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Sex pheromones: Made with a little help from my (bacterial) friends

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Males of the olive fruit fly *Bactrocera dorsalis* team up with *Bacillus* bacteria in their rectal glands to synthesize 2,3,5-trimethylpyrazine (TMP) and 2,3,5,6-tetramethylpyrazine (TTMP) from glucose and threonine. The bacterially produced TMP and TTMP are utilized as sex pheromones to attract virgin females.

Efficient communication is essential for a biologically successful life. However, finding the right medium to communicate is not trivial as most organisms live in a vibrant biotic environment where each individual from the multitude of coexisting species shouts its message out into the world. Luckily, organisms can employ a variety of different acoustic, visual, haptic, gustatory, and olfactory signals. Chemical signals, however, represent the oldest form of communication[1]. Microbes have been using chemical communication since long before complex, multicellular life evolved, and chemical communication is still widely used across the tree of life. In insects it represents the most common way of communication, especially in intraspecific communication[2]. Intraspecific-communication substances are called pheromones[3], regardless of their structure or origin, and they serve diverse functions including attracting conspecifics and mating partners, warning them about dangers, and conveying information about individual identity, directions, or food sources. Retaining a clear communication channel with closely related species amongst the chemical chatter of the animate world represents a challenge. Sharing a long evolutionary history with other species also includes sharing a chemical 'language'. Thus, young species are under strong selection pressure to modify their chemical vocabulary to ensure specific communication, requiring either signals of unique compounds or a unique blend of compounds. However, evolving novel signals is not necessarily trivial. Although existing signal molecules can be, in principle, easily modified with single novel enzymatic steps[4], their evolvability might be constrained by multiple functions[5] and de novo evolution of completely novel pathways is a highly complex process[6]. Thus, insects have been repeatedly reported to employ an alternative strategy to gain access to novel informative substances: they capitalize on the unique biosynthetic metabolism of microbes that provide an entire arsenal of potential signals novel to the animal world[7]. As a consequence, they often engage in stable (symbiotic) associations and coevolve these pre-existing compounds and their perception into specific signals, or pheromones. A new study by Ren *et al.*[8] published in this issue of *Current Biology* describes such a case: *Bacillus* bacteria harbored in the rectum of the oriental fruit fly *Bactrocera dorsalis* (Tephretidae) are the source of a male sex pheromone containing the two compounds 2,3,5-trimethylpyrazine (TMP) and 2,3,5,6-tetramethylpyrazine (TTMP) (Figure 1).

Both sexes of Tephretid flies are known to employ pheromones to attract the opposite sex, including the male-produced compound TMP[9–11]. Ren *et al.*[8] first conducted a deeper analysis of extracts from mature male recta that proved to attract virgin females. Gas chromatography coupled to mass spectrometry revealed TMP as well as TTMP, 2-ethyl-3,5-dimethylpyrazine, and 2,3,5-trimethyl-6-ethylpyrazine. A test called ‘electro-antenno-detection’ was utilized to investigate whether females were able to detect any of these four compounds. In this assay the native receptors in the antennae of an insect are employed to investigate whether they can detect a certain compound[12]. The antennae are cut off the insect and immediately attached to a pair of electrodes that can measure the electrical signal of the nerves leading from chemosensitive cells to the brain. These cells are covered with different proteins sensitive to various compounds. When these antennae are exposed to a single pure compound or complex blend, an electrical signal can be measured if the proteins on their surface are sensitive to at least one of the compounds. When Ren *et al.*[8] tested the male rectal extracts on female antennae, only two of the above-mentioned compounds, TMP and TTMP, proved to elicit such a response in female antennae. However, this insight still did not prove that they are the actual attractants from the complex extracts — other compounds could be present in the extract that were just not discovered in the chemical analysis. Thus, Ren *et al.*[8] also gave females the choice to move in the direction of either TMP, TTMP, or their combination versus a control containing only the solvent to confirm their pheromone activity. Females preferentially moved towards each of the single compounds as well as their mixture, confirming an attraction and thereby their function as pheromones.

TPM and TTPM were previously reported to be produced by bacteria of the genus *Bacillus* by metabolizing glucose and threonine[13]. As *Bactrocera* flies are also known to harbor a functionally important gut microbiota, and the male rectum continuously fills and becomes bloated when reaching sexual maturity, the authors hypothesized that bacteria in the rectal gland are responsible for the synthesis of these male pheromones[14]. They sequenced the gut microbiota of flies at different life stages by amplicon profiling of the 16S rRNA gene. They detected multiple *Bacillus* species to be present in the male rectal gland at various ages, with an increasing proportion toward sexual maturity at 12 days. In addition, they confirmed a heavy bacterial load in the rectal gland via fluorescence in situ hybridization. The gold standard to verify the bacteria as the source of both pheromones would be to completely remove them from flies, expecting a complete absence of the pheromone, and a rescue of this effect by re-inoculation with single isolates. However, this approach did not prove fruitful. Instead, Ren and colleagues were able to reduce *Bacillus* titers with different antibiotic treatments and correlate this with a reduction of pheromones. This finding supports, but does not unambiguously prove, a bacterial origin of the pheromones, as antibiotics can also have side effects on the health of the animals that may affect pheromone biosynthesis. However, the authors were able to substantiate their hypothesis by isolating multiple *Bacillus* species in axenic cultures. After fermenting single *Bacillus* isolates with the previously identified precursors of the alkylpyrazine synthesis pathways[13], glucose and threonine, they detected both pyrazines.

The microbial contribution to intraspecific communication of insects either via the modulation and supplementation of the hosts’ own biosynthetic pathways or by direct, de novo synthesis of compounds has been demonstrated before[7]. The study by Ren *et al.*[8] is interesting insofar as they identified the involved microbial partner and in addition the specific biosynthetic pathway. This insight, along with the established cultivation of both host and bacteria, opens the possibility to test further hypotheses experimentally. One open question is how these pheromone-synthesizing symbioses evolve in the first place[7]. Do they only provide these pheromones or are there other, yet unknown benefits that have to be considered to understand their ecology? Environmentally acquired microbes from oviposition sites or fermenting food are likely origins for the association of insects with bacteria that release attractive volatiles[15,16]. Both would also represent a suitable medium for transmission of a symbiont, but also remnant nutritional benefits. Insects would initially already benefit from such an attraction to nutritious diets and could co-opt the associated microbes and their biosynthetic capabilities for novel, informative volatiles. The association with males, like in this study, poses a special challenge as males usually do not transmit their microbiota to the next generation. However, for a stable association, transmission must be ensured and possible costs of harboring the microbial partner within the gut, or possibly other reservoirs, must not outweigh their benefit[7]. An experimentally amenable

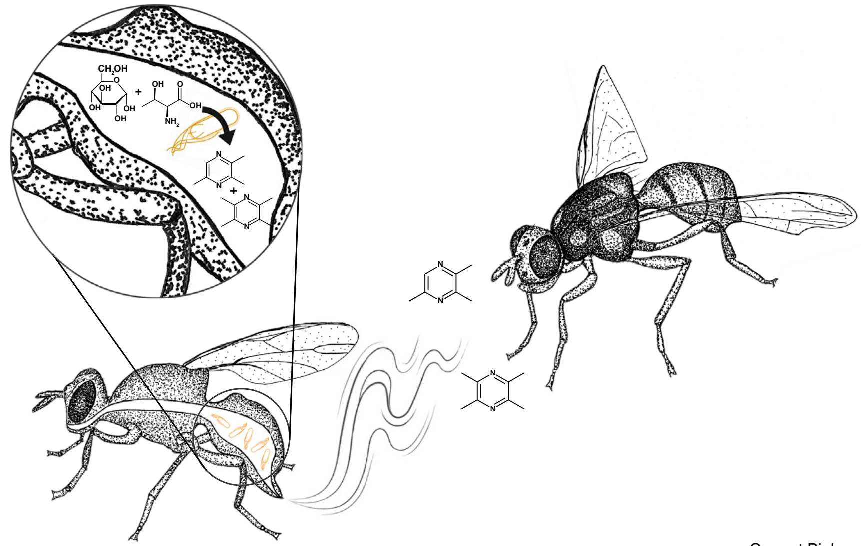

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Figure 1. Conceptual representation of bacterial pheromone synthesis in oriental fruit flies.
Bacillus sp. bacteria in the rectum of mature oriental fruit fly males, *Bactrocera dorsalis*, convert glucose and threonine into 2,3,5-trimethylpyrazine (TMP) and 2,3,5,6-tetramethylpyrazine (TTMP). Once released, these compounds act as sex pheromones, attracting conspecific females. (Drawings of flies © Benjamin Weiss; used with permission.)

system could address these questions, for example, via extended manipulation of the host–symbiont association, potentially even by genetic manipulation of the symbiont, introducing costly traits or mitigating beneficial ones. Ren *et al.*[8] already raise one possible cue: females seem to harbor the *Bacillus* species as well, raising the possibility of additional functions, including a possible benefit on female vigor or reproduction that has been suggested, but not tested, for other microbiota members of the female reproductive tract[17].

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Neuroscience: Boosting the brain

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A therapeutic effect of vagus nerve stimulation has been reported for a wide range of neurological, medical and psychiatric conditions. New research provides evidence that this effect results from extensive increase of physiological arousal and brain activation.

The vagus nerve is a mixed nerve of the autonomic nervous system. Anatomically, it runs from the brainstem to the abdominal cavity, passing through the neck and thorax. Along this path it constructs a complex network of ramifications with many body organs (hence its name, which means ‘wanderer’ in Latin)[1]. This complex system contains substantially more afferent (80%) than efferent (20%) fibers[2]. The latter provide mainly parasympathetic innervation to the heart, lungs, stomach, liver, pancreas and kidneys while also projecting to the striated muscles of the pharynx and larynx. The former carry a large array of visceral and somatic signals to the nucleus tractus solitarus, the first relay in the brainstem. These signals are then transmitted to most cortical regions, through various midbrain nuclei and thalamic pathways[1]. A new study by Collins, Boddington *et al.*[3] reported in this issue of *Current Biology* provides important insights into how this transmission is affected in response to direct electrical stimulation of the vagus nerve (VNS).

Historically, because of its accessibility and the richness of its interconnections, the vagus nerve has long been

