide reduces vector abundance in treated and neighbouring untreated houses: Implications for control of *Leishmania infantum*.** PLoS Negl Trop Dis 2021. 3:15(2):e0009080.

- **In trials in selected houses, the authors demonstrate the entomological impact of a male aggregation pheromone combined with insecticide in reducing the population density of the sandfly *Lutzomyia longipalpis* vector of the parasite *Leishmania infantum*, that causes human and canine visceral leishmaniasis in the Americas.**

44. Virant-Doberlet M, Čokl A. **Vibrational communication in insects.** Neotrop Entomol 2004, **33**,121-134.
45. Shymanovich T, Faw L, Hajhashemi N, Teague J, Schal C, Ponnusamy L, Apperson CS, Hatano E, Wasserberg G. **Diel periodicity and visual cues guide oviposition behavior in *Phlebotomus papatasi*, vector of old-world cutaneous leishmaniasis.** PLoS Negl Trop Dis 2019, 13(3):e0007165.
46. da Rocha Silva FB, Machado VE, Pinto MC. **Light-emitting diodes as visual attractants to phlebotomine sand flies (Diptera: Psychodidae): A mini-review**. Acta Trop. 2023, 239:106828.
47. Araki AS, Brazil RP, Hamilton JGC, Vigoder FM. **Characterization of copulatory courtship song in the Old World sand fly species *Phlebotomus argentipes*.** Sci Rep. 2020, **10**(1):5116.
48. Vigoder FM, Araki AS, Carvalho AB, Brazil RP, Ritchie MG. **Dinner and a show: The role of male copulatory courtship song and female blood-feeding in the reproductive success of *Lutzomyia longipalpis* from Lapinha, Brazil.** Infect Genet Evol. 2020, **85**:104470.
49. Staniek ME, Hamilton JG. **Odour of domestic dogs infected with Leishmania infantum is attractive to female but not male sand flies: Evidence for parasite manipulation.** PLoS Pathog 2021, **17**(3):e1009354.
    •**The authors demonstrate that *Leishmania infantum* enhances the attraction of dog odor to the sandfly *Lutzomyia longipalpis* highlighting the importance of parasite-induced volatile cues in leishmaniasis transmission ecology.**
50. O'shea B, Rebollar-Tellez E, Ward RD, Hamilton JG, El Naiem D, Polwart A. **Enhanced sandfly attraction to Leishmania-infected hosts.** Trans. R. Soc. Trop. Med. Hyg 2002, **6**(2):117-8.
51. da Rocha Silva FB, Miguel DC, Machado VE, Oliveira WH, Goulart TM, Tosta CD, Pinheiro HP, Pinto MC. **Influence of *Leishmania* (Viannia) *braziliensis* infection on the attractiveness of BALB/c mice to *Nyssomyia neivai* (Diptera: Psychodidae).** PLoS One 2019, **14**(4):e0214574.
52. Magalhães-Junior JT, Mesquita PR, Oliveira WF, Oliveira FS, Franke CR, Rodrigues FD, de Andrade JB, Barrouin-Melo SM. **Identification of biomarkers in the hair of dogs: new diagnostic possibilities in the study and control of visceral leishmaniasis.** Anal Bioanal Chem 2014, **406**:6691-700.
53. Magalhães-Junior JT, Oliva-Filho AD, Novais HO, Mesquita PR, Rodrigues F, Pinto MC, Barrouin-Melo SM. **Attraction of the sandfly *Lutzomyia longipalpis* to possible biomarker compounds from dogs infected with *Leishmania infantum*.** Med. Vet. Entomol 2019, **33**(2):322-5.
54. Tabbabi A, Mizushima D, Yamamoto DS, Kato H. **Sand flies and their microbiota.** Parasitologia 2022, **2**(2):71-87.
55. Bassene N, Niang EH, Fenollar F, Doucoure S, Faye O, Raoult D, Sokhna C, Mediannikov O. **Role of plants in the transmission of Asaia sp., which potentially inhibit the *Plasmodium* sporogenic cycle in *Anopheles* mosquitoes.** Sci. Rep 2020, **10**(1):7144
56. Maffo CG, Sande MM, Fadel AN, Tchouakui M, Nguete DN, Menze B, Kusimo MO, Njiokou F, Hughes GL, Wondji CS. **Molecular detection and maternal transmission of a bacterial symbiont *Asaia* species in field-caught *Anopheles* mosquitoes from Cameroon.** Parasit Vectors 2021, **14**(1):539
57. Hamilton JG. **Sandfly pheromones: their biology and potential for use in control programs.** Parasite. 2008, **15**(3):252-6.

Fig. 1


Olfactory cues
Blood feeding
Nectar foraging
Oviposition site selection
Mating and reproduction
Microbial-sand fly interactions
Host leishmanial infection
Microbial symbionts
Physical cues
Visual
Thermal/humidity
Tactile & acoustic
Courtship