

One simple molecule regulates sexual behavior in *Drosophila*

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Common vinegar flies (*Drosophila melanogaster*) during mating on a kiwifruit. The pheromone methyl laurate triggers courtship behavior in males and is responsible for mating success. Credit: Anna Schroll

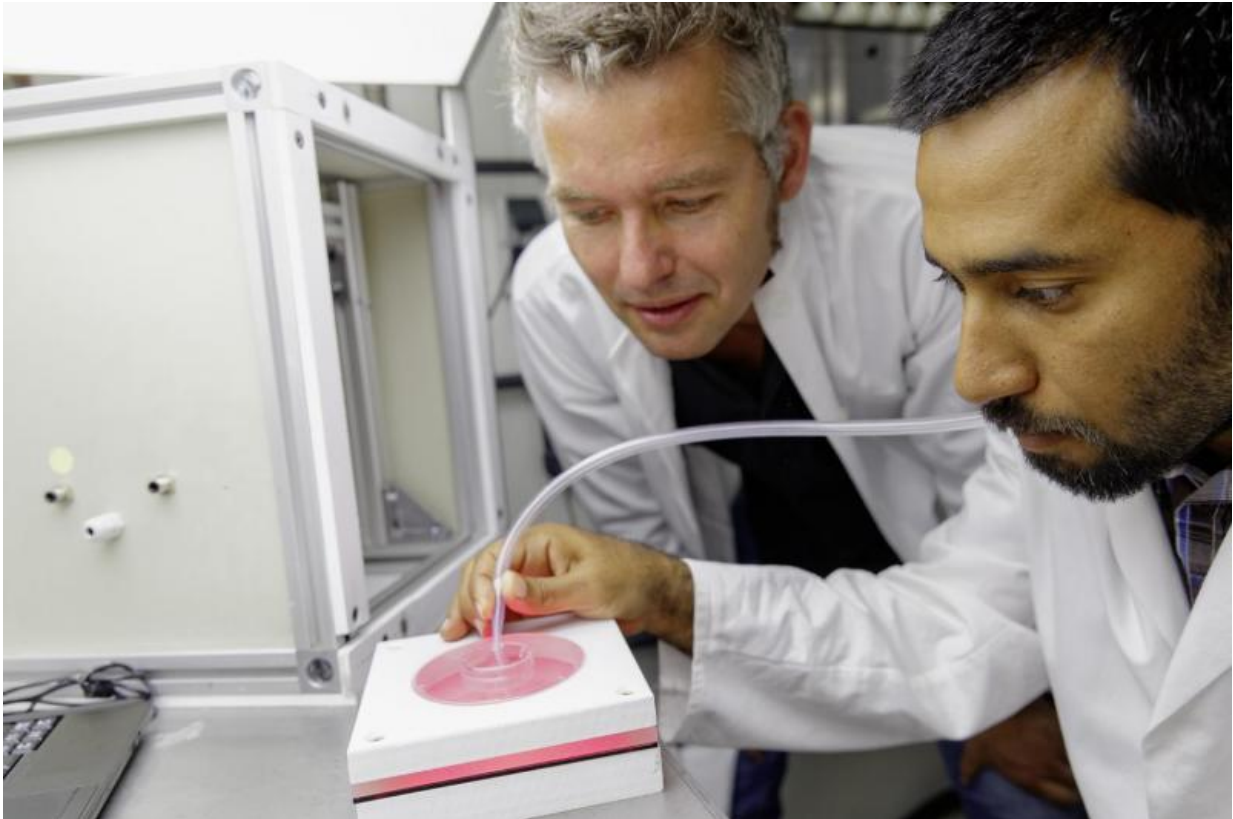
The common vinegar fly *Drosophila melanogaster* is a very well-studied

animal. For decades, the fly has been used as a model organism in genetic research; its genome was fully sequenced in 2000. However, until now researchers have failed to identify the specific pheromone in this species that leads to mating success. Although the pheromones that inhibit mating in *Drosophila* were known, the positive pheromone signal that elicits courtship behavior and mating remained a mystery. Scientists from the Max Planck Institute for Chemical Ecology in Jena, Germany, have succeeded in identifying a relatively simple molecule that is able to regulate complex mating behavior in vinegar flies: a fatty acid methyl ester called methyl laurate. Verification was a result of the combination of state-of-the-art chemical analytic techniques, physiological measurements in the fly brain, and behavioral assays.

Pheromones are signal molecules that pass information from individuals of one species to their conspecifics. In addition to aggregation pheromones, which prompt insects of the same species to come together, or alarm pheromones, which warn conspecifics about imminent danger, sex pheromones are quite well-known. Female insects emit sex pheromones in order to attract potential mating partners. The sexual attractant used by female silkworm moths, bombykol, was the first pheromone to be identified and its effect on males proven (this occurred in the late 1950s). Since then various [sex pheromones](#) in other insect species have been identified. Pheromones have also been used successfully for pest control purposes: the female attractant is employed as bait to lure males into pheromone traps. Yet the female sex pheromone ? the signal molecule which attracts males and triggers [mating behavior](#) of the vinegar fly *Drosophila melanogaster* remained until recently unidentified.

Hany Dweck, Markus Knaden, Bill Hansson and their colleagues in the Department of Evolutionary Neuroethology started their research by making a mistake: They had assumed that the odor of virgin females was especially attractive to males and were therefore looking for odors that

are exclusively found in unmated females. However, these studies did not in the end help identify the substance that would trigger mating behavior.



Markus Knaden and Hany Dweck prepare a mating experiment with vinegar flies. Experiments are performed under red light, which is not perceived by the flies. Their behavior is therefore triggered entirely by odors. Credit: Anna Schroll

Physiological and genetic data indicated that the flies must have a neuron type that responds to a specific, yet unidentified, compound within odor collections from flies. When this neuron type, which expresses the specific olfactory receptor Or47b, was missing, mating behavior in

males was inhibited. Therefore Hany Dweck started to collect odors from thousands of vinegar flies, not only from virgin females but also from mated females and even males. In order to analyze single odor components, he used the new technique of Thermal Desorption GC-MS. This combination of gas chromatography (GC) and mass spectrometry (MS) also uses the advantages of thermal desorption, which help to measure and identify even the tiniest amounts of volatile substances. Hany Dweck then performed electrophysiological measurements to test all identified compounds with respect to their ability to activate the pheromone-specific neuron. From all tested volatile compounds vinegar flies emit, only one substance triggered a strong response in this neuron: methyl laurate, a substance which analyses revealed to have a relatively plain molecule structure. "From our perspective as chemists, we were almost disappointed that a molecule which conveys something important as sex has such a simple structure," says Aleš Svatoš, who performed the chemical analyses to identify the pheromone.

Methyl laurate occurs not only in virgin females but also in mated females and even male flies. The compound is detected by a neuron which expresses the olfactory receptor Or47b; this receptor responds exclusively to methyl laurate. In males, methyl laurate triggers courtship behavior. The odor of virgin females is most attractive to males, because during mating males transfer the male-specific pheromone cis-vaccenyl acetate to females, making these mated females unattractive to other males. Methyl laurate is also detected by another sensory neuron type that expresses the olfactory receptor Or88a. Flies which lack the olfactory receptor Or47b are still attracted by the odor of other flies; however, their mating behavior is considerably reduced. Flies which lack the [olfactory receptor](#) Or88a are no longer attracted to fly-specific odors; however, their mating behavior is uninhibited. "The novel pheromone activates two different circuits: one is involved in courtship and mating of males and females, the other one in aggregation," Markus Knaden, who led the studies, explains.

Interestingly, the scientists were able to show the presence of methyl laurate in all tested *Drosophila* species. Furthermore, the same pheromone-specific neuron types which activate the olfactory receptors Or47b or Or88a respond to methyl laurate. Methyl laurate transmits a positive mating signal that seems to be conserved within many drosophilid flies. In order to circumvent hybridization, each species seems to carry species-specific cuticular hydrocarbons that inhibit mating.

The close collaboration of scientists from different disciplines was the secret of this successful basic research project. "Our study would not have been possible without this interdisciplinary approach. It is great that we can combine the results of different techniques, chemical analytics, electrophysiological measurements, imaging methods and last but not least behavioral assays. We can team up and make such a comprehensive and exciting story out of it," says Bill Hansson, director of the Department of Evolutionary Neuroethology.

In further experiments, the scientists want take a closer look at the compound methyl laurate. They are particularly interested in knowing whether the fly produces the substance itself or whether symbiotic bacteria may be involved in its production. [AO]

More information: Dweck, H. K. M., Ebrahim, S. A. M., Thoma, M., Mohamed, A. A. M., Keesey, I. W., Trona, F., Lavista-Llanos, S., Svatoš, A., Sachse, S., Knaden, M., Hansson, B. S. (2015). Novel pheromones mediate copulation and attraction in *Drosophila*.

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