# Insect pheromone research: some history and 45 years of personal recollections

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**Abstract**: Since when have men known of chemical communication? The essay will begin with a speculation on a possible "prehistoric" scene when early human hunters observed animals. This will lead directly to a more than 300-year-old description of a rather modern experiment with dogs. From here we come to the situation about 120 years ago, when sexual attraction of male moths by their females was described. Even the use of female moths for trapping pest species males was already considered 100 years ago. The so far final development began in the 1950s with the chemical identification of the sexual attractant of the silkmoth *Bombyx*. Such substances were then named pheromones and bombykol, the sexual attractant of the female silkmoth, was the first pheromone (1959). With chemically pure pheromones and their derivatives available, corresponding physiological studies began in this period and led to the knowledge of the sensory specificity, sensitivity and adaptation of receptor cells and eventually of stimulus-induced behavior. During the following decades, many insect attractants were identified and their application in "integrated pest control measures" is well developed. Important physiological, microanatomical and biochemical investigations were still more recently done. Even the processing of pheromone information in a special area of the olfactory brain of moths and cockroaches, and the anemotactic orientation which is elicited by pheromone odor, are now widely understood. The complex biochemical mechanisms of sensory transduction, however, are not yet fully clarified and no receptor molecule was identified until now.

**Key words**: history of insect pheromone research, *Bombyx*, electroantennogram, EAG, EAD, olfactory brain processes, transduction biochemistry

This essay overviews the development of pheromone research of insects with a focus on the author's long personal experience in this field. Two introductory comments on the general pheromone history will open the scene.

The first comment is my favorite speculation on a prehistoric scenario when our ancestors might have taken notice of what we now call pheromone communication. I imagine that early hunters knew what it meant when their female prey animals (or their tamed female dogs) were in heat and the males sniffed the female odor message which was carried by the wind. Such vision overcomes me when I see the spectacular cave paintings, some of which are thirtythousand years old.

In historical times, even until 150 years ago, very little clear description of the phenomenon in question can be found, although Francois Rabelais (1565) mentions that the ancient Greeks knew of the powerful olfactorial attractivity of bitches in estrus. This writer described in an amusing burlesque how a vulval smear of a bitch attracts many male dogs. This imaginative description must either be based on observations of the writer himself or of earlier

authors. Clearly, not only the bitch or her glandular tissue, but her isolated biochemical product was attractive. All this looks very modern and compares well with similar experiments which we now do with experimental animals. Rabelais thus was, as far as I know, the first reporter of a modern pheromone experiment.

With my second comment I come to my favorites, the Lepidoptera. Experimental studies of sexual scents which attract moths have first been done little more than 100 years ago. Female moths, seemingly luring their mates with an odor signal, found particular interest among biologists and claims were raised that the males came from many kilometers distance (Fabre 1879; Forel 1910; Mell 1922). These truly pioneering studies were, however, not yet done under sufficiently controlled conditions and some of the results thus remained ambiguous. The problem was not only the poor control of the behavior of the moths, but also that neither the human nose, nor the then available art of chemical analysis allowed to identify, let alone to quantify, the suspected female odorant. In this situation, some authors proposed that the luring signal might not be an odor but some unknown radiation. The answer to such questions came with the identification of bombykol (Butenandt et al. 1959) and with many following isolations of attractants which were then called pheromones (Karlson and Lüscher 1959). Physiological and wind tunnel studies could now be done under proper conditions. But the clarification of long-distance luring remained a problem. A little known "side chapter" of a study by Priesner et al. (1986) gave a clear answer. This team probed luring effects of synthetic pheromone blends with diurnal sesiid moths and could even follow the flight-path over a bare, harvested field. The starting point of the males was an isolated berry-bush area where the moths had emerged. The distance of one kilometer was covered in a 12 minutes upwind flight.

After these two introductory stories, I will now report on our *Bombyx* work in Tübingen-Munich-Seewiesen. After a Göttingen doctorate, I was offered a joint position by the University and the Max-Planck-Institute for Biology at Tübingen. Here I taught biology, continued nerve studies, but also started morphogenetic experiments with marine bryozoa, moss animals. My first year in Tübingen was interrupted by a short postdoctoral time with R. Granit and B. Frankenhaeuser in Stockholm, where I learned some modern electrophysiology.

It then so happened that my housing-neighbor Peter Karlson, told me of Adolf Butenandt's then already long going project to identify the sexual attractant of the silkworm-moth, following unsuccessful attempts of other groups to do the same with the gypsy moth (Götz 1951). One of the problems which the chemists faced was the behavioral biotest which was done in the same building where finally one half million female *Bombyx* glands were eluted und the fractions analysed. Peter proposed 1953 that I try to develop an electrophysiological odor test to probe the efficacy of their analytical fractions. This idea stimulated my curiosity. Could one ever do this? How could I overcome my ignorance and naivity with respect to this challenge? I certainly knew of the spectacular sexual attraction in moths, but I did not know the details of the original descriptions. Journal reports soon informed me of behavioral work, of speculations on antennal sensilla (e.g. in the gypsy moth), but no *Bombyx* studies were available. The total number of even distantly relevant papers which I found, was about thirty. My plan was to record the initial receptorial reaction of the suspected olfactorial organ, the antenna, quite in analogy to the electroretinogram the ERG, with which I

was familar. My workplace consisted of a stereo-microscope, a micro-manipulator, a simple amplifyer, an oscilloscope and a camera. The isolated antenna of the male moth was mounted between electrodes, the AC-amplifier switched to a long time constant range and the enriched eluate of female glands (still 5 years before bombykol) was available as stimulus. The experimental odor source was the tip of a glasrod which had been dipped into the extract of the female luregland and a puff of air was now directed over the rod to the antenna.

With this arrangement I hoped to synchronously activate a sufficient number of receptor cells to show me a response. No doubt, you can understand my excitement, when I saw a deflection of the electron beam of my oscilloscope, lasting for the time of the stimulus. I praised the chemists to give me such a powerful extract since I thought, naively, that the natural emanation of a fresh female gland would never suffice to elicit a visible response in my recording system. But I learned soon that the *Bombyx* gland was a more powerful stimulus than the extract. In analogy to the electroretinogram, I named this odor-induced electrical response of an insect antenna "electroantennogram", EAG (Schneider 1957). I deliberately avoided the term "olfactogram" because I was not sure whether other modalities beyond olfaction (mechanoreception-thermoreception?) were also involved.

But was this now really the odor response of a "biological" receptor or just an artefact? A critical physicist and a physiologist of the University suspected that I was only recording artifacts, namely electrode potentials. Such physico-chemical odor-induced phenomena are in fact known when silver-chloride electrodes contact saline, like the hemolymph of the antenna. My now serious personal problem was that these critics were charged to judge my promotion to the rank of a lecturer at the university. Unfortunately, the critics overlooked that my thesis also presented the proof for a "biological" EAG: (1) only the antenna of the male but never that of the female moth responded to the gland odor while the antennae of both sexes responded to other odorants; (2) an antenna can be reversibly anaesthetized and (3), a dead but fresh antenna gives no response. However, this unjustified critique of the two professors sufficed for the Tübingen Faculty of Science to reject my application. Fortunately, my findings were soon verified and widely accepted by better informed colleagues.

You may now think that the EAG method offered me an interesting, wide open research field. Remember, this was about the time when the "Silent Spring" movement was in full swing and pheromones were thought to be the escape from the application of insecticides. But I was with my research planning in a serious conflict because I had just discovered a striking phototropic growth-reaction of the buds of my bryozoa which deserved much attention. How could I live with two "priorities"? Somehow I survived for quite sometime with both projects. Luckily, I was now supported by the Science Foundation (DFG) and K-E Kaissling, then still a young student, helped me in the laboratoy. Interestingly, Karl-Ernst, whom you may know as an insect physiologist, later got his doctorate with an important thesis on the rhodopsin-type reaction spectrum of the bryozoa (Kaissling 1963).

What did we learn from the early EAGs before bombykol? (1) themale antenna is the receptor organ for the attractant; (2) female antennae are anosmic for their own product; (3) anaesthetics reversibly suppress the EAG; (4) cyanide vapor irreversibly cancels the EAG; (5)

the EAG amplitude follows the intensity of the stimulus exponentially and recovers soon after the end of the stimulus; (6) antennae of both sexes respond to a variety of odors.

The simple and safe EAG-methods should now have enabled the chemists to establish their own EAG laboratory for the daily tests of the stimulatory power of their extracts and thus of the "purety" of their fractions. But this was apparently now too late since the chemists were in the meantime close to knowing that hexadecadienol was the *Bombyx* attractant (Butenandt et al. 1959). After all, natural product chemists are craving for the identificaion of new, exciting and even "useful" molecules, but not so much for the detailed roles which the molecules play in living systems. Soon, bombykol and all its isomers were fully known. The work of the Tübingen-Munich chemists was done. These rather personal comments are describing the difficulties of interdisciplinary interaction, but I hasten to say that the availability of bombykol and its isomeres was a phantastic gift for us biologists. Basic research on olfaction and odor-induced orientation profited directly from the bombykol analysis, and entomologists and chemists, who needed attractants of pest species, were quite encouraged.

About two years after my affair with the Tübingen faculty in 1956, I was lecturer in Munich and doctoral students joined the group. Already in Tübingen, K-E Kaissling and myself started to study also the morphology of the antennae (Schneider and Kaissling1957). We found several morphologial types of complex sense organs, sensilla, which are all formed by a fixed number of cells (Keil 1997). But we could not yet answer the "prize questions", namely: (1) which sensillum serves which sensory modality and (2) how specific are the receptor cells in the olfactory sensilla? Clearly, all the sensory nerve endings in the sensilla are bathed in sensillum lymph and covered by cuticle. We suspected that the walls of an odor-sensitive sensillum must have holes in its cuticle to allow the molecules to reach the receptive membrane. But vital stain, comparable to E Slifers' (1961) work, showed that only the wall of the sensilla basiconica, yet not the thick wall of the sensilla trichodea allowed the dye to stain the nerve endings, the dendrites. At this point, we even thought that the sensilla trichodea might be mechanoreceptive organs since we saw no dendrites in the hair.

In all our early papers, we therefore depicted the long hair sensilla as solid tubes with dendrites ending at the base of the hairs and had difficulties to think of them as odor receptor organs. This conflicting state of our knowledge was later corrected by electron-microscopical studies which began in the early sixties (Schneider et al. 1964) and resulted in the detection of dendrites, of pores and pore tubules in the walls of thin- and thick-walled olfactory sensilla (Ernst 1969; Steinbrecht 1973). Another important development started about this time. The ultrathin sectioning for electron microscopy required equally perfect histological fixation of both, the soft cytological material, and of the walls of the cuticule of the sensilla. Eventually, the development of new fixation and cryo-substitution techniques satisfied our request (Steinbrecht 1980).

The electrical recording from single sensory cells is, since the times of Lord Adrian, a must for a deeper understanding of the receptor process. My early attempts to record from the sensory cell bodies inside the antenna did not yet bring reliable results, but recordings from the base of a trichoid hair sensillum of beetles and moths gave useful impulses (Boeckh 1962; Schneider et al. 1964). Much later, Kaissling (1974) invented the optimal extracellular

recording technique. He clipped the tip of a single sensillum and contacted this opening with a capillary electrode. This was of course a modern version of the epochal recording of impulses from the open tip pore of taste receptor cells of the fly, first done by Hodgson, Lettvin & Roeder (1955).

With the availability of the synthetic bombykol isomeres, we could now compare the "efficiency" of the sterically different molecules. The EAGs indicated this already and the single cell reordings showed that one and the same cell of the pheromone reaction type responded, albeit with different sensitivity (different chemical affinity), to these stimuli. The now clearly determined exponential stimulus-response characteristics of the EAG confirmed my early prediction that it is a "summated" potential of many synchroneously activated receptor cells.

With our methods of recording from single bombykol receptor cells and with properly designed behavioral tests, we were now able to tackle the problem of the sensory threshold. Such classical physiological questions were raised for all sensory modalities - just think of the dispute on the finite visual sensitivity and the minimal number of quanta required for the activation of the eye. In olfaction, the phantastic claims of long-range luring of male moths encoraged earlier authors to speak of mono-molecular (single odor "quantum") effects. The necessary precondition for our study was the availability of labeled bombykol for the calibration of the stimulus. G Kasang tritiated the bombykol (1968) and K-E Kaissling & E Priesner (1970) performed the critical experiments and evaluated the data. All this needed to be combined with bombykol adsorption measurements on the antennae, surface diffusion evaluation of molecules and extensive statistics (Steinbrecht & Kasang 1972). The result was, in short: the receptor cells respond to single molecule hits, but the male moth only reacts if ca. 300 cells are activated in a period of one second.

The identification of bombykol was worldwide understood as a model for the search for the attractants of insect pest species and the EAG was the simple biotest. Over the years, with rapidly improving methods for chemical analysis, pheromones of the major pest insects became known. Twenty years after the EAG technique was described, Arn, Staedler and Rauscher (1975) developed the "Eletroantennographic Detector System" (EAD), which is now widely used. Gaschromatographic fractions of an extract of a pheromone gland are directly tested for their EAG power and their GC peaks lead to mass spectra. This elegant combination often allows to identify a pheromone in the course of days or weeks, instead of years, as with bombykol. But the critical preliminary condition for a successful use of the EAD is of course a sufficient number of antennal receptor cells which are tuned to the biologically relevant odor, such as a pheromone.

Insect pheromone research developed well until now (Kaissling 1987; Schneider 1992). The functional properties of many receptor cells which are sensitive for pheromones, and of other cells reacting to other odors, were analyzed and the high receptorial specificity for the pheromone corroborated. Pheromones of male insects occur in many groups, but found less interest since they are of no visible use for pest control measures. These male signals are mostly used in the final courtship phase and might be important for the "female choice" (Boppré 1984; Birch et al. 1990; Schneider 1992). Electrophysiological recording techniques were in the meantime rapidly developing and functional localization of central nerve cells became routine.

This technique opened a whole new field. Nerve fibers of antennal pheromone receptor cells of the males were found to end in a separate region of the olfactory brain, the macroglomerular complex (MGC), while the fibers of cells sensitive for general odors end in both sexes in the normal glomeruli. Generally, the fraction of pheromone sensitive receptor cells on the antenna of the male moth and cockroach is between 75% and 25% of all odor receptor cells (Boeckh and Boeckh, 1979; Boeckh et al.1984; Homberg et al. 1989). After processing of the odor messages in and between the glomeruli, only a very small number of projection neurons transfer the odor message to the higher brain areae (Ernst and Boeckh 1983; Christensen et al. 1989; Boeckh et al. 1990; Hansson 1995; Hildebrand 1995; Mustaparta 1996).

Is it now at all possible, to compare the "recognition" of an odor by an insect with our human scent perception? I think it is possible, and here is my argument: Our senses image the physico-chemical situation of our habitat, of our specific outer world. Such images, for instance blends of scents in particular situations, are not only well remembered but may also elicit specific behaviors. If such reactions are caused by pheromones (and we are no exception), they may be innate and are thus instinct reactions. Would it also be permissible to speak of olfactory "imaging" in an insect? The answer is again yes, since only the proper, the specific blend of components of the attractant elicits the innate anemotactic upwind flight, an alarm reaction or trail following. But insects can also learn scent-images as is well known for honeybees since Karl von Frisch's work (1919). More recently, odor learning in bees and in *Drosophila* could be localized in the antennal lobes and also in the higher brain areas, including the mushroombodies (Davis 1993; de Belle and Heisenberg 1994; Menzel and Müller 1996: Faber et al. 1999). From here, motor commands must go out to execute the complex instinct reactions (Olberg 1983; Olberg and Willis 1990) and eventually motivate the insect for its anemotactic upwind flight (Kramer 1996; Kaissling 1997).

Biochemical insect studies of olfactory transduction began some twenty years ago and are presently an active field. The physical and biochemical processes from the adsorption of the odor molecule on the cuticle of the sensillum to the induction of the dendritic generator potential are of high complexity. The sensillum lymph which surrounds the dendrites, is full of rather specific binding proteins for pheromones- and general odor components. The probably multiple role of these proteins is not clear yet. They may: (1) act as carriers of the odorants; (2) be selective filters; (3) present the odorant to the (still unknown) receptor in the dendritic membrane; (4) clean the lymph space; (5) deactivate the odorants after the transduction (Kaissling 1996; Steinbrecht 1998).

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