

The fruit fly (*Drosophila melanogaster*) provides a model for the researchers working on olfactory research with Bill Hansson.



# Olfactory Research Is a Precision Business

Have you ever wondered how fruit flies manage to zoom in on a fruit bowl or glass of smooth red wine in the blink of an eye? Although their test subject measures little more than half a millimeter, a research team working with **Bill Hansson** at the **Max Planck Institute for Chemical Ecology** in Jena is hot on the scent of the tiny fly's olfactory system with the help of some highly sophisticated measurement technologies.

TEXT **MARCUS ANHÄUSER**

The life of the basic researcher is not an easy one. At the meeting of Nobel laureates in Lindau in 1951, the wife of one of the scientists asked the famous pheromone researcher Adolf Butenandt how she could get hold of these "sex-attractant substances" he was working on. When Butenandt explained that he had studied the pheromones of butterflies, the lady replied with disappointment: "Oh, Dr. Butenandt, why did you waste your time with butterflies?"

One can only imagine what this woman would have said to Bill Hansson. The Director of the Department of Evolutionary Neuroethology in Jena is not "wasting" his time working on butterflies, but on even less impressive flies with a penchant for rotten fruit. These pests, which can be found in every fruit bowl in summer, are commonly known as either fruit flies or vinegar flies. The fly, sometimes also referred to as the laboratory researchers' pet, is probably also the only creature whose Latin name, *Drosophila melanogaster*, is almost as well known as its common one.

Bill Hansson, who is Swedish by birth, has been studying this small insect's sense of smell for a total of nine years, and since 2006 at the Max Planck Institute for Chemical Ecology in Jena. The decision to focus on this test subject is, of course, no coincidence: "No other animal offers us so many possibilities as *Drosophila*," says Hansson. The fly can be modified genetically and therefore manipulated like a model kit: "You can remove building blocks, reshape building blocks, or exchange a red block for a blue one," says the Max Planck Director, somewhat oversimplifying the processes involved.

## ODOR COMPASS WITH KNOBBY SNOOTS

It must be said that, at first glance, the fly does not seem to be a particularly ideal subject for researching olfaction, compared, for example, with the silk moth, the laboratory animal traditionally used in pheromone research. The fruit fly is smaller and its behavior is not as effusive as that of the moth: "When a male moth smells a single molecule of the female's attractant,

whoosh, it's off and nothing else matters," says Hansson. In *Drosophila*, the reaction tends to be less "clear" for various reasons, including the fact that pheromones don't play any major role for flies over great distances.

This difference can also be observed in the olfactory organs of the two species. The male moth's finely forked and – relative to the size of the insect – large antennae sit enthroned on the animal's head like a cross between fern and antlers and demonstrate with impressive clarity the significant role played by olfaction in the moth's sex life. The corresponding organ in *Drosophila* takes the form of two conical knobs that sit between the comparatively enormous compound eyes. The term olfactory bulb couldn't be more appropriate. Somewhat lower down, just above the proboscis, sit two other small knobs, the maxillary palps, which are also used for smelling.

Hundreds of fine olfactory hairs, the sensilla, sprout from the antennae and maxillary palps. An odorant molecule must make its way into these hairs to be recognized. "In principle, each olfactory hair functions like a miniature



top | The fruit fly breeders from Jena analyze microscope images: Silke Sachse presents the latest insights into the fly's brain to her boss Bill Hansson and colleagues Dieter Wicher and Markus Knaden (l-r).

bottom | The incubator in the olfactory researchers' laboratory provides a nursery for the next generation of flies.

nose," says Hansson. The odorant molecules submerge into a protein-containing solution, the sensillum lymph, through pores – insects have an otherwise impermeable exoskeleton. The molecules are received in the sensillum lymph by odor-binding proteins that accompany them to their final destination: the olfactory receptors.

### DEDICATED LINE TO THE OLFACTORY CENTER

The odorant molecule docks at a receptor, which sits on one of the one to three dendrites, the extended arms of a nerve cell. There, the message "odorant molecule docked" is transformed into an electrical signal. The signal migrates across the nerve cell's dedicated line, the axon, directly into the fly's olfactory center. "Just as in humans and all other animals, the olfactory receptors are the only peripheral receptors that conduct signals directly to the brain," says Hansson.

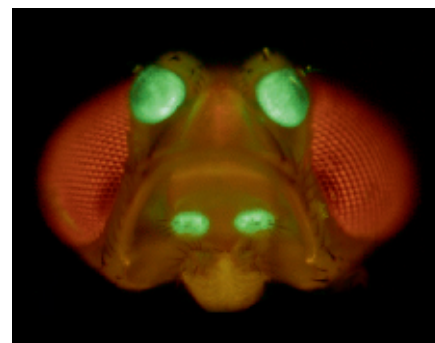
The fly's olfactory brain, the antennal lobe, consists of numerous spherical nerve nodes. These nodes, known as glomeruli, look like a group of small balloons. Each of the 1,200 nerve pathways with a total of 45 different receptor proteins innervates a corresponding balloon. The "olfactory balloons," in turn, are interconnected by neurons and are linked with higher areas of the brain via projection neurons – thus making it possible, perhaps, for the smell of an over-ripe apple to conjure up, in the fly's inner compound eye, an

image that "makes its proboscis water," as it were. "We are quite familiar with all of these morphological elements of the olfactory system," says Hansson, "but we do not know exactly what each of them does."

Hansson's team needs an entire arsenal of microscopes to be able to study all of this process in the tiny flies. There is hardly a room in the laboratory that doesn't have one of the high-tech devices, and anyone who wanders through the laboratory will see that these microscopes have little in common with the traditional ones we all remember from our school days. The day of the simple optical microscope is long gone; every possible technical option is now used to push the old system to the limit, increase the resolution or make only certain areas of the object visible. The names – confocal laser scanning microscopes, multi-photon laser scanning microscopes, fluorescence microscopes and inverse microscopes – merely hint at how it all works.

### NOT ALL MOLECULES IN AN ODOR EXCITE NEURONS

Various devices are stacked up to the left and right of each microscope workstation. Hansson stops at one of them: "This is our specialty," he says. The object in question is a gas chromatograph, a device that splits a smell into its molecular components and analyzes them. The chromatograph prints the result on paper as an odor curve that



Observed while smelling: The illuminated points show active nerve cells in the fly brain, which glow neon green through the exoskeleton.

shows the peak values for each individual component. The device also blows the odorant molecule directly at the fly in a gentle stream of air.

The researchers record in real time under the microscope how the flies – or, more precisely, their receptor-equipped nerve cells – react to the smell. Each microscope table has a computer and a monitor beside it. "We combine the odor analysis with the spectra of neuronal activity and allow the antennae to tell us, so to speak, which of the hundreds of molecules transmitted by a banana actually work on the fly," says Hansson.

Silke Sachse, head of the Optical Imaging Research Group, literally observes the flies as they smell. In order to obtain focused images of a live animal the size of an apple seed, she clamps the animal to a 3-millimeter-wide copper slide. Sachse pushes the



narrow part of the fly between the head and chest into a 0.1-millimeter-wide gap in the slide, making it look as though it were wearing an oversized neck brace. She secures the head in place with a little wax. The animal must be kept completely still to ensure that the microscope images are not blurred. The Max Planck scientist then pushes the slide under the microscope lens. A video camera records the image, enlarged by a factor of between 100 and 400, which can then be examined in comfort on the computer screen.

### A MUTANT AS A TEXTBOOK EXAMPLE

For her tests, the biologist uses a mutant fly in which the olfactory process in the antennae, palps and brain can be made visible almost by magic with the help of a dye: "This transgenic *Drosophila* produces a fluorescing dye that colors all of the olfactory sensory neurons," explains Sachse. Nothing special can be seen here in normal light conditions. However, as soon as the scien-

tist switches to fluorescence, the window becomes dark and the antennae and palps glow neon green. "We can measure through the cuticle," says Sachse. The dye shines so brightly that it shows through the paper-thin exoskeleton of the olfactory bulbs.

It is not quite so easy to observe the olfactory brain. "To do this, we cut open a window in the head cavity," explains Sachse. And all that in micrometer-sized dimensions. *Drosophila's* brain is just a little more than half a millimeter across, an antennal lobe is one tenth of a millimeter, and a glomerulus is ten times smaller. "It really is incredibly small." Even after many years of research, Silke Sachse is still awed by the dimensions in which she works.

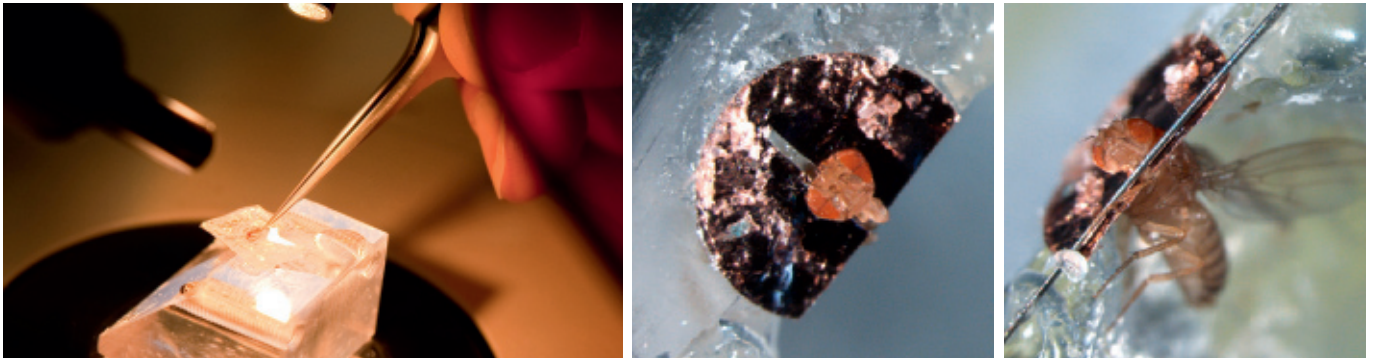
When there is something to be smelled, the olfactory brain also glows bright green. But the dye, which is called chameleon, can do much more – it can indicate changes: "The dye binds calcium. The more calcium it binds, the brighter the antennal lobe glows," says Sachse. The greater the stimulus to a nerve cell, the more calcium ions

stream into the cell. The more calcium streams into the cell, the better it binds with the dye and the brighter the cell glows in the fluorescent light.

### GREEN SHEET LIGHTNING OVER THE OLFACTORY BRAIN

The entire process as seen in the film of the recording is reminiscent of sheet lightning erupting over the olfactory brain: a brief flash here, a garish glint there, depending on which odorant molecule the antenna receive. In this way, Sachse obtains activity patterns that are typical for every smell and that can be matched up with the peaks in the olfactory curve: "I can now see exactly which neurons are active with which smell and how strongly activated they are." She extracts individual shots from the short films and transposes the activity into other colors: red for intense activity, green for average activity and blue for no activity.

While Silke Sachse observes and analyzes the olfactory system of the fly without ever touching it, her colleague



left | Fly researcher Silke Sachse prepares her miniscule laboratory animal for video recording.  
 above | To ensure that the recording is as clear as possible, Sachse pushes the head of the fruit fly through the 0.1-millimeter-wide gap in the slide. Once it is secured in place by this "neck brace" and a drop of wax, the animal cannot blur the image.

Dieter Wicher penetrates to even smaller dimensions. He works his way through to where the odorant molecule comes into contact with the fly: the olfactory receptors in the sensilla of the antenna. "This is particularly interesting because insects are known for their ability to perceive odorants in very small concentrations," says Wicher. The fruit fly needs only ten or a hundred molecules in a cubic centimeter of air to sniff something out. "That is a million times more sensitive than the human sense of smell," says Hansson: "We usually need a few hundred million molecules or more to actually smell something."

The extreme sensitivity of the insect nose is partly explained by the structure of the receptors and the way in which they transmit the "odorant molecule arrived" signal. Wicher and his fellow scientists identified a previously unknown receptor type using the patch-clamp method: using pipettes, which act as electrodes and whose finely polished tips have an internal diameter of just one hundredth of a milli-

meter – a human hair is five to ten times thicker – the researchers measure the current flowing through individual ion channels in the range of billionths of amps directly on the nerve cell. Hansson, Wicher and their colleagues presented their findings in the journal *NATURE* in April 2008.

The receptor usually transmits the signal in a multi-phase process via so-called G proteins located on the inside of the cell membrane. At the end, the messenger substance cAMP (cyclic adenosine monophosphate) pours into the cell. This transmits the signal on to ion channels located in a different part of the membrane. But this takes time. The channels open up, eventually developing an electrical potential with calcium, sodium and potassium ions. From there, the information is transmitted electrically as far as the olfactory brain.

Wicher and his colleagues discovered that the receptor and ion channel in the membrane of the insect neurons are immediate neighbors and are not distributed across the membrane as is the case in humans. In insects, they

form a two-part entity known as a dimer. "We already knew that this was a dimer, but the fact that the second protein was the ion channel is something our measurements proved for the first time," says Wicher.

### INSECT RECEPTORS ARE DESIGNED DIFFERENTLY

The immediate proximity of the receptor and channel offers key advantages: the signal can take a shortcut. Instead of taking the long route via G protein and cAMP production, the receptor and ion channel can short-circuit. They do this if large concentrations of the odorant molecule flood the receptor. However, if the odor trail is weak, the receptor switches to the traditional biochemical path via G protein and cAMP messenger substance, an option that is still shorter than it is in the mammalian cell. "Stimulus transduction in insects is thus a far more sensitive process than that involving the olfactory receptors of other animals," explains Wicher. >

The researchers still do not fully understand the system. A number of questions remain unanswered, such as: What form do the individual steps in the G protein signal chain take? What regulates the receptors?

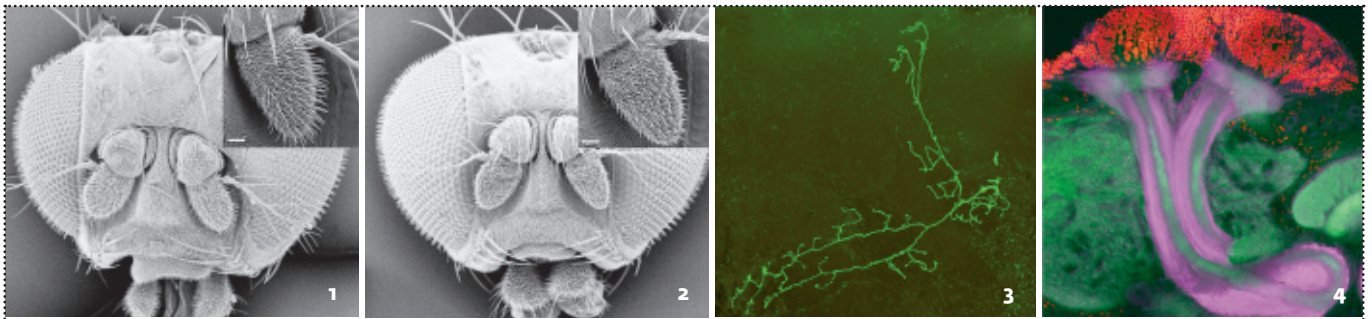
One thing appears to be clear, however: the different design of the olfactory receptors in insects as compared with mammalian receptors is an indication of the fact that the two olfactory systems developed independently of each other and do not – as previously assumed – have a shared

origin. “It appears that this really is a special design found only in insects,” says Wicher.

**WHY ODORS MAKE FLIES GET A MOVE ON**

Once the fly has smelled something with its uniquely constructed receptors, Hansson’s other colleague Markus Knaden takes over. Knaden needs none of the numerous microscopes standing around the lab. He is not interested in looking inside the fly, preferring to ob-

serve the entire animal instead. Knaden studies the behavior of the flies and thus the last link in *Drosophila’s* olfactory system. The questions he would like to answer are fundamentally simple ones: What does the fly do when it senses a smell or a component of a smell? Does it follow the odor trail, does it fly away or does it remain disinterested? The behavioral ecologist discovered in his initial experiments that this is not as easy to research as might be assumed with such a supposedly simple creature as a fly.

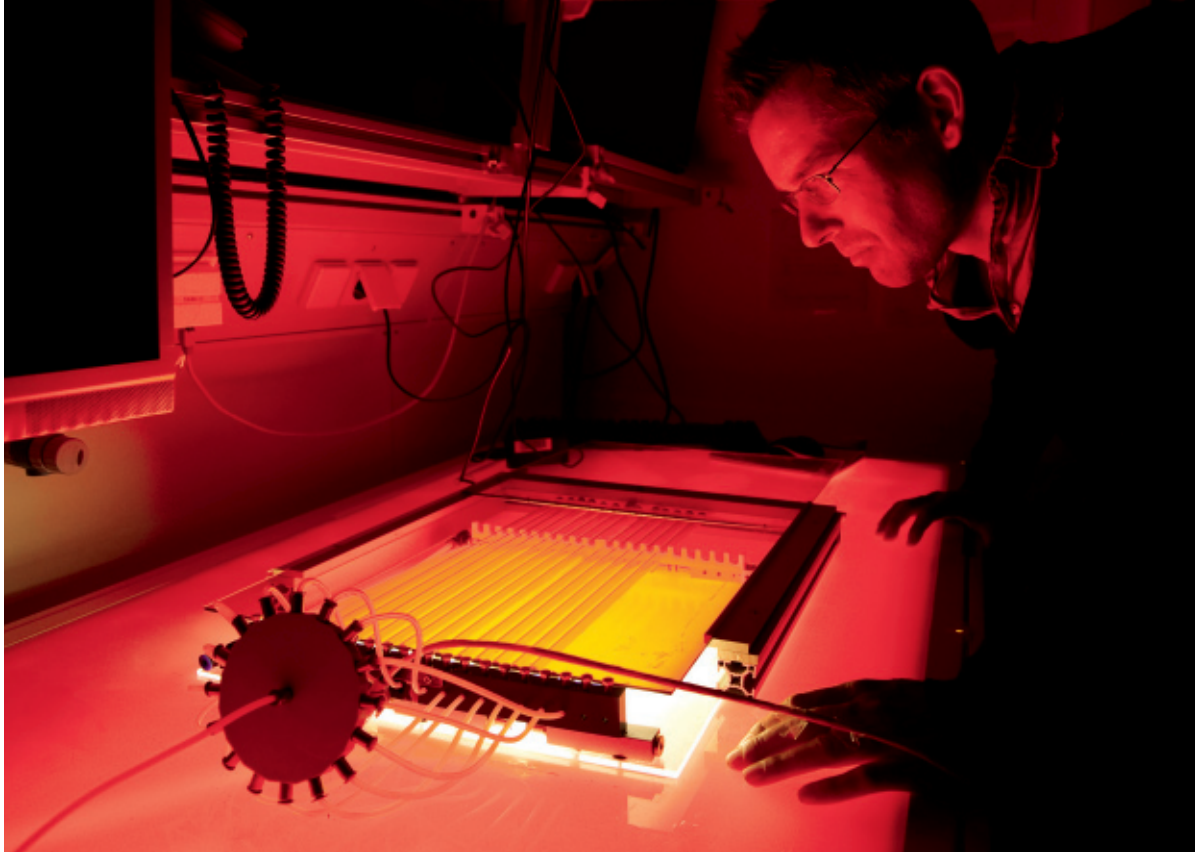


1. The smell of noni fruit repels *Drosophila melanogaster*. 2. *Drosophila sechellia*, on the other hand, is attracted to it. 3. Flies store odors in the mushroom body. 4. The mushroom body consists of Kenyon cells.

**DROSOPHILA AND THE NONI FRUIT**

In order to find out how *Drosophila’s* sense of smell adapted to different living conditions in the course of evolution, Bill Hansson’s team compared several *Drosophila* species. However, “from an evolutionary perspective, the olfactory system of *Drosophila* is very conservative,” says Hansson. In reality it has hardly changed at all – except in the case of one species in which it was dramatically different. A particularly fussy fruit fly lives in the island world, in the tropical climate of the Seychelles, north of Madagascar. *Drosophila sechellia* loves noni fruit and noni fruit alone. The fly not only loves to eat the fruit of the Indian mulberry tree *Morinda citrifolia*, it also lays its eggs there. “The fruit has a very particular smell,” notes Hansson, “a mixture of pineapple and Gorgonzola.” The cheesy smell is a sign of a high acid content. It is so high in the yellow noni fruit that other flies normally die

when they eat it. Not so the Seychelles variant, which is fully adapted to the exotic fruit and, indeed, highly dependent on it. “The dependency is reflected in its olfactory system,” says Hansson. Most of the neurons in the antennae are calibrated to the two odorants found in the special noni smell. They are also incredibly sensitive. The flies can sense the noni smell when only a billionth of a milligram is present in the air. Even the olfactory brain has adapted to the delicacy, which also acts as a nursery for the species: two of the glomeruli receive all of the signals from the noni neurons from the antennae. “And they are three times bigger than the corresponding glomeruli in *Drosophila melanogaster*,” says Hansson. While their relatives take flight when they encounter very high concentrations of the noni smell, *Drosophila sechellia* simply cannot resist the yellow fruit.



Increased efficiency: With his specially designed wind-channel model, Markus Knaden can flood ten *Drosophila* with smells simultaneously, as opposed to a single fly.

Such questions are usually approached using a standard experimental setup. The fly sits in a wind tunnel at the foot of a rod that it can climb up as required. A smell then floods the channel and the fly's reaction is observed. "But only a single animal can be studied with this setup. This is very time consuming, given that we want to test a number of odor components on different individuals," says Knaden. With this in mind, the biologist devised a setup to enable him to examine ten flies simultaneously in parallel channels.

### RESEARCH CARRIES NO GUARANTEE OF SUCCESS

He can then introduce whichever odorant he chooses using an elaborate system. The behavior of the flies is recorded by a camera and later analyzed on the computer. On paper, and when everything was ready, it looked perfect. However: "When we put the flies in the tubes for the first time and introduced the odorants, nothing happened," says the behavioral researcher, his frustration still obvious. Knaden and his two colleagues tried every-

thing they could think of, but the lab animals refused to play ball.

Now he has to come up with another idea. Perhaps observing each animal individually again? Or altering the test setup in some way? He doesn't know what he is going to do yet. The entire process from planning to the initial experiments has already cost him eighteen months. But that's how it goes with research sometimes – there is no guarantee of success. Even Nobel laureate Adolf Butenandt and his fellow scientists had to deal with numerous setbacks before he finally succeeded in isolating the "sex-attractant substances" of the butterflies.

Was it all a waste of time, as the shocked lady said? Butenandt's research marked a pioneering advance in the understanding of the olfactory process. And if a justification of basic research of this kind were required, it can be found here: every single bark beetle trap in use today is based on the olfactory research carried out on insects. In this instance, however, the smell in the traps does not lure the male beetles to their loved ones, but rather to their death. ◀

### GLOSSARY

#### Dendrite

Branching of a nerve cell, often similar to the crown of a tree. The contact points that a nerve cell receives from other nerve cells sit on the branch-like extensions.

#### Axon

The long fiber-like extension of a nerve cell that transmits electrical nerve impulses from the cell body.

#### Fluorescent dye

Dye with the capacity to absorb energy-rich light (such as UV light) and emit low-energy light.

#### Patch-clamp method

A method that makes it possible to measure how an individual ion channel can change its form and thus the current flow in the course of a few millionths of a second. The term patch refers to the small section of membrane under the pipette that also acts as a measurement diode. The membrane area is kept at a specified potential during the measurement process.

#### Sensillum

Small sensory organ in or on the cuticle of insects. It consists of sensory cells, each with a hair-like extension (cilia).