Mechanism and plasticity of vectors' host-seeking behavior

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The host-seeking behavior of disease vector insects is central to the transmission of pathogens. In this context, an improved understanding of the mechanisms that allow vectors to detect, identify and locate a potential host will be crucial to refine existing control strategies and invent new ones. Host-seeking is mediated by the integration of cues that are processed by multiple sensory modalities, and provide robust information about host location and quality. Responses to these cues are plastic and vary as a function of the vector's internal state, age, and previous experience. Vectors also integrate other factors such as time of day, or even the level of defensiveness of the host. Here, we review the most recent advances on the molecular basis of host-seeking behavior, with a particular emphasis on disease vector mosquitoes.

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Introduction

Vector-host interactions are largely dependent on the ability of vectors to present effective responses to olfactory, visual, mechanical, acoustic, hygric, and thermal host cues [1,2,3[•],4]. Although they can be detected in an isolated way, it is the spatial and temporal combination of these cues that provide vectors the most robust evidence of the presence and location of a potential host [5–7]. Understanding the sensory processes mediating host-seeking is therefore crucial as it bears the potential for revealing neural and molecular targets that could be used for vector control. In this context, a common approach is to quantify behavioral responses to a given host cue, identify the molecular components that

underlie its detection and processing, and demonstrate that when these components are knocked down or knocked out, the behavioral response is reduced or abolished (e.g. [8[•]]). In the olfactory realm, this has led to the identification of so called 'labeled lines' where olfactory circuits are tuned to specific odors and, when activated, lead to predictable behaviors [9]. Recent advances in the field of vector sensory physiology and neuroethology have brought invaluable insights on the neural and molecular mechanisms of host-seeking, and how these latter are modulated by physiological and environmental conditions. With a particular emphasis on disease vector mosquitoes, we will review here some of these recent advances and make an attempt at identifying outstanding questions in the field.

Neurobiological perspectives: from sensory receptors to olfactory processing
Identifying key sensory receptors

In the yellow fever vector mosquito, *Aedes aegypti*, both males and females rely on their sense of olfaction to identify humans (females for blood-feeding, males to increase their chances of finding mates) [10]. To avoid unnecessary exposure to hosts that could kill them, they need to be responsive to host cues only in the right context. Therefore, they only respond to these cues when they are physiologically ready. A combination of transcriptomic and behavioral approaches showed that these behavioral changes in host seeking behavior are correlated with changes in the expression levels of genes coding for odorant binding proteins (OBP), chemosensory proteins, as well as odorant and ionotropic receptors [11[•]]. A similar study in the malaria vector *Anopheles coluzzii* linked these transcriptomic changes to changes in antennal sensitivity, reflected in the modulation of host-seeking behavior [12]. In addition to sensory gene expression, peripheral modulation of olfactory sensitivity appears to be mediated by local (i.e., antennal) expression of neuromodulatory and endocrine genes, as demonstrated in the Chagas vector, *Rhodnius prolixus* [13,14]. Given that some of the OBPs, chemosensory proteins, and receptors identified are yet to be deorphanized in vectors, how this peripheral modulation is reflected in odor processing and representation remains to be fully unraveled. In particular, as the ratio between compounds in an odor blend reflects the valence of the blend [20[•]], modulation in the sensitivity to a subset of these compounds could be sufficient to lead to drastic modifications in how this blend is perceived by the insect. Nonetheless, several recent efforts conducted in mosquitoes have set

the stage for identifying the contribution of specific odorant receptors (e.g., sulcatone sensitive Or4 [15,16]), gustatory receptors (e.g., CO_2 sensitive Gr3 [17]), and ionotropic receptors (e.g., carboxylic acids and lactic acid sensitive Ir8a [8•]) in the detection of key volatiles that are signature of a human host.

Processing of complex odorant blends

In most instances, resources present a significant amount of variability in their emission of cues, potentially reflecting variations in their quality [18,19]. In a recent study investigating the role of mosquitoes as pollinators of an endangered orchid species, *Platanthera obtusata*, the ratio between nonanal and lilac aldehydes was shown to determine plant odor valence, that is, triggering attraction or not [20•]. The sign of the ratio between these volatiles is encoded by the GABA-mediated differential activation of the LC2 and AM2 glomeruli, which drives mosquito preference for *P. obtusata* over other orchid species that co-occur in the same sedge. Remarkably this phenomenon was also observed in mosquito species that are not naturally present in the distribution area of these plants, including the vectors *Ae. aegypti* and *Anopheles stephensi* [20•]. Although not directly related to host-seeking, this study of mosquito-plant interactions provided new perspectives on the neural mechanisms of olfactory preference, that are relevant in the context of host-seeking. Despite sharing overlapping chemical composition, each host species, and each individual host within a species, is also defined by a unique odor profile. These signatures result from inter and intra specific differences in host diet and metabolism [21], microbiota [22], but also associated skin bacterial community [23]. But how these differences serve as a substrate for individual host discrimination and preference is yet to be determined. In the context of malaria transmission, *Plasmodium* infection enhances the attractiveness of infected hosts to *Anopheles* vectors, by increasing the production of aldehydes, therefore leading to higher transmission rates [24•]. But how these changes in host odor profile are encoded by the central olfactory centers in the brain of vectors, that is, the mechanisms by which this host odor alteration by the parasite is interpreted as a more salient cue, remains an outstanding question in the field.

Plasticity in olfactory behavior

In addition to variation in the profile of emitted host cues, the individual experience of vectors can further modify how they respond to these cues, enabling them to select a subset of least defensive hosts which are the easiest to feed on [25]. Host olfactory signatures can be learned by mosquitoes and associated with the intensity of host defensive behavior, that is, serve as conditional stimulus [26,27]. Comparing four mosquito species (*Ae. aegypti*, *A. stephensi*, *Toxorhynchites amboinensis*, *Culex quinquefasciatus*) revealed that learning performance was better for chemical compounds predominantly associated with the

host species preferred by each species [27]. In the antennal lobe of these mosquitoes, stronger dopaminergic innervation and odor-evoked responses are associated with glomeruli sensitive to the odors that were most learnable by each species [27]. Mosquitoes and triatomine bugs (kissing-bugs) present daily modulation of olfactory sensitivity, which allows them to respond to host cues in the proper temporal context (i.e., when hosts are available and easiest to feed on; [28,29]. In triatomines, peak responsiveness to host cues also corresponds to the time of peak learning performance [30], but how biological clocks regulate behavior and learning abilities remains unknown.

Multimodal integration

Given the energetic cost of host seeking, tracking the most reliable sensory cues is expected to be adaptive for vectors. Therefore, olfaction is not the sole sensory modality to mediate host seeking and, at different ranges and timing during host approach, vision, thermosensation, and mechanoreception interact with olfaction to provide a complete and reliable set of sensory inputs. In isolation, warm objects [31], host odors [8•,26], and visual cues [7,32] can elicit attraction. Free-flight experiments in the presence of CO_2 demonstrated that the addition of a dark contrasting visual cue to a warm object enhanced its attractiveness [6]. In fixed-tethered experiments, CO_2 modulated the response to smaller translating visual objects, for example, stripes and squares, but not to wide-field translational visual stimuli presented under open-loop conditions, for example, expanding and regressing objects, patterns of optic flow [32]. However, when attached to a rotating magnetic tether, which allows them to control their flight turns more naturalistically, mosquitoes did not show any modulation when fixating a dark stripe with or without CO_2 [7]. In this last example, the criteria used to define fixation was for the mosquito maintained an offset between the stimulus and its orientation within $\pm45°$, it is possible that the modulatory effect could have been below the detection threshold in this particular study. Another possible explanation for this discrepancy between experimental paradigms is that the modulatory effect of CO_2 on visual responses is dependent on the behavioral context. In the studies mentioned above, mosquitoes were tested in either free flight and responding to a static visual object, rigid-tethered and responding to an object in motion, or fixed to a magnetic tether and stimulated with objects following a sinusoid trajectory, respectively. Combined with potential differences in the visual attractiveness of the dark objects that were used, these differences in the experimental paradigms could have led mosquitoes to interpret cues differently.

Two independent studies added heat to the equation and reported that visual cues enhanced the attraction of *Ae. aegypti* to objects presented at host temperature [6,7]. In

the same species, mutation of the *AaegGr3* gene, coding for a subunit of the CO_2 receptor, led to an inhibition of the response to heat sources at host temperatures, suggesting that CO_2 is required for thermotaxis [7,17]. However, in Y-tube choice assays, female *Ae. aegypti* responded to thermal cues and could discriminate between heat sources at ambient, host, or deleterious temperatures in the absence of other host cues [31,33]. A difference between the assays described above is the temporal dimension of the thermal stimulus. Gating of the thermal response by CO_2 occured when the temperature of a Peltier element was raised from ambient levels to host-range temperatures for brief heat bouts (3−5 min) [7,17]. Mosquitoes released in the Y-maze responded to heat sources already present and maintained at target temperatures [31]. The difference in the timing of the thermal presentation could have led to differences in the thermal gradient established by convection. Here again the experimental paradigm seemed to play a crucial role on the expression of mosquito responses. Altogether, these results suggest that mosquitoes will respond to a steady, continuous, heat source at host temperature [31], but will only investigate a heat source that appears suddenly in their perceptual field, contingent upon CO_2 detection [7,17]. This hypothesis is supported by results from wind tunnel assays where mosquitoes were more attracted to visual objects at ambient temperature in the presence of CO_2, but preferred the warmer of two otherwise identical objects regardless of the presence/absence of a CO_2 plume [6]. Addition of CO_2 in the wind tunnel, however, increased the proportion of mosquitoes that approached either object (i.e., ambient or warm temperature), indicating that multimodal integration triggers more robust responses towards hosts.

Host defensive behaviors

Obtaining a blood meal is essential for female mosquitoes to acquire nutrients needed for producing progeny. Their ability to survive host encounters is therefore directly driving their fitness. The particularity of vector–host interactions is that a host can, at any point, turn into a predator (via defensive behaviors) [1,5]. Surprisingly, there is only little information regarding the cues vectors use for selecting hosts based on their level of defensiveness, and even less regarding how they evade defensive host behaviors. As a general rule, a negative correlation between feeding success and mosquito density has been observed across studies, with higher densities of mosquitoes being more likely to trigger host defensive behaviors [34]. In an experiment using a tethered cow as a potential blood source, mosquitoes displayed what authors called a 'pre-biting resting' behavior, where mosquitoes of different *Anopheles and Culex* species would approach the host but find a nearby location to land, rest and wait before flying towards the host [34,35]. Given that the amount of blood available from a single host is not a limiting factor for mosquitoes, it is feasible for mosquitos to wait until host defensive responses

triggered by excessive attacks have ceased [34]. The sensory mechanisms that regulate their decision to take off and bite are, however, yet to be identified. Analyzing the swinging patterns of multiple mammals' tail, and creating a robotic mammal tail simulator, Matherne *et al.* [36•] showed that the mechanical perturbations generated by host motion were sufficient to lower mosquito landing success by up to 50%. But these mechanical cues would only be perceived once the insect is in proximity to the host. Visual cues on the other hand would likely be perceived from longer distances, where a defensive host is not yet a threat. Fur color and patterns influenced the attractiveness of horses and zebras to tabanid flies [37,38], in particular at close range, where stripes prevented flies from making controlled landings on the skin [38]. However, whether vectors use visual cues (e.g., rapid movements, general agitation) to identify threats associated with host defenses remains to be fully understood.

Concluding remarks

To obtain the blood meal they require to sustain their metabolism or produce progeny, disease vectors rely on constantly fine-tuning their sensory system to account for variations in physiological and environmental conditions. Host emitted sensory cues also vary amongst and between individuals and species. In other words, vector–host interactions represent a multimodal and dynamic system that allows vectors to maximize their chances to detect, locate, and reach the mobile and defensive organisms that transport their food and, then, get their share satisfactorily. Understanding the mechanisms and modulatory pathways that regulate behavioral decision-making in the context of vector–host interactions therefore offers the promise of revealing new direly needed targets for their control.

Conflict of interest statement

Nothing declared.

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