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Insect Olfaction: Once Swatted, Twice Shy

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While some mosquitoes are known to have an innate penchant for human hosts, new research details that they can learn, what they can learn and how they can learn.

"*From his beginning man has been prey to the lusts and appetites of hordes of insects. Very early in his history he devised methods of combatting these pests to which he was host. More often than not manual dexterity in the form of slapping and picking, as practiced in true anthropoid fashion, constituted, as it does in large measure to this day, the prime instrument of insect control.*" (Vincent G. Dethier, 1947 [1])

For as long as our species has been around, mosquitoes have been a curse — directly, because of their unpleasant and painful bites, but even more so indirectly, because of the diseases they transmit. Hence, considerable human ingenuity has been spent on repelling these age-old foes. As early as half a million years ago, our ancestors rubbed themselves in red ochre to save their skins from bites, and as human culture advanced, ever more sophisticated ointments were devised, up to today's synthetic insect repellants. But there's an even simpler insect defense, used by humans and other animals: swatting. Whenever an insect approaches us, we will instinctively swat it away, just as will cattle with their constantly moving tails. Swatting is incredibly effective, and its efficiency has even been scientifically tested: goats, for instance, when they are sedated and unable to perform defensive behaviors, receive fifteen times more insect bites than unrestrained goats [2]. But whereas for a goat it is just an automatic flick of the tail or for us an unconscious reflex-like swing of the hand, for a mosquito a swat can very well mean the end of everything. So, how do mosquitoes deal with the mechanical shocks they receive from defensive hosts? This is the starting point of a new study in this issue of *Current Biology* in which Clément Vinauger, Chloé Lahondère, Jeff Riffell and colleagues [3] investigate the effects of aversive learning on how mosquitoes pick (and prick) their hosts.

Overcoming a Fatal Attraction

Vinauger, Lahondère and colleagues [3] study *Aedes aegypti*, the yellow fever mosquito (Figure 1), which besides the eponymous disease, spreads dengue, zika, chikungunya and several other virus-borne illnesses that plague millions of people worldwide. *Ae. aegypti* is an opportunist that will lay eggs in even the tiniest puddles of water — a bottle screw cap may be enough — which has made habitats shaped by trash-producing humans their second home. Along with this 'domestication' came a preference for indoor settings and the human food that lives within them, at least for some strains of *Ae. aegypti* [4]. This 'urbanized' subspecies, *Ae. aegypti aegypti*, prefers to feed on human blood, even when other animals are around, and originally stems from a West-African forest-dwelling population that laid its eggs in water stuck on leaves and fed on monkeys, rodents and reptiles [5,6]. Like most mosquitoes, *Aedes aegypti* is a heavily smell-driven creature, known to primarily rely on olfactory cues — mainly carbon dioxide in combination with other host odors — to locate and identify suitable hosts. The shift from biting animals in general to biting humans specifically has also been accompanied by distinct shifts in odor preference, with *Ae. aegypti aegypti* strongly preferring human odors over all other animal smells. Linked to this shift is the increased expression and sensitivity of a single odorant receptor, tuned to sulcatone, a signature human volatile [7]. It is also known that other mosquitoes can draw on experience when deciding from which hosts to draw blood — after a single meal of rabbit blood, for instance, females of the malaria vector *Anopheles coluzzii* will avoid rabbits for the rest of their life cycle [8]. While it is clear that mosquitoes have an innate olfactory preference for certain hosts, this attraction can easily become fatal. In the case of the small mosquitoes attacking a much more powerful host, what nourishes the mosquito can readily kill it. This makes it potentially dangerous if the attraction were not only innate but also inflexible. So, is the innate preference for human hosts something that can be modulated?

In an exaggerated, lab-style version of the trauma experienced during swatting, Vinauger, Lahondère and colleagues [3] subjected mosquitoes to 30 seconds of vortexing in the presence of certain odors. As it turns out, pairing a blend of human smells with vortexing has a profound effect on the mosquitoes' host-seeking behavior: they lose their innate attraction to human hosts, acting just as though no odor was present at all. Given that naive mosquitoes show such a strong preference for human odors, the fact that they can overcome their natural urge after a single swatting experience is striking. Of course, the smells of a human are a complex cocktail of compounds made by ourselves and by commensal bacteria. One such compound is 1-octen-3-ol, or octenol, a well-known mosquito attractant that is regularly used in traps, and to humans smells a bit like mushrooms. When the vortexing experiment was repeated with octenol, mosquitoes not only lost their attraction, but even became repelled by octenol, to the same extent as naive mosquitoes are repelled by the all-powerful mosquito repellent DEET. Octenol is oozed by many mammals, but not by birds. Fittingly, using odors from rats and chickens, Vinauger, Lahondère and colleagues [3] show that mosquitoes (which in their

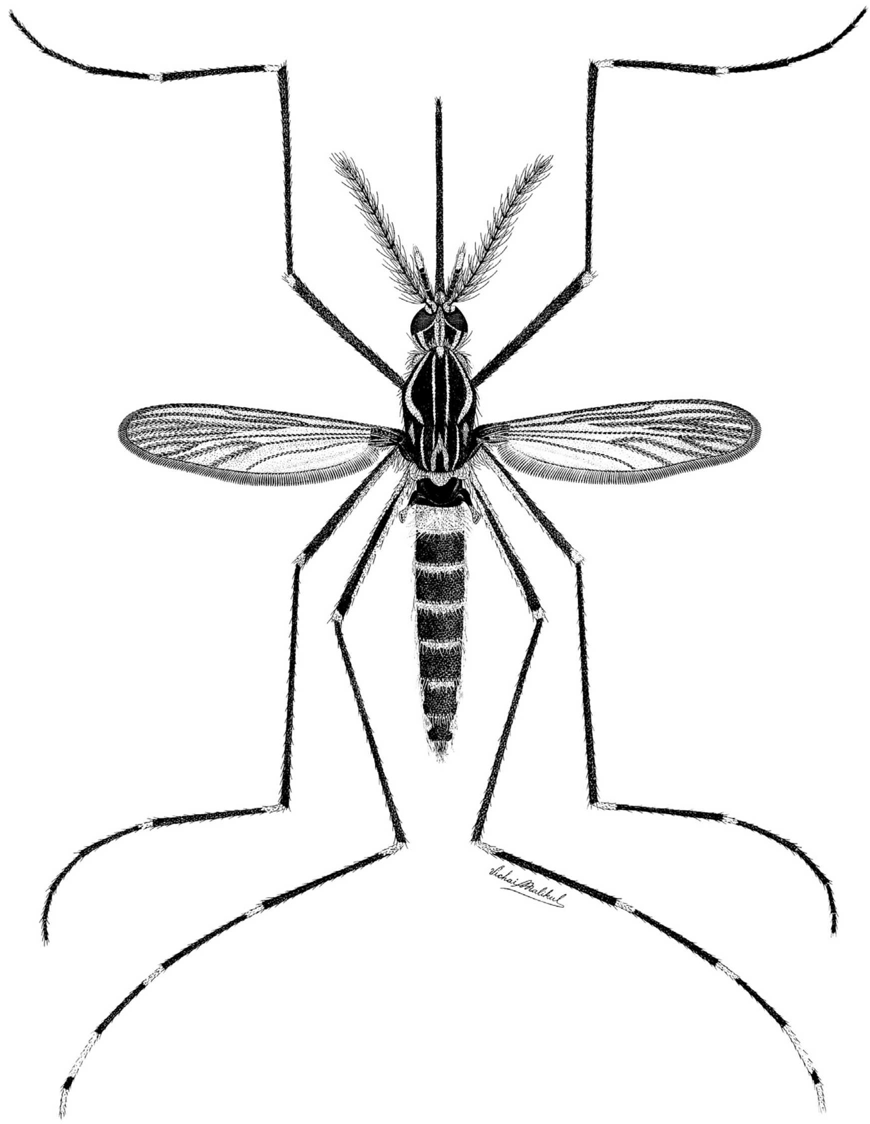

Figure 1. A fast learner.
Although the yellow fever mosquito *Aedes aegypti* — a curse in many tropical and subtropical regions — is innately attracted to humans, aversive learning can modulate this preference. Mosquitoes simultaneously exposed to human host odors and mechanical stress stay away from those odors on repeat encounters. Illustration: Vichai Malikul.

naive state equally like chicken and rat odors) can unlearn rat odors, but not chicken odors when they are paired with mechanical shocks.

This kind of aversive learning appears to make intuitive sense. For a female mosquito, the blood meal is a key event in her life — it provides the nutritious boost that enables her to lay eggs and reproduce. But a defensive host can easily end that mission in one bloody swat. Therefore, it may be important for the mosquito to not try an already irritated host again for some time (in their experiments, the memory lasts for at least a day). It would be interesting to know what the longer lasting consequences of such aversive learning are [9]. Do mosquitoes after such experiences generally shy away from humans and switch to other hosts? Or do they instead just avoid certain individuals, particularly eager swatters for instance? Of course, on the species level, learning has evidently not taken place — after all, even after millennia of swatting, these mosquitoes still attack us. The supply of hosts and the reward a blood meal offers is just too high. However, mosquitoes do tend to feed on body parts that are hard to swat, such as the ankles, and have also evolved a matching olfactory preference for smelly feet, as manifested by the mosquito's fondness for carboxylic fatty acids of the same kind that bestow Limburger cheese with a smell uncannily similar to that of unwashed feet [10].

Dopamine Does It

Vinauger, Lahondère and colleagues [3] also investigated what mediates the learning ability in the mosquito brain. The result will come as little surprise even to the neuroscientifically naive: dopaminergic neurons are required for learned aversion in mosquitoes. A number of studies have shown that dopamine signals both reward and punishment in the insect brain, through distinct sets of neurons [11]. Accordingly, mosquitoes in which the dopamine receptor DOP1 has been inactivated, through injection of dsRNA or via CRISPR-mediated gene editing, are unable to learn to avoid odors — either individual compounds or mixes — that were paired with vortexing. They behave entirely like naive mosquitoes. The DOP1 mutant mosquitoes, unable to learn, should also provide a neat tool to address what effect learning has on mosquito host seeking and avoidance in semi-natural settings, and most importantly how learning behavior and ability might affect disease-agent transmission.

To dig deeper, the authors looked at dopaminergic innervation in the mosquito brain, specifically the antennal lobe. As in all insects, the antennal lobe is the first-relay station for olfactory information in the mosquito brain, and its organization mirrors that of the olfactory bulb in their mammalian hosts in that the neurons expressing a specific odorant receptor that can recognize one or more odors all converge into a specific antennal-lobe glomerulus. Each glomerulus thus responds to one or more odors. Vinauger, Lahondère and colleagues [3] find that some glomeruli — among them the one responding to octenol — receive much more dopaminergic innervation than others. And application of dopamine changes the way these glomeruli respond to odors, causing some odors, including octenol, to become more distinctly represented in the antennal lobe, presumably affording the mosquitoes with an increased ability to identify these odors.

The differential dopaminergic innervation in the mosquito antennal lobe might provide the anatomical underpinning for the observed differences in learning ability. Not all odors are equally learnable. Previous work from these authors had shown that mosquitoes can learn to associate certain odors with blood meals much more easily than others [12]. Innately neutral odors, L-lactic acid for instance or octanol, can be learned when paired with a blood meal; the same is true for some aversive odors, like Z-3-hexen-1-ol, but not others like β-myrcene. Likewise, when different odors are paired with the swatting shock, octanol can be learned to be avoided, but nonanol cannot. Curiously, lactic acid when paired with aversive reward even becomes attractive [3]. Whether this has a real-life significance or is an oddity of the behavioral paradigm remains to be seen.

Like for any animal, life for a mosquito is a fine balance between responding in the right, reliable way to pertinent stimuli, and being able to remain flexible to adjust to inherently unpredictable situations. The mosquito has at the same time to be able to immediately recognize the smell of a promising human host and has to learn to avoid a human vigorously swatting it away. For the mosquito, whose particular ecology means that a bountiful food source can also be a deadly killer, striking this balance must be an especially formidable challenge. Learning is what makes brains flexible. But, as the work on mosquitoes shows, not every stimulus can be learned as easily as the next, and not all stimuli can be learned in the same way. Naturally, a brain limited in size and energy, like that of a mosquito, is not set up to learn every possible stimulus and every possible association. Instead, it appears that learning ability in the mosquito brain is a carefully allocated capability. Untangling how this capability is implemented in the brain — whether through differential dopaminergic innervation in the antennal lobe or through higher-level processing — and which evolutionary forces hone the mosquito's learning ability towards certain odors but not others, will be a fascinating and long endeavor, and by no means just a swat.

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Neuroscience: The Rhythms of Speech Understanding

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An understanding of the precise role played by certain characteristic brain rhythms in facilitating speech comprehension has been elusive. New research adds important insight by showing that manipulating these rhythms leads to systematic changes in how brains respond to speech.

"To be or not to be, that is the question." So begins one of the most famous speeches in all of literature. While listening to Hamlet's haunting contemplations, one can be forgiven for failing to notice the physical act that he is performing to produce these words. Indeed, speech is so central to human life that it is almost comical to pause and consider that its