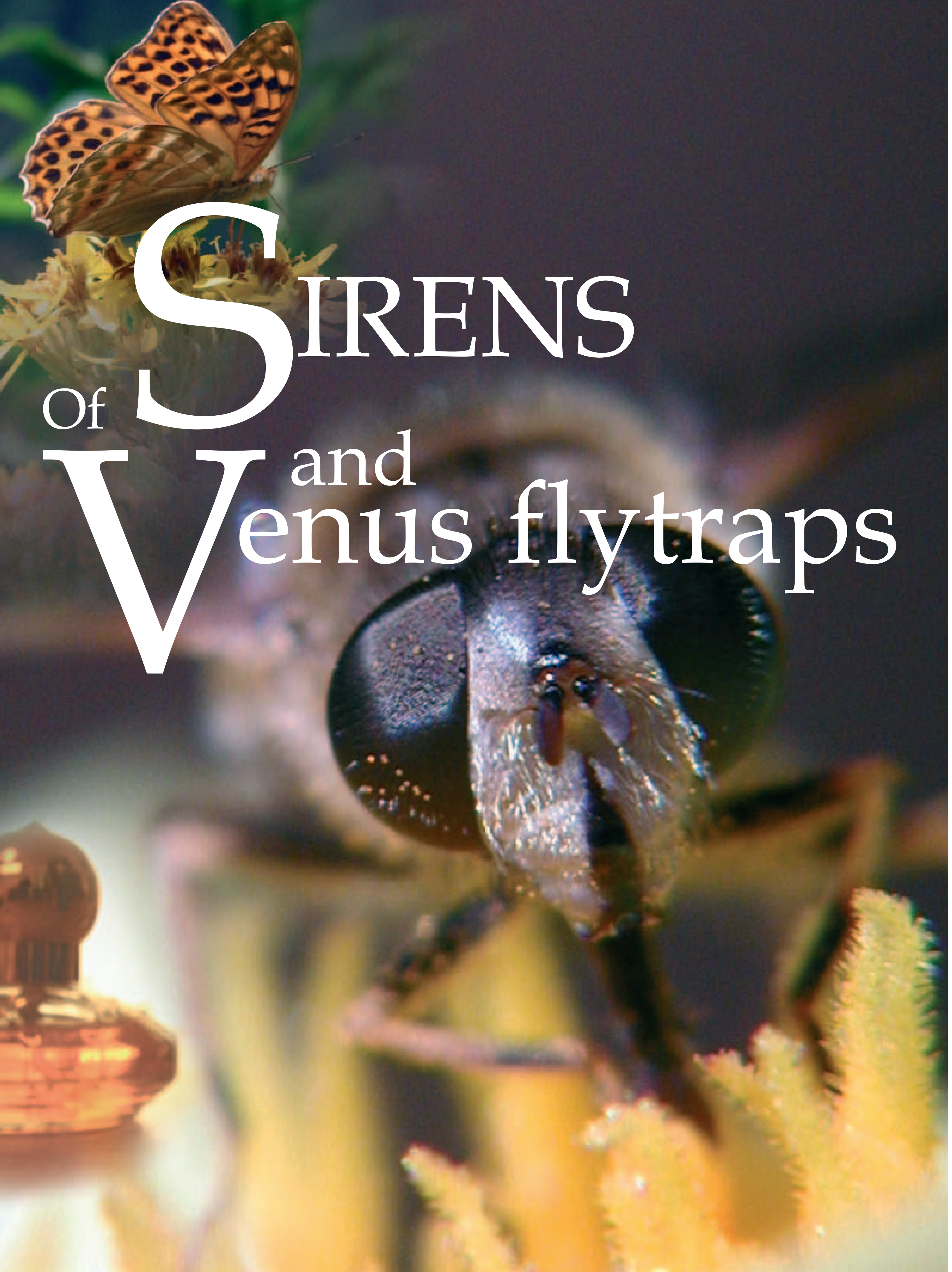


Of Sirens and Venus flytraps

A relatively new field of research into natural products and one that has a great deal of development potential is chemical ecology. It is a research area of a decidedly interdisciplinary nature and is based on chemical and biological approaches in equal measure. Chemical ecology describes the complex mechanisms influenced by chemical signals which are involved in controlling the many and varied interactions between living beings. So chemical ecology deals not only with the identification and synthesis of ***semiochemicals*** and questions related to their ***biosynthesis***, but also with investigating structures of the ***chemoreceptors*** involved and how they are interconnected when passing on and processing signals in the nervous system of the recipient. Finally, questions relating to phenomena which form part of evolutionary biology, behavioural research and ecology as well as aspects of molecular-biology also belong to the field of chemical ecology. In short – it's about the impact of low-molecular natural products ("small molecules") in their natural environment. The Max-Planck-Gesellschaft values this field of research so much so that it went to great lengths to found an Institute for Chemical Ecology several years ago in Jena.

Semiochemicals communicate information

In addition to optical, acoustic and tactile stimuli, animals, plants, and micro-organisms – whether they live in water, on land or in the air – use a wealth of chemical signals to communicate information. The basis of these specific messages are chemical substances which act as a chemical channel of communication and as such are tools in what is undoubtedly the oldest system for the transfer of information. Molecular recognition – so the interaction of chemical substances with other molecules from their environment and the ensuing, reproducible consequences this has - is at the very source of life.

Insects, which as small life-forms in a vast environment – often also in the dark – have to find sources of food, mates and hosts but also have to evade predators, have developed a highly sensitive sense of smell. The receptor organs are their "antennae" or "feelers", which are equivalent to the nose of mammals and which react to even just a few molecules of specific semiochemicals. The chemical structures of such compounds have been particularly well researched in insects. Communication within an insect species is generally based on mixtures of several (volatile) substances, which thanks to their qualitative and quantitative composition form a code unmistakable for that specific species and which can certainly be said to indicate elements of a "chemical language". Usually these signals, which are used especially in sexual attraction, in the context of social behaviour, and for defense, are released from special glands, and the biologically active compounds, similar to the cosmetic formulation of a perfume, are embedded in a matrix of attendant substances such as solvents, fixatives or stabilisers.

Attempt by a male bee to copulate with a flower ("pseudo copulation") which attracted him to pollinate it by imitating the scent of the female bee.

Signals effective intraspecifically – so within a species – are called ***pheromones***. Those effective interspecifically – so between different species – are subsumed under the term ***allelochemicals*** and are called ***allomones***, ***kairomones*** or ***synomones***, depending on their respective biological function.

Allelochemicals
Substances which transmit chemical messages between different species of life-forms.

Allomones
Allelochemicals which only serve the producing organism itself. Chemical weapons such as tetrodotoxin, the poison of the Japanese puffer fish which has spelt the untimely end of many a gourmet, come under this category.

Biosynthesis
Formation of metabolic products from simple precursors with the help of enzymes; for instance, the production of sugar from carbon dioxide and water.

Chemoreceptors
Proteins usually found on the surface of the cell, or glycoproteins, which can interact with smaller-sized molecules (semiochemicals q.v.), thereby changing their shape. The transformation on the cell surface is recognized by the inside of the cell, enhanced by a cascade of further reactions and turned into nerve impulses or metabolic activity.

Kairomones
Allelochemicals which serve solely the recipient. For example, bark beetles are led to sources of food and breeding places by components of the tree resin. The bark beetle's predators locate their prey by their scents.

Pheromones
Chemical signals which enable communication between life-forms of the same species and which trigger a reaction in the recipient (usually a change of behaviour).

Semiochemicals
Chemical substances (many are volatile) containing specific information of importance to the recipient.

Synomones
Allelochemicals which benefit both communication partners.These include scents used by flowers to attract pollinating insects.

Sticky trap with sex pheromone dispenser (centre) to monitor population dynamics of the codling moth. Some trapped males can already be seen.

Insecticides
Insect-killing agents.

Contact poisons
Poisons which take effect as soon as the body surface of the target organism comes into contact with it.

Pheromones and other semiochemicals – not just for environmentally-friendly pest control

Pheromones can be used successfully in plant protection (integrated pest management). For example, several species of moth which as pests cause severe economic damage to agriculture can be controlled using synthetic pheromone traps. If dispensers loaded with female pheromones are spread out over a large area, the male moths' orientation is confused when hunting for their females because of the overdose of the substances. This leads to far fewer offspring as a result. This method has proven its worth above all in the protection of cotton fields and viticulture. In the center of Burgundy (France), where the world's most expensive red wine is produced, the growers take great pride in being able to protect the entire winegrowing area from grapevine moths using this "confusion technique" (confusion sexuelle).

A different technique is used in so-called monitoring systems. Here, pheromone traps are distributed regularly across the area which is to be monitored, and the population density as well as the reproductive success (gradation) of the target insects are monitored in order to determine the best time to use *insecticides* and subsequently to check their efficency. Finally, pheromones – especially for bark beetles and scarab beetles – have been used successfully to trap insects en masse. There are different approaches to this. Specific locations can be targeted (for example using trap-like boxes) to deploy a combination of pheromones and *contact poisons* (trap-and-kill) or pheromones containing micro-organisms harmful to insects (trap-and-affect) in order to achieve the desired protection.

Furthermore, the use of pheromones to trap insects is by no means an original invention of the human intellect. Certain species of spider do not spin webs to catch their prey but instead produce a component of a female sex pheromone particularly widespread amongst moths and therefore a substance that attracts males of many different moth species. The spiders trick their victims into their vicinity (like the famous sirens on their rocks) to then kill them with the help of a sticky thread skilfully hurled at them. Many types of orchids imitate the pheromones of insects to attract the males of these species to pollinate them. For the same reason, or also in order to catch insects, some plants imitate the odour of rotting meat (Venus flytraps).

TYPES OF CHEMICAL COMMUNICATION

Knowledge of the "chemical language of insects" is important because:

insect pheromones enable selective pest control reducing the use of insecticides by deliberately and specifically disrupting the relevant communication channels;

insect pheromones are the ideal model substances to study neurophysiological aspects of smell;

as components of overarching communication systems, insect pheromones contribute to our understanding of ecological interconnections.

Mammals, too, make use of chemical signals to transmit information; however the structures of the relevant compounds are still largely unknown to us. One indication of the archaic nature of chemical signals is the fact that the structure of the pheromone, which female Asian elephants produce to attract the bulls for mating is identical to the principal component of the sex pheromone of many moth species. And that's not all – frontalin, a "typical" bark beetle pheromone, is also released by elephants. The enantiomeric composition of this chiral substance changes depending on the physiological state of the animals and thereby indicates readiness to mate.

AND WHAT ROLE DO PHEROMONES PLAY IN HUMANS?

Saying someone "stinks" as a way of saying we don't like them in general may well have more to do with the hygiene standards and class differences of former times than anything else as there is no evidence of the existence of semiochemicals that influence human behaviour as directly as in the insect world. This is something that has been studied very carefully by the perfume industry. What is remarkable, however, is that the sexual pheromone of the boar, a substance structurally closely related to human sexual hormones, is also present in the armpit sweat of men. The same substance, incidentally, is also contained in celery and truffles, known aphrodisiacs – and is the reason why "truffle pigs" are used successfully to track down the precious mushrooms.

What is striking about humans is that they take great pains to get rid of their own body odour, which certainly exists and is mainly produced by micro-organisms on the surface of the skin, to then spray or rub on a mixture of foreign substances (perfume) onto the then anonymous and "odour-free" body to give it an "individualized" odour or fragrance. This desire among humans to change and determine their odour themselves has been known from as far back as antiquity. Nowadays, the advertising industry uses this particularity any subtle way it can to boost the sales of an entire range of economic branches of activity.

More recent studies now indicate, incidentally, that human pheromones do indeed play a role in attracting the opposite sex – but on a completely different, microscopically small level. Here the female ovum seems to guide the male sperm with a seductive violet odour to then block entry to stragglers with a deterrent substance. This discovery could be used in developing natural contraceptives which confuse the sperm in the hunt for their target.

Peaceful (?) coexistence of a peacock butterfly and a bumble bee on a flower – in reality they are competing for food.

The amazing structural coincidences amongst semiochemicals in living beings from completely different areas indicate that they are the products of widespread, fundamental metabolic flows and that micro-organisms may also be involved in their biosynthesis.

Identifying semiochemicals – a special challenge

In nature, most chemical signals are only emitted in extremely small quantities. As you may be wondering how the chemical composition of a signal code can be deciphered, here we would like to briefly outline approaches to structure determination of a "biologically

A male butterfly spreads its feelers to detect scent signals, e.g. pheromones from the female.

A female moth with a raised abdomen and protruding pheromone glands (calling behaviour) attracts the male by emitting sex pheromones.

Electrophysiology
Study of the mechanisms of nerve-stimulus conduction at macroscopic and molecular level (neurophysiology) and tracking of the electrical impulses involved.

Gas chromatography
Standard method of separation (without decomposing) of volatile substances. Also routinely used for quality control (e.g. perfume) and in environmental analysis.

Mass spectrometry
Analytical method to determine the atomic composition of molecules and molecular fragments. The relevant ions are recorded after having passed magnetic and electric fields. Combining this technique with gas chromatography is the most common method to analytically investigate complex mixtures of naturally occurring volatile substances.

Solid Phase Micro Extraction (SPME)
A plastic fibre with the dimensions of an injection syringe is covered with a polymer which adsorbs (and concentrates) volatile substances emitted by the subject of investigation. It suffices to leave the device in the vicinity of the source of the scent (e.g. insect or flower) for a time or to touch the subject briefly (e.g. in order to study substances on its surface). Subsequently, the device can be directly inserted in the injection port of a gas chromatograph. This allows several tests to be run on the same subject, for instance depending on its physiological condition (age, copulation), the time of day or a situation (attraction, deterrence etc.).

active compound" in the laboratory. The substances released into the environment by living animals can, for example, be captured by an adsorbent. Alternatively, the relevant glands can be prepared and extracted with the right solvents from freshly killed animals. A particularly efficient, non-invasive method has proven to be ***Solid Phase Micro Extraction (SPME)***. In a subsequent test, the behaviour of the animals is used to test whether the sample extracted really does trigger the biological phenomenon being investigated, so attracts the animals, for example. This is then the key indication that the extract does indeed contain the target semiochemical. To investigate volatile compounds, combinations of ***gas chromatography*** and (high-resolution) ***mass spectrometry*** [GC/(HR) MS for short] or Fourier Transform ***infrared spectroscopy*** [GC/FT-IR for short] are the tools of choice as they combine high separation efficiency with high detection sensitivity and important information on the structures of the target substances. The small quantities of pure individual components that can be isolated are usually not sufficient to use other spectroscopic methods such as NMR.

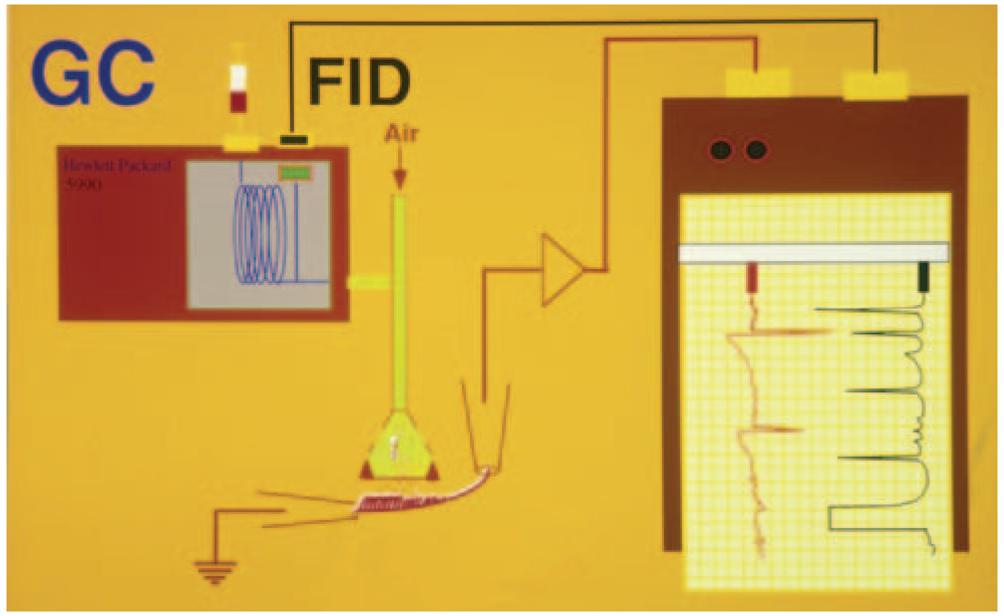
A cockchafer attentively spreads its feelers (antennae) to detect scents (signals) from members of its own species or plants.

Which of the many compounds detected in the gas chromatogram are of biological significance, however, and which belong to the matrix? Here the combination of gas chromatography and ***electrophysiology*** [GC/EAG for short] helps. The stream of compounds eluting from the column installed in the gas chromatograph is split; one part is channelled to a conventional detector which usually uses physico-chemical properties of the substances leaving the separation system, the other is analysed using a biological system. If semiochemicals of insects are being investigated, an insect antenna is removed from the insect's head and prepared so that it is fixed between two electrodes. This preparation can then be used 20 to 200 minutes. Whilst the conventional detector indicates every substance leaving the gas chromatographic separation system, with the antenna only those compounds that actually have receptors give rise to a signal, whereby the potential jump triggered through the nervous conduction of the stimulus can be up to several millivolts.

The semiochemicals detected by the antenna must then be investigated for their chemical structure. The steps required for this are part of the standard of modern organic-chemical microanalysis. The mass spectrum of the target substance is compared with the data saved, and it may be possible to identify the semiochemical immediately using its fragmentation pattern. What helps here is that even from picogram amounts of the target compound both the molecular mass and the exact atomic composition can be determined. If the substance is not recorded in a database or is entirely new (which analysts enjoy), chemical micro-reactions can be used to determine certain structural features and their position and spatial arrangement in the molecule. This then allows a structure proposal to be developed which must then be tested using synthetic compounds. If all the data of the synthetic product match that of the natural substance then it counts as identified.

Of particular significance in this context is the so-called absolute configuration of a compound, which describes the exact spatial orientation of the atoms in a molecule: two otherwise identical molecules can behave geometrically like the left and right hand. The absolute configuration of a molecule is particularly important for its biological efficacy because of the interaction with the receptors this requires: just like the right hand really only fits properly into the right glove and the left into the left glove. When all of the parameters of the natural substance now match those of the synthetic product, the ***experimentum crucis*** must be performed to prove its function as a semiochemical under natural conditions. This tests whether in the biological test with synthetic material the same phenomenon can be triggered as with the original extract or with the living animal. So, to stay with the example we selected, to attract a particular insect species. If this is the case then it's drinks all round.

Wittko Francke



GC/EAG apparatus: the right-hand curve plotter shows all the volatile substances of the sample. The left one shows only those detected by the insect antenna (here: one in a higher, the other a lower concentration)

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Links on the Web

The International Society of Chemical Ecology
www.chemecol.org/society/society.htm

Infrared Spectroscopy (IR spectroscopy)
Analytical procedure to identify the composition of substances and, where appropriate, their quantitative composition in mixtures. When energy is added the atoms within a molecule can start vibrating. If this so-called excitation energy (e.g. IR radiation) is continually changed, the so-called IR spectrum is created through absorption of the energy of the corresponding wavelength, which shows specific groups of atoms present in the molecule through characteristic bands. Similar to gas chromatography coupled with mass spectrometry combining IR spectroscopy with gas chromatography allows complex mixtures to be studied.

A naive Blue Jay is sick after eating a conspicuously-coloured monarch butterfly because it contains considerable quantities of defensive compounds (allelochemicals). The next time it encounters a monarch butterfly the bird will remember ….