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Multi-Cue Integration: How Female Mosquitoes Locate a Human Host

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To reproduce, the female yellow fever mosquito has to find a human host. There are many potential cues available to guide such navigation: exhaled carbon dioxide, a plethora of skin odors, the host's visual and heat signatures and, close by, moisture. Recent work is shedding new light on how these are integrated by the mosquito in targeting a human host.

The yellow fever mosquito, *Aedes aegypti*, occurs in most tropical and sub-tropical areas worldwide and its range continues to expand into suitable regions. Females are highly anthropophilic and transmit three globally important arboviruses: dengue, yellow fever, and chikungunya. Not surprisingly then, this mosquito has long been a model for studies of mosquito host-finding behavior, in part to facilitate discovery of effective repellents. Among the established cues these mosquitoes use to locate a human host are CO_2 from exhaled breath, body odors, the host's visual silhouette, and, presumably close by, elevated levels of heat and humidity [1] (Figure 1).

In recent work, McMeniman et al. [2] were able to assess if a female mosquito's reactions to heat and skin odor were modulated by contact with CO_2 by comparing the responses of normal mosquitoes with ones engineered to lack a subunit of the CO_2 receptor (and therefore to be insensitive to CO_2). In a screened-cage assay, mosquitoes exposed to a 20 second pulse of CO_2 landed on a heated target, whereas mutated mosquitoes were insensitive to this stimulus. Similarly, in a port-entry assay, normal mosquitoes were more apt to enter a port releasing human odor plus CO_2 over human odor alone, but mutated mosquitoes were not influenced by added CO_2. Thus, in these trials, CO_2 seemed to gate the response to heat and skin odor. In a semi-field setting, however, these mutated mosquitoes had only marginally impaired (about 15%) orientation to humans compared to wild-type mosquitoes, indicating that, the in absence of CO_2 detection, other

combinations of odorants and cues can induce orientation to a host and landing.

A further study published in a recent issue of *Current Biology* by van Breugel et al. [3] using a wind-tunnel setting found that the presence of a CO_2 plume gated highly directed flights to, and landings on, an otherwise unattractive 20-cm-diameter black dot placed against the white background of the tunnel's floor. Evidently, the dark object's contrast with background was important in this reaction, because, in another wind-tunnel study [4], a dark visual target that was not highly contrasted with background failed to induce landing in the presence of a CO_2 plume. In the work of van Breugel et al. [3], this mosquito only oriented to a thermal mimic of human in close proximity and this salience, in contrast to findings of McMeniman et al. [2], seemed independent of recent contact with the CO_2 plume. The differences evidenced in these three studies [5–7] suggest that the context of stimulus presentation can determine behavioral outcomes and interpretation of stimulus integration and valence.

The distances over which these cues guide orientation, the sequence of their encounter, and how they interact have long posed challenges to exploration with diagnostic experiments. Most analyses of mosquito attraction assume that initial recognition of an upwind human host occurs over distances of perhaps 10 meters when a flying mosquito detects fluctuations in CO_2 concentration above ambient ($\sim$0.035% or 350 ppm) caused by the addition of a plume containing $\sim$4% CO_2 from human breath. The mosquito then tracks the plume upwind toward its source using optomotor anemotaxis, first

demonstrated by Kennedy [8] some 75 years ago. Kennedy used *A. aegypti* as a model to establish that flying insects navigate upwind by gauging their displacement relative to the wind flow by visual feedback and not (as many assumed) by mechanoreception of wind flow. A front-to-rear image flow indicates a course aligned with the wind flow. When Kennedy introduced his breath into a wind tunnel, mosquitoes flew upwind by gauging their displacement relative to movement of a projected floor pattern.

As an odor plume is carried downwind, turbulent forces tear the plume into odor filaments [9]. Mosquitoes are exquisitely attuned to detect the slight elevations of CO_2 in odor filaments amidst its ubiquitous background via receptors on their maxillary palps. These alter their firing rate in response to fluctuations in concentration of only 50 ppm [10]. In *A. aegypti* reiterative encounters with filaments of CO_2 induce the mosquito to surge upwind [7] while simultaneously lowering its threshold of response to skin odorants [5]. *Aedes aegypti* do not surge upwind, however, in response to individual filaments of skin odor, but instead skin odor seems to require longer intervals of plume contact as occurs within a relatively homogeneous cloud [6,7]. As mosquitoes are drawn to within a meter or so of a prospective host, skin odorants rather than the combination of skin odorants and CO_2 may guide further orientation [8] and landing, especially as they may land on body regions well removed from our exhalations [11]. The presentation of odorants including CO_2 at concentrations and in spatial distributions that reflect their occurrence in nature is a necessary prerequisite to establishing
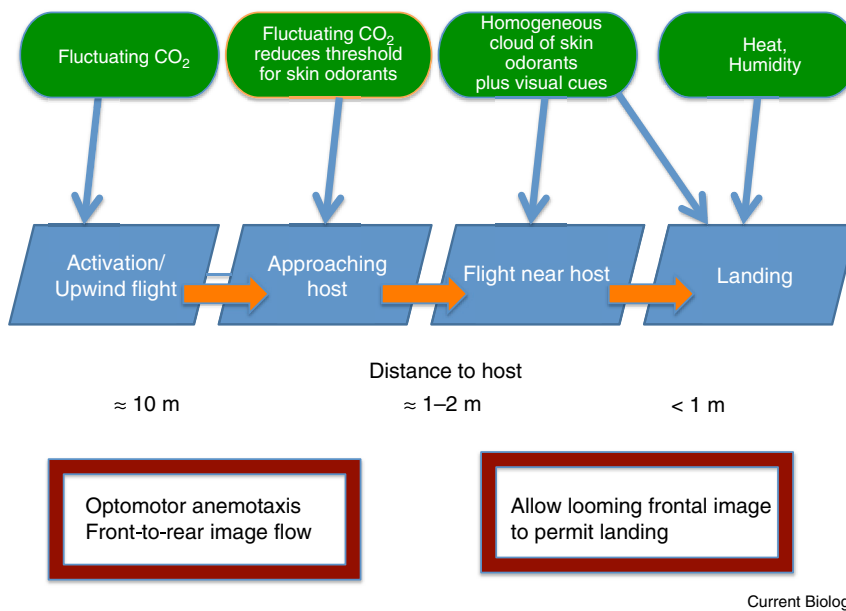
Figure 1. Presumed sequential encounter of host cues and navigational inputs in *Aedes aegypti*.
Well downwind of a human host, mosquitoes detect fluctuating concentrations of CO_2 above ambient levels, and use cues from their visual surround and optomotor anemotaxis to head upwind along the CO_2 odor plume. Repetitive contact with filaments of CO_2 sets an upwind course and also lowers the threshold of response to human skin emanations. As the mosquito nears a prospective host, it may leave the CO_2 plume and only rely on skin odorants and visual cues for close approach and landing. Landing also can be gated by contact with a CO_2 plume and by other human odorants. Heat and possibly humidity also influence landing. The relative valance and sequence of interactions of these cues in a natural context remains to be established.

their contribution to host finding. Many bioassays score activation — a change in behavioral state from quiescence to flight — as a positive response and CO_2 alone certainly induces this response in *A. aegypti* [12].

The human skin emanations that modulate orientation potentially include hundreds of compounds [13]. Individual humans vary in their attractiveness to mosquitoes and, based on the comparative attractiveness of identical twins versus fraternal twins to *A. aegypti* [14], this attribute has a heritable component. Although certain odorants such as L-lactic acid, carboxylic acids, and others induce attraction in lab assays [15], which combination of the many odorants humans emit govern orientation of *A. aegypti* (and other anthropophilic species) remains enigmatic. Likely there is no fixed signature bouquet for host finding that is common to all humans, suggesting that mosquitoes must be somewhat plastic in their response to host odorants and that other non-chemical cues assist in host location.

For a mosquito to bite, it first must land, a maneuver that requires the mosquito to allow the frontal image to expand and requires at a minimum specific visual cues [1]. Under some bioassay conditions, visual cues alone can direct orientation and landing. Kennedy [8] found that females orient toward dark stripes in evident absence of stimulation by CO_2 or skin odorants and it has long been recognized that *A. aegypti* (and many other) mosquitoes orient to and land on dark objects [16]. Another study [17] that confined mosquitoes in cages that excluded odorants found that females landed preferentially on dark rectangles.

Not all orientation scenarios need involve upwind orientation from a distance as depicted in Figure 1. Some East African populations of *A. aegypti* exhibit 'house-entering' behavior [18]. This may shelter mosquitoes from environmental extremes or it may foster a 'sit-and-wait' strategy wherein some mosquitoes use skin odorants to locate and enter an unoccupied human dwelling, settle, and then attack when

fluctuating CO_2 levels signify the presence of a living host [7]. The malaria mosquito, *Anopheles gambiae*, also exhibits house entering behavior and CO_2 gates its landing response in the presence of skin odor [19].

In controlled observations sensory inputs are often presented for many minutes at close range and decoupled from other salient cues [2,3,12]; subsequent orientation of initially quiescent mosquitoes may be opportunistic and truncate the sequence of cue encounter that occurs in nature. Disentangling which odorants besides CO_2 govern orientation and how they interact with the other host-associated cues of visual presence, heat and humidity thus remains a continuing challenge. The highly anthropophilic nature of *A. aegypti* points to a pivotal role of skin odorants in finding and selecting a suitable host [20], but CO_2 alone can induce orientation at long range and then gate response to darkly colored objects. We still have much to learn about how these multimodal interactions are orchestrated in *A. aegypti* and other mosquito vectors of human disease such as malaria. Both decoupling of stimuli and the presentation of multimodal cues in the order and at a spatial scale mimicking how these would be encountered naturally should aid in this quest. The possibility of strain differences in behavior within *A. aegypti* needs to be evaluated as well. From the perspective of preventing this dangerous mosquito from biting, the adage of wearing lightly colored clothing stands, and the use of a repellent to interfere with response to skin odorants remains the most practical tactic.

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Locomotion: Why We Walk the Way We Walk

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The way we walk determines the energetic investment needed. Humans spontaneously alter their walking style to exploit energetic opportunities. New research demonstrates the sensitivity and timing of this optimization and opens the door to discovering the underlying mechanisms.

The substrate that we walk across defines the surface landscape we have to contend with. But when walking we are also tied to another landscape, the energy landscape of 'metabolic cost' [1]. Negotiating the everyday world effectively can require using a range of walking modes. As every movement we make involves an investment in metabolic energy, the myriad combinations of stride lengths, frequencies and speeds that constitute our potential repertoire of walking motions combine to generate a surface, the metabolic cost landscape (Figure 1). Certain points on this surface will provide the best solution under a given set of circumstances. For instance, our preferred walking speed is located

near the global minimum [2] and the best combinations of stride frequency and stride length to walk faster or slower run along the valley perpendicular to the speed axis (Figure 1) [3]. Optimization of energy use is to be expected and could arise from a variety of forms of adaptation, such as adaptation of the species over evolutionary history, or of the individual over a lifetime's experience with walking. It is uncertain, however, whether this cost landscape is utilized on a moment-by-moment basis. How would an individual respond if the shape of this cost landscape suddenly changed? A new study by Jessica Selinger and colleagues [4] in this issue of *Current Biology* sheds new light

on this aspect of locomotion coordination.

Locomotion is initiated by the motor control centers of the brain, and is subsequently influenced by various ascending and descending features of the neuromuscular and mechanical systems of the body [5]. However, our bodies move in a manner that cannot neglect the influence of the physical environment. This is a complex issue, doubtless with a variety of key inputs. How does the brain choose the best strategy to drive the motion and placement of the limbs? Even for constant speed locomotion, such as walking or running on a treadmill, this question currently remains open. Although an interesting and fundamental