On the Trail of Love of the Silk Moth

The females of many moths and butterflies attract the males with a beguiling scent. In 1959, **Adolf Butenandt** unraveled the chemical nature of the attractant he had recently investigated at the **Max Planck Institute of Biochemistry**.

TEXT **BIRGIT FENZEL**

This is also a long-distance relationship of sorts: over distances spanning several kilometers, the males of many butterfly species find females that are willing to mate. How they accomplish this was long a well-kept secret of the butterflies, and one that spurred on insect lovers and entomologists in equal measure. It was almost exactly 50 years ago that a Nobel laureate in chemistry found the right approach to solving this mystery. A year before Adolf Butenandt took office as the third President of the Max Planck Society, he published the chemical structure of the scent with which female butterflies attract their suitors.

The question of how male butterflies find their way to the females had already long fascinated insect lovers and entomologists alike. And evidently not just them – the words of Adolf Butendandt in his research report reveal an early enthusiasm for this subject: "Even today we enjoy reading the fascinating accounts by I.H. Fabre, written more than 50 years ago, which tell of a single female Emperor moth kept in a wire cloche at an open window from 8 pm to 10 pm attracting a total of 150 males over a period of 8 days, some from 'kilometers away'."

Chinese researchers provided more precise details about the range of this mysterious attractant of the female butterflies by releasing males from a train traveling away from a gauze cage containing attracting females. A good quarter of the male butterflies found the females over a distance of eleven kilometers – an achievement bordering on the miraculous as the Münchner Merkur newspaper commented at the time.

It is no coincidence that many different theories regarding these enigmatic, silent siren calls of the female butterflies were circulating at the time. Some assumed that "vibrations that can travel over incalculable distances in connection with actual diffusion of matter" were behind this phenomenon – and indeed Butenandt mentions this theory in an article dating from 1959 in which he presented his knowledge about the love-life of butterflies, but probably simply for the sake of completeness.

As a renowned expert in hormonal questions, he was more interested in another approach that other researchers were also following. In a series of experiments, they had already found out that the females of certain butterfly species produce sexual and species-specific substances in special glands that are used to attract males. Experiments were conducted in a number of places and with various butterfly species in order to isolate the scents for more detailed chemical analysis. The knowledge gained from 30 years of butterfly pheromone research was, however, "discouragingly modest."

With this as their starting point, Adolf Butenandt and his colleagues set off on the track of the scent at the Kaiser Wilhelm Institute in Berlin-Dahlem in 1939. The researchers wanted to identify the biochemical properties of the gland secretions and determine the threshold at which the enticing substances began to take effect. They focused on the silk moth, *Bombyx mori* – a furry white type of moth with a squat body shape and a pronounced propensity for lethargy. Butenandt anticipated that this exotic laboratory species (it had not been used for such experiments before) would have certain advantages. The species is easy to breed, and the creatures are undemanding in terms of food, climate and light conditions. In addition, mature specimens cannot fly – a fact that greatly facilitated the experiments in open dishes.

The other behavior of the fat moths also made them a suitable test species in the search for the sexual attractant of lepidoptera. First of all, the females emitting the scent carry their secretion store around with them in two yellow sacs at the end of their abdomen, just like perfume bottles in a handbag. Second, because of their extremely phlegmatic lifestyle, the males make excellent indicators for the beguiling substances. In fact, they only move if they detect the presence of a female or her scent. Then they are transformed into living spinning tops with shimmering wings and circular searching motions gravitating in the direction of the higher concentration of the glandular scent.

To begin with, the researchers plundered the attractant scent sac of around 7,000 female moths. At 100 milligrams, the yield of a waxy substance was not exactly opulent. Further examination showed that this must be a "lipid-soluble, neutral and unsaponifiable alcohol" that is "resistant to diluted acid and alkali, but sensitive to oxidants." They named the new substance Bombykol.

In order to determine the quantity of substance required to trigger arousal in the males, Adolf Butenandt and his colleagues placed the fat *Bombyx* males in flat dishes.

What might the silk moth attractant look like? This question interested Adolf Butenandt, seen here in the 1950s at his desk at the Max Planck Institute of Biochemistry.

They then held glass rods moistened with a petrol ether solution containing various concentrations of the tantalizing pheromone in front of the olfactory organs of the test moths.

In these experiments, the scented rod proved to be a genuine magic wand. Beginning at a certain threshold, it brought the male moths – that had been decidedly inert until then – to life and even made them dance at higher doses. After several years and many experiments, the researchers had developed a solid qualitative test for the comparative evaluation of attractant preparations of varying degrees of purity.

Münchner Merkur from July 9/10, 1960

» Professor Adolf Butenandt had approximately 500,000 hormone glands processed in order to obtain a full 12 milligrams of this hormone and to explain its chemical structure. (...) He set out the following perspective: With the aid of the attractant, all male insects within a certain area can be enticed into a lethal trap so that the females remain unfertilized.

According to Butenandt, the researchers defined the unit of attractant as "the quantity by weight of substance (...) contained in 1 ccm of a petrol ether solution, with which (...) a clear positive reaction can be triggered in at least 30 out of 60 animals." Experiments showed that the original version of the pheromone obtained from the female butterflies "had to be enriched by several powers of ten to produce the pure attractant."

Years would pass before Butenandt and his colleagues succeeded in identifying the precise chemical structure of the pheromone. The war years had their impact on the work of the researchers, and although larger quantities of butterflies could still be obtained via Japan and Spain, the resulting yield of the substance was far too low to solve the mystery. Nevertheless, the researchers were able to accumulate valuable knowledge about the "behavior of the attractant in the application of chemical and physical separation methods."

The 1950s heralded the return of fruitful times for butterfly research at the institute. In 1953, Butenandt and his colleagues had 313,000 female moths available that had hatched from 1,000,000 cocoons. The researchers enriched the glandular secretion to such an extent that they obtained three milligrams of a preparation of which a hundred-thousandth of a microgram – a millionth of a gram – was sufficient to send half of the tested males into raptures of love. According to Butenandt, this quantity was not sufficient for a full analysis, "but it was concluded from their behavior that the attractant must contain two conjugated double bonds and more than ten carbon atoms."

Six years later, the researchers finally isolated enough substance for a more precise examination. From around 500,000 glands of female silk moths, they obtained 12 milligrams of a crystallized colored ester from which the colorless attractant itself is extracted. "It is a double-unsaturated alcohol of the formula $C_{16}H_{30}O$ and contains the attractant in 10^{-10} γ!" wrote Butenandt. At that time, the unit γ was used for a microgram. In the preparation, the attractant was thus 100,000 times more concentrated than in the product of the previous analysis: just one ten-billionth of a microgram bewitched half the males used for the scent test.

After 20 years, Butenandt had finally found the substance that constituted the love navigation system of moths and butterflies and produced it in pure form – a pioneering achievement in the field of pheromone research that would influence generations of researchers to come. Although designed as basic research, Butenandt had already been thinking of the practical benefits, such as combating pests with specific sexual attractants. "But that is still all in the future," he wrote at the time. Now, 50 years later, scent traps for moths are found in the kitchens and wardrobes of many households. At least in this respect, practical applications have become a reality.

In other respects, scents have been used only in literature. Luckily, one might say – as everyone who has read Patrick Süsskind's novel *Perfume* will know. That might have also comforted the American researcher's wife who met Butenandt at a Nobel laureate congress in Lindau in 1951. In his article, he recalls her question about when the "sex-attractive substances" could be used. The woman's response to his comment that the substances affected only butterflies was a disappointed: "Oh, Doctor B., why did you waste your time with butterflies?" ◄

Photos: Archive of the Max Planck Society, Berlin-Dahlem