100 Years of Pheromone Research

An Essay on Lepidoptera

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Communication with species-specific chemical signals – pheromones – is a common phenomenon in the world of organisms. Lepidoptera are good examples because they show a variety of mechanisms and are experimentally well suited for such research. Many male-attractant odor blends of female moths are chemically known and their biosynthesis understood in principle. The morphology and physiology of the corresponding male receptor system is well studied and the biochemistry under investigation. It is supposed that the whole system is of monophyletic origin: female lure glands, male receptor organs, and perhaps even the central nervous machinery are homologous. This is in contrast to the as yet not well understood biology of male scents of Lepidoptera which fall into serveral structural, chemical, and functional categories. Many different communicative goals are met with male scents: attraction of females to males, of males to males, or even both. Futhermore, recognition of sex, competitors, and group partners seem to be involved. Male scents might even be used for defense.

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* Dedicated to Prof. H. Autrum on the occasion of his 85th birthday.

Communication is one of the elements of life. Sender and receiver, messages, and their recognition are basic biological functions and must have been already operating between early organisms. At its highest level, communication is the essence of human social interactions. Sexual and social life require recognition of partners.

Chemical communicative signals are surely the original ones and are still used by micro organisms, unicellular eukaryotes, and most animals. The basis of signal-receptor interactions is molecular bonding which requires that the two molecules involved attain the necessary proximity to allow physical forces to act. Molecular diffusion and convection carry the signals which the sender emits, active searching behavior and rheotaxis are the mechanisms which bring the partners together. This is already apparent in bacterial chemotaxis and in plant and animal sperm cells when they proceed toward an egg.

Man must have suspected the existence of odorous sexual attractants long before our time, either when hunting or by observing the males of their domestic animals when these encountered females in estrus. In 1565, François Rabelais attributed such knowledge to the ancient Greeks in his amusing narrative of Panurg who put a vulval smear from a bitch in heat on the garment of a particular lady who was then harassed by 600014 male dogs [1].

But how has our knowledge of odorous signals in the insects developed? Anecdotal reports about the attractive status of a freshly emerged female moth which lured males have appeared a number of times in the literature. Thirty-three years ago, the first of such interspecifically acting signals was chemically identified and the biological class of these substances named *pheromones* [2]. My dating of the beginning of pheromone research to 100 years ago is somewhat arbitrary but it was then that female moths were reported to attract their males even when hidden in the house but not when they were tightly covered by a glass cup (Fig. 1 [3, 4]; for male attractants, see [5]). This set the focus on the question of the physical property of the attractance and, should it be an odor, on the localization of the insects "nose".

Since we know that we sniff with our nose there is little doubt about the organ of smell in our fellow mammals, but many pages have been filled concerning the localization of the insect nose. Proposals stretched from the tracheal breathing openings (sic!), to the mouthparts and to the antennae. Again, about 100 years ago, it was unequivocally shown that insect antennae bear olfactory organs [6] (see also [4, 7]). Later, von Frisch [8] found in his detailed study that the honeybee fails to react to a learned odor if the outer antennal segments are occluded.
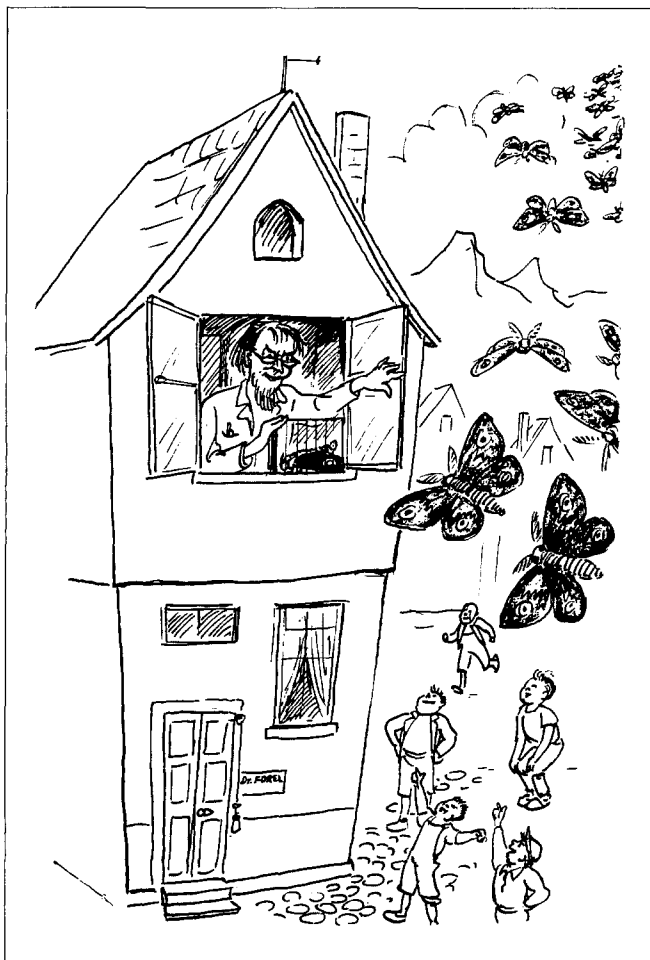
Fig. 1. August Forel (1848 – 1932) with a female wild silk moth in his studio while numbers of male moths (unknown in the town) approach the female to the amazement of neighborhood boys [4]. Drawing by A. Egelhaaf (Cologne 1982), from [15]

In this context, the attractiveness of female moths was particularly puzzling because the observers could not detect the expected luring odor [3, 4]. Although we all know how difficult it is to accept natural forces beyond our sensory powers, one wonders why "radiation" instead of a subtle odor was considered as the moth's sexual signal. After all, the males even came to the empty hatching box of the female [3]. What else other than such behavioral studies could our colleagues have carried out 70 or even 100 years ago to elucidate the phenomena of insect chemical communication and the related production and reception of scents? Analytical chemistry was not sufficently developed nor was a handy electrophysiology available to address such questions.

In addition to basic scientific interest, entomologists concerned with the control of pest insects were interested to learn about and obtain the attracting chemicals of certain moths, cockroaches, bark beetles, and other insects. Already in 1893, traps with luring ("call-ing") females of the dreaded gypsy moth were used to catch males [9, 10]. Could biologically active substances perhaps be identified with a reliable test and the analytical chemistry then available? Would it be possible to catch most males of a given pest species with a now synthesized attractant knowing little of the functional background [10]? By comparison, already before the invention of modern chromatography and spectroscopy, a number of vitamins and hormones were chemically known and clinically used without detailed knowledge of functional aspects.

Early attempts to identify the attractant of the Eurasian gypsy moth, which also became a severe forest pest after its introduction to North America, failed [10]. Later, A. Butenandt chose the domesticated silk moth Bombyx mori as the model insect in his quest to determine the chemical nature of an attractant in order to have an ample supply of female glands for the chemistry and of male moths for the biotest. Eventually, the alcohol bombykol was identified as the luring substance [11]. This was the first chemically known pheromone (Fig. 2a). Now, 33 years later, hundreds of attractants of female moth species have been described [12, 13]. Altogether 5×10^5 female glands needed to be extracted to clarify the chemistry of bombykol. To date, with advanced methods, a few hundred glands would suffice.

My essay focuses on old and new facets of the pheromone biology of Lepidoptera with emphasis on those studied in Tübingen, München, and Seewiesen. This research field and its application in agriculture and forestry grew slowly until about 40 years ago and then rapidly developed during and after the identification of bombykol. A number of useful books and reviews on pheromone research and closely linked insect olfaction studies have appeared over the last 10 years [14 – 21].

Female-Produced Lure Pheromones

Female-produced attractants have been found in all phylogenetically higher families of moths. The majority of these groups are mostly active at night, but even diurnal moths rely on female attractants (examples: Sesiidae, Ctenuchidae, and Zygaenidae [13]). In contrast, little is known of such scents in the females of the day-active butterflies. They find one another visually, but this does not always suffice for close recognition (see below). The lower Lepidoptera (Monotrysia) probably also operate with female attractants [22], although many of the quite primitive hepialids, e.g., use attractants of either (or both?) sexes [23]. In principle, attraction of males by the mostly heavier, less mobile females seems to be an economical choice, yet one wonders why a number of species in some groups have also, or only, attractants produced by males?
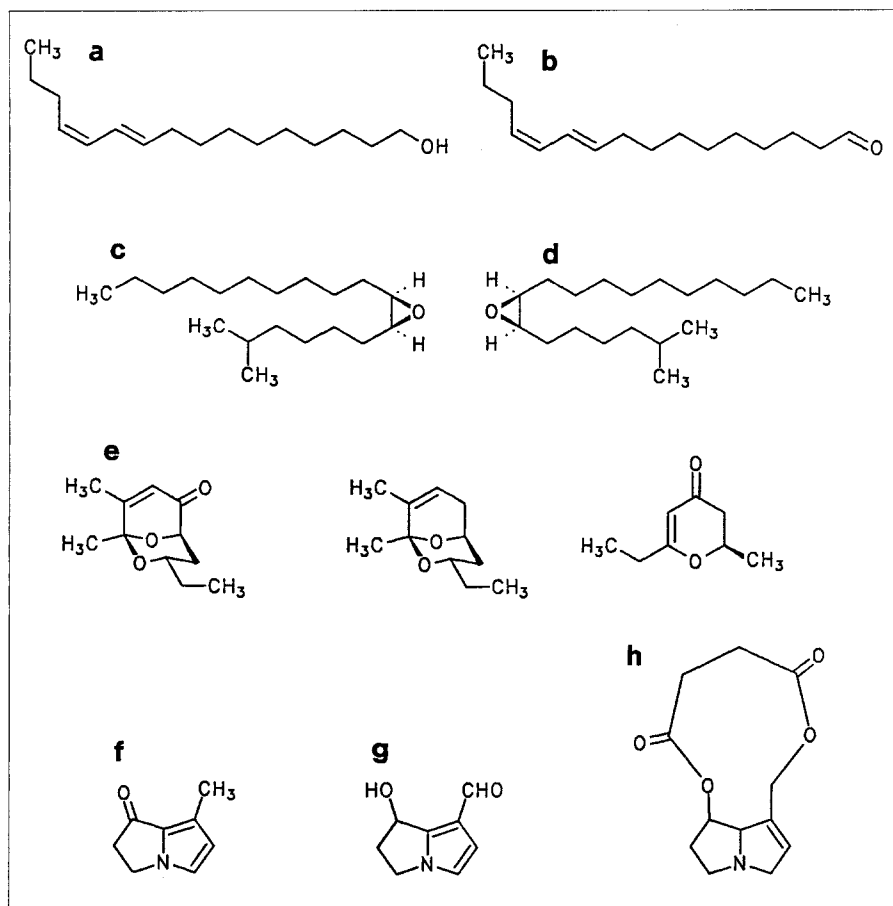
Fig. 2. Chemical structure of pheromones, *a* bombykol, *b* bombykal, *c* (–)-disparlure, *d* (+)-disparlure, *e* three types of male *Hepialus hecta* pheromone components, *f* danaidone, *g* hydroxidanaidal, *h* pyrrolizidine alkaloid (PA)

Studies of pheromone chemistry and biology flourished after the identification of bombykol. The reason for this was not only interest in basic mechanisms but also concern from pest controlling agencies and, further, the result of growing environmental awareness. Following the "Silent Spring" [24] warning against misuse and side effects of insecticides, attractant pheromones were hoped to become a species-specific and environmentally tolerable means to *mass-trap* the males. However, such goals only rarely were attained: pheromones are no panacea. The main reason for this failure is that the pest insect populations of forests, orchards, and fields inhabit a wide, "open" biotope where a multitude of physical and biological factors must be ideal in order to successfully control the pest [14, 25 – 30]. Under closed, insular conditions, e.g., in storehouses, mass trapping was much more successful [31]. Another, in some cases successful, use of pheromones is the *mating suppression* technique, where a biotope is virtually polluted with the female attractant, with the aim of disturbing the orientation of the males [30]. However, the main use of the attractant pheromones is now the *early monitoring* of pest outbreaks which allows a timely application of insecticides.

Since bombykol was and still is the only attractant of the silk moth, the original thought was that each species uses only one specific attracting substance [2]. However, as a general rule, it soon became clear that blends of chemicals, often in precise quantitative ratios, are the species-specific lure [32]. All these compounds are produced and dissipated by one and the same subcuticular glandular zone of the female abdomen. The "sacculi laterales" glands of *Bombyx* are expandable versicles [33], while the respective organs of most moths are much less visible [34 – 36]. In the female arctiids and ctenuchids, these glands are permanently invaginated (inner) tubes which are ventilated by abdominal pumping [37, 38, 85]. Presumably, all these organs, which are composed of unicellular gland cells, are homologous.

Surprisingly, the gland cells have neither individual nor collective excretory ducts, which means that the pheromone molecules, after traversing the often enlarged cell membrane [33], must pass through the cuticle to reach the outher surface from where they evaporate. It is uncertain whether lipid filaments in the cuticle assist the passage of the lipophilic pheromones [33, 36]. During their pupal time, these gland cells also secreted the overlying cuticle. All female moth attrac-
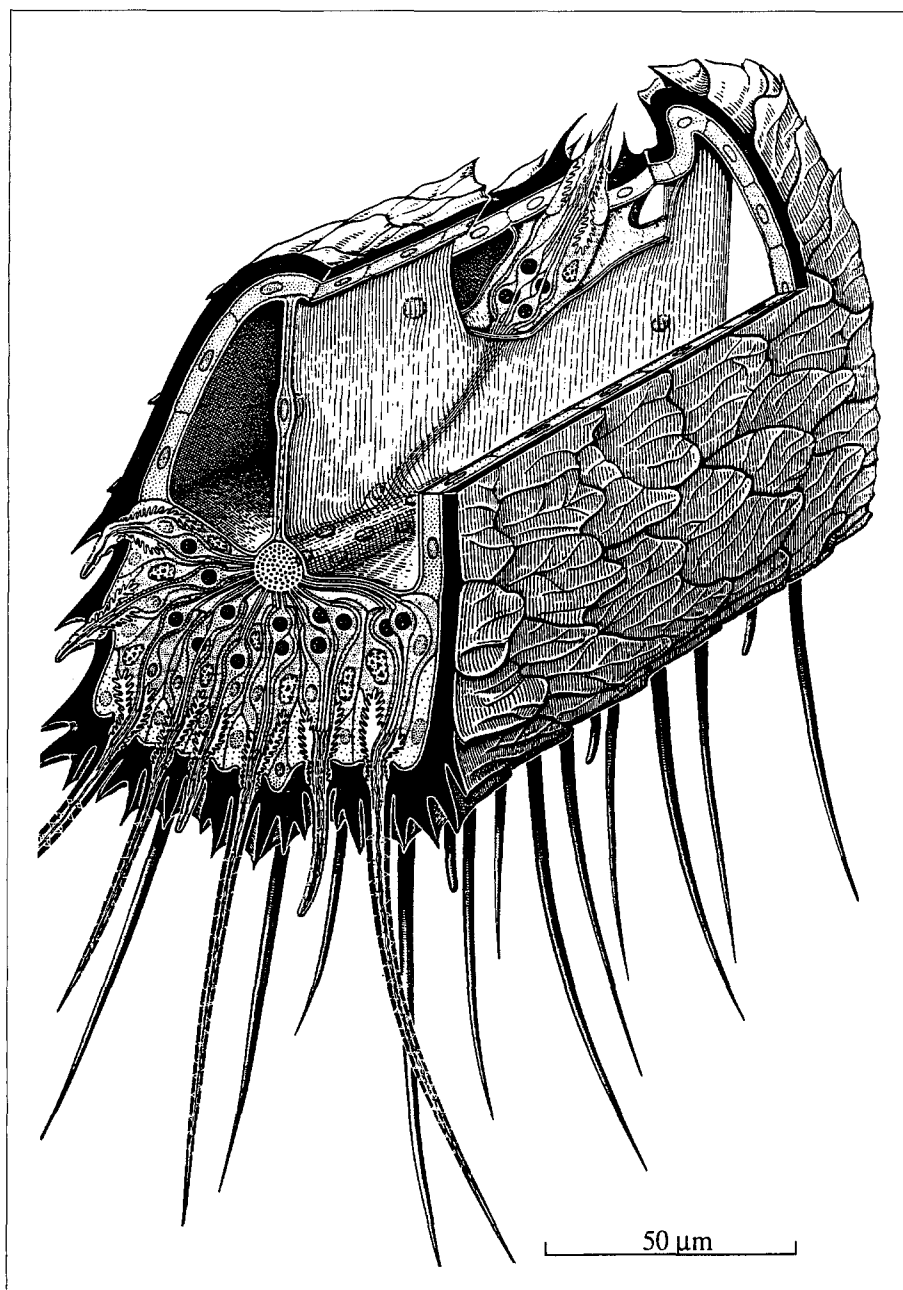
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Fig. 3. Male silk moth *Bombyx*, section of an antennal branch with cuticle (black), sensory epithelium, and hemolymph space. The lymph space is divided by a septum with a sensillum coeloconicum (of unknown function). On the other side, the long, thick-walled sensilla trichodea (bearing the bombykol/al-sensitive receptor cells: only in the male) are prominent. Between these hairs, thin-walled sensilla basiconica are positioned which house receptor cells sensitive to nonpheromone odors. Sensory nerve cells are depicted with a dark nucleus. This is a new version of the original drawing [15, 44] which the artist E. Freiberg (Tübingen) adapted (1984) to the actual state of knowledge: Electron microscopy revealed the complex cellular relationships and, most important, the porous cuticle of the odor receptor sensilla [45 – 47]

tants are de novo products, derived mostly from a Δ-11-desaturation of fatty acid precursors and are not dependent on the uptake of dietary precursor compounds [22, 39, 40].

Chemically, the female lure pheromones are mostly unbranched, unsaturated (in *E* or *Z* configuration), even-numbered chains of between less than 10 and more than 20 carbon atoms with a terminal alcohol, aldehyde, or acetate group [12, 13, 41, 42]. Epoxides are rare, the methyl-branched gypsy moth's *disparlure* (Fig. 2 c, d) is the best-known example [43]. Many of these compounds were synthesized in sufficient quantity to allow their application and to enable physiolo-

gists to study the function of the receptor system on the male antennae.

The antennae insert on the head capsule and are in many moths sexually divergent with larger (and/or sex-specific) structures in the males. The antennae bear a variety of sensory mini-organs, *sensilla*, which can detect chemicals, changes in temperature and humidity, mechanical distortion, and vibrations [18, 44 – 47, 64]. A sensillum consists of a cuticular structure, such as a hair, and formative and auxiliary cells, and is innervated by one or several sensory nerve cells. The cuticle of the olfactory sensilla has many tiny pores and continuous pore tubules [45 – 47]. All the cells
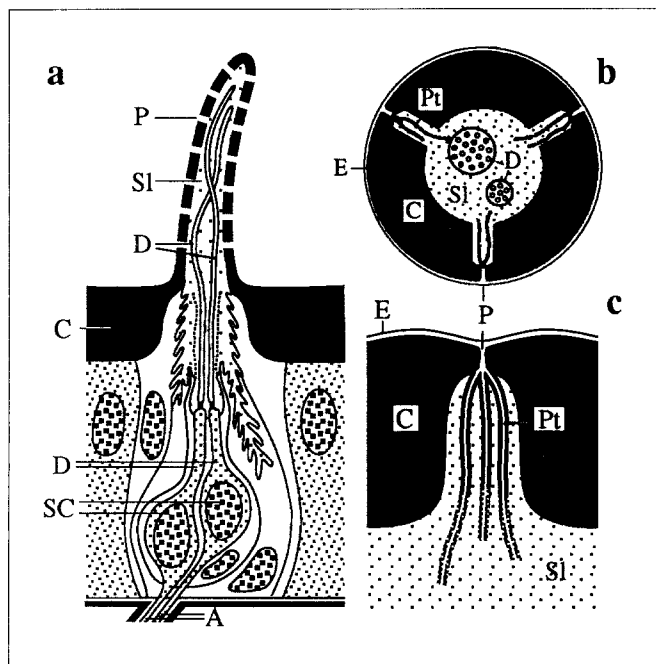
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Fig. 4. Schemata of a) an odor-sensitive sensillum of a moth, b) cross section of an olfactory hair sensillum with two receptor cells (with microtubules in the dendrite), c) wall pore with pore tubules. *A* axon, *C* cuticle, *D* dendrite, *E* epicuticular surface layers, *P* pore, *Pt* pore tubule, *SC* sensory cell, *Sl* sensillum lymph (courtesy R. A. Steinbrecht; see [57])

Fig. 5. Schematic view of the first μm of odorant-adsorbing and transducing sensory structures. Left: outer layer of olfactory mucosa of the vertebrate nose with cross sections of the terminal portion of the olfactory cilia. Right: portion of a cross section of an olfactory hair of the moth antenna. Cuticular hair wall (dark) with pore tubules in contact with cross-sectioned dendrite of an olfactory receptor cell (modified from [55])

of a sensillum belong to the epidermis (Figs. 3, 4). The majority of the receptor cells on the antennae serve the olfactory sense. From the soma of an olfactory neuron, a dendrite reaches into the *sensillum lymph* space and its axon projects to the olfactory lobe of the brain [48]. The set of different types of sex-pheromone receptor cells on the male antennae comprises about 50 % of all its odor receptor cells. Female moths do not have such cells and are thus unable to detect their own attractant odor. The high number of pheromone-sensitive cells explains why stimulation with the female odor allows one to record an overall receptor potential, the electroantennogram (EAG) [49], which is analogous to other sensory reactions such as the electroretinogram (ERG) [50]. In subsequent studies, individual odor receptor cells on the antennae were successfully tapped with microelectrodes and extracellular slow potentials, as well as impulses, were recorded. The good technical accessibility of these cells made them a favored system for basic olfactory studies [18, 51, 52].

When animals conquered dry land they faced many problems of adaptation. One of these was to protect the sensitive parts of the olfactory organs from desiccation while keeping them accessible to scent molecules. The vertebrate olfactory epithelium is, by comparison, situated deep in the nasal cavity and covered by a wet mucus on which the odorants adsorb. The
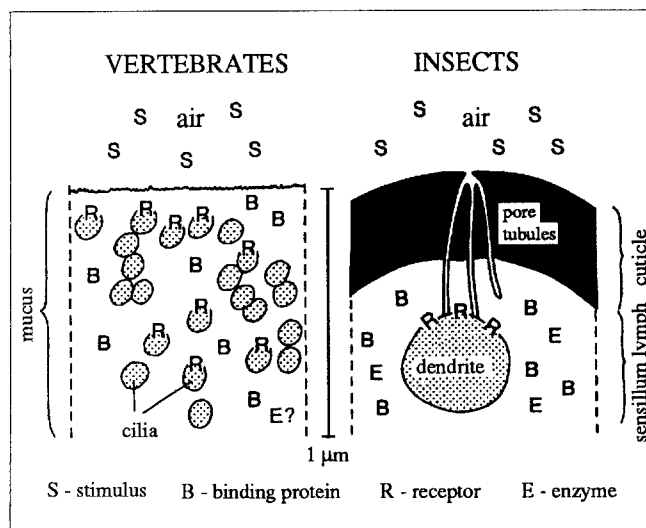
mucus contains odorant-binding proteins which are supposed to act as carriers, transporting the odor molecules to the sensitive cilia of the receptor cells [53]. The sensillum lymph of the insect olfactory sensilla contains, in analogy to the mucus, pheromone-binding proteins (PBP) and pheromone-degrading enzymes [18, 53, 54] (Figs. 4, 5).

How do the odor molecules reach the receptors on the dendritic membrane and what is their fate during and after transduction? There is general agreement that the first step is adsorption on the cuticle of the sensillum. The "catching" of airborne molecules is most effectively done by the long hair sensilla which house the pheromone-specific receptor cells in male moths [18, 64]. From the cuticle, the molecules diffuse into the pores and pore tubules. The subsequent processes are only partly understood and a matter of some speculation. Two alternative interpretations have been proposed (reviews [18, 55 – 57]):

1) The *contact model* [18, 55, 57] assumes that most pore tubules contact the dendritic membrane so that the lipophilic odor molecules reach the receptor without passing the aqueous lymph. The "used" odor molecules are released into the sensillum lymph where the PBPs immediately bind them. This is thought to explain the fast recovery of the receptor cell after the end of the stimulus. Finally, the pheromones themselves are slowly degraded by enzymes [54].

2) In the *carrier model* [56], the odor molecules are released into the sensillum lymph from the ends of the pore tubules where they bind to the PBPs which trans-

port them to the receptors. This would serve molecule transfer through the aqueous lymph if too few pore tubules reach the dendrite. After the transduction, degrading enzymes inactivate the pheromones.

The biochemistry of the transducing channel-opening mechanisms in olfaction is presently under intensive investigation. In vertebrates, different classes of odorants seem to activate different second-messenger cascades: stimulation of either an adenylate cyclase or phospholipase C is observed [53]. Patch-clamp recordings from olfactory cilia of frogs showed the opening of ion channels by cAMP and cGMP [58].

In contrast, up until now, no adenylate cyclase has been found to be involved in insect olfactory transduction. The pheromones appear to stimulate the phosphoinositide turnover via G-proteins, followed by a rapid increase in the inositol triphosphate (IP$_3$) level [53]. In addition, patch-clamp recordings from dendrites of pheromone receptor cells of the moth *Antheraea polyphemus* show that protein-kinase C activates a cation channel by phosphorylation [59].

Interestingly, electrophysiological recordings of elementary receptor potentials allow the assumption that pheromone transduction could operate without a second-messenger cascade in *Bombyx* and other moths. The receptor molecules would then act directly as ion channels [18, 55, 60].

Receptor molecules were elusive until recently. Now, in mammals, a novel, large multigene family of proteins has been found which fulfills several criteria of odorant receptors [61]. In the silk moth, *Antheraea polyphemus*, a receptor candidate in the dendritic membrane is reported which binds the PBPs and a pheromone component [62].

The specificity and sensitivity of the pheromone receptor cells are high. Geometrical isomers or enantiomers of the correct odor molecule are much less effective stimuli. This is probably due to properties of the receptor molecules, since the specificity of the binding proteins might be insufficient [18, 53, 56, 57]. The "dark"-adapted, fully rested receptor cell for bombykol responds with an impulse to a single pheromone molecule, but the male moth itself does not react to the stimulus until after about 300 such cells are activated. In the *threshold* situation, a 1-s exposure to an air current of 60 cm/s, with ca. 1 000 bombykol molecules per cm^3, suffices to excite a male moth [18, 63, 64]. The blend of the luring components induces the male to start his anemotactic upwind search flight [14]. Whether or not particular components play special roles, such as an initiation of landing, is an open question. The odor is only a "go upwind" command for the male. The essentials of the anemotaxis are now understood thanks to flight compensator studies [65, 66]. The obvious zigzags of the upwind flight of the

males (which are still mostly interpreted as the result of central nervous turning commands [20]) are, rather, correcting turns. Under natural conditions of free flight with uneven, turbulent air currents and a complex odor pattern, however, "chercher la femme" is a most difficult task for males. Claims as to which distance the males cover in their search flight reach from several meters to kilometers [14]. One of the most reliable values was recently reported from sesiid moths flying over a stubble field to the pheromone source: they covered 1 km in 12 min [67].

In some moths, the odor blend also contains components which reduce the attractive power of the rest of the blend. This is not a biochemical inhibition of the receptor site of those cells which respond to the attractant, but an activation of another cell type which is sensitive to the antagonistically acting compound. The inhibition is thus the result of central-nervous interactions. In *Bombyx*, the male hair sensilla are innervated by two cells, one specifically responding to bombykol, the other to bombykal (Fig. 2a, b). The latter compound is also produced by the female gland (but in lower amounts) and inhibits the attraction in a manner as yet not understood [68]. Such substances appear in other cases to discourage males of neighboring species (with an equal or similar attractive blend) to approach the luring females and therefore serve reproductive isolation [69]. But why does the male of the calling female also have such a receptor cell type? Is this a "leftover" from a shared ancestor of the two, now competing species? Another example for such a behavioral inhibition is provided by the gypsy and nun moths (*Lymantria dispar*, *L. monacha*) which live sympatrically in parts of Europe and share (+)-*disparlure* as attractant (Fig. 2c, d) [41, 70]); but only the female nun moth produces in addition ten times more (−)-*disparlure* which keeps the male gypsy moths from approaching her [41, 71]. Female gypsy moths are attractive for males of both species but only male gypsy moths have specific receptor cells for each enantiomer, while nun moth males only have receptors for (+)-*disparlure* and thus find their female in spite of her inhibitory odor component. This unilateral inhibition of interspecific attraction is still unexplained [71, 72].

What is the immediate effect of "pheromonal" nerve impulses when they reach the brain? The first projection area of these nerve fibers is the (male-specific) macroglomerular complex (MGC) of the deutocerebrum. Modern neurophysiological methods have revealed much information about the function of this area. It is only here that all the nerve fibers which carry the pheromone message have synaptic contact with olfactory interneurons, while the receptor fibers which signal the non-pheromonal "general" odors proceed to the normal glomeruli [48, 73, 74]. In this

area, the numerical 500 > 1 conversion of receptor fibers to interneurons greatly improves the signal-to-noise ratio and thus explains why as few as 300 pheromone impulses (as in the *Bombyx* behavior at threshold) are "understood" to be a significant message and eventually lead to a behavioral reaction [18, 63, 73]. In the meantime, even more details of this antennal-brain projection have been detected in a male moth: i.e., four types of receptor fibers which specifically respond to different pheromone components end in separate parts of the MGC [75]. In the olfactory lobe of the brain, interneurons integrate the peripheral messages and projection neurons bring them to the higher centers [76]. Deciphering the integrative processes of a set of messages such as the pheromone blends is a particular challenge to neurophysiologists. So far, two basic types of output neurons which leave the olfactory brain have been found: neurons which signal individual pheromone components and neurons which signal the blend [77]. Because the flying moth can only visually detect the upwind direction, olfactory and visual information [63 – 66] need to be integrated before descending neurons carry the flight command to the thoracic ganglia [78, 79].

It is tempting to consider the female-luring, male-responding system of the Lepidoptera to be an evolutionary early and uniform development, whereby the glands, their products, receptor sensilla, and neuroethological machinery would, respectively, have the same origins. Initial insight into the genetic base of this system indicates that the receptor, pheromone production, and behavior are dependent upon different, unlinked gene complexes [80] and pheromone polymorphism could have evolved in a "coevolutionary" manner [22]. The extension of such studies might reveal how similar the corresponding genes (and their expression) are in more or less related species.

Pheromones and Related Scents Produced by Males

Communication mediated by scents of the male Lepidoptera, when compared to that of female moths, is of bewildering complexity. The scent-producing organs of the males are found on different parts of the body, the chemistry of the volatiles is not uniform, and their biological meanings belong to a variety of categories, from attraction of one or both sexes to seduction, species recognition, male-male antagonism, and even defense [16, 81].

Many of these scents are, in contrast of the female pheromones, detectable by the human nose [5, 82], often with a pleasant note. With few exceptions, the scent-producing cells are also trichogen cells which
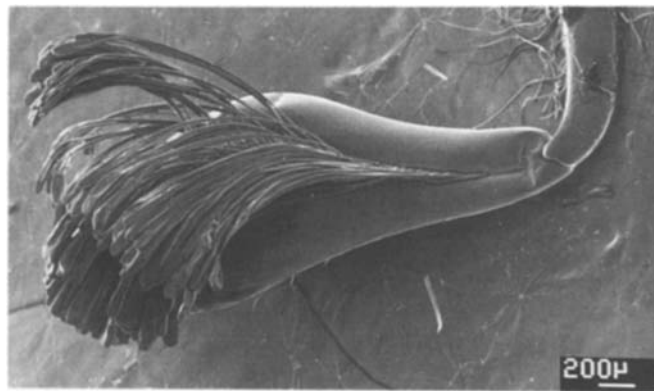

Fig. 6. *Hepialus hecta* male, scent brush on the tibia of the third leg; scanning electron micrograph. The scales which emit the attracting odor are fastened with a hinge mechanism in the tibial pocket. This allows an orderly erection of the scales out of the pocket and later their proper return after use [86, 90]. This mechanism secures the preservation of the volatile odorant when the brush is not used. The total amount of the major pheromone components (see Fig. 2 e) is approx. 20 μg [89]. The leg has no tarsi and is not used for walking (original scanning electron micrograph, courtesy R. Kittmann)

form a scale or hair into which the odorants must be transported before evaporation [83 – 85]. The delicate, spongy structure of these scales (often called hairs) is ideally suited to first accept, and then dissipate, the scent, e.g., from scale fields on the wings of a male cabbage-white (*Pieris* [16]), from the hindleg brushes of a primitive ghost moth *Hepialus*, or from the giant, extrudable brushes of some tiger moths (Arctiidae). Male scent-producing (*androconial* [16]) organs have been described from many families and are not rarely unevenly represented in a given genus.

Because a generalization of the functional anatomy, chemistry, and behavioral meaning of male scents is not possible, three better-known examples will now be presented: 1) a local ghost moth, 2) Monarch butterflies of the tropics, and 3) two Asian arctiid moths.

1) *Hepialus hecta* is a classical example of an insect which produces a male pheromone. Already 100 years ago, the function of this pineapple-like odor was thought to be an attractant for the females [5, 86, 87]. In a short activity period at dusk, groups of males engage in a hovering, pendulating flight above low vegetation while they expand their scent brushes from the hindlegs (Fig. 6). The formation of these groups is based on interactions between males mediated by optical stimuli and by male pheromones [88 – 90]. A female, which is olfactorially and visually attracted to the males, touches one of these in flight, then escapes to settle on a nearby plant. The contacted male follows and mates with her. The major components of the odor of the brush are heterocyclic substances (Fig. 2 e) which, after synthesis, were available for behavioral and electrophysiological tests [89]. Both sexes have an-

tennal receptor cells for the male odor components [89, 90]. This species is thus an example of pheromonal male-male/male-female attraction. Many of the male hepialids do have such tibial scent brushes. In the genus *Hepialus*, six species have male scent brushes, 13 have none [23]. Interestingly, behavioral observations on those hepialids without male scents indicate that they use female-produced attractants. Whether or not the ones with male attractants (such as *H. hecta*) are, in addition, also using female scents is an open question [23, 90].

2) All males of the highly developed Danainae (Nymphalidae) have extrudable, scent-dissipating hair pencils close to their abdominal tip which they present to the female during the courtship flight. The crucial pheromonal components of these organs are heterocyclic derivatives of pyrrolizidine alkaloids (PA, Fig. 2f−h). Males (e.g., *Danaus gilippus*) are not accepted by the females unless they present this odor [91]. Unexpectedly, these insects were unable to biosynthesize this scent *de novo* but need to ingest a PA as the pheromone precursor. Wilting PA-containing plants (e.g., *Heliotropium*) emanate a PA metabolite, which the males detect with their odor receptors and anemotactically locate the plant. They now settle, wet the plant surface with saliva, and re-ingest the fluid which now contains dissolved PA [92, 93]. In the typical case of *Danaus chrysippus*, the pheromone biosynthesis is additionally dependent upon behavioral contacts of the hair pencils with pocket glands on the hindwings [94]. Several species produce cuticular granules (*pheromone-transfer particles*) on the hairs of their pencils or inside the wing pockets. The granules are impregnated by a sticky substance and the pheromone [91, 95] and are "dusted" on the female antennae during courtship. The male odor stimulus is thus maintained even if he is no longer flying in front of her [16, 91, 95, 96]. In this subfamily of ca. 160 species, a variety of mechanisms assist the pheromone production and presentation [16, 97]. Most hair pencils also have species-specific scents besides the dihydropyrrolizidine pheromones. These odor bouquets are thought to serve species recognition, male-male interaction, and competition when similarly patterned species live in mixed groups in suitable localities [98 − 100].

3) In the large family of arctiid moths, many species have male odor-producing organs. The most impressive ones are found in the Asian species, *Creatonotos gangis* and *C. transiens*. At dusk, males take position on exposed places and extrude four abdominal hair-covered tubes (coremata) with a pneumatic mechanism (see cover picture). The 3 000 hairs dissipate a PA derivative (Fig. 2 g) [85, 101, 102]. This scent is an attractant for other males and also for females, which directly approach (olfactorially and probably also visually guided) a male in the group and mate with him. The alkaloidal precursor of the pheromone is derived in this case as a consequence of larval feeding on PA-containing plants and the quantitiy of consumed PA controls the amount of pheromone produced [85, 102]. The quantity of pheromone might thus be a measure of the fitness of the male, which the females could probe with their odor sense, thus making their mate choice. Fitness would here mean: higher PA content and thus an increased unpalatibility for a predator (see below) [103, 104, 106]. In *Creatonotos*, we are still searching for evidence of a PA-related female choice which has not been observed in the laboratory but has been reported from a related moth [105]. *Creatonotos* also possesses powerful female attractants, which lure unmated males later in the evening [85, 102]. The polyphagous larvae have a feeding preference (like the adult danaines) for these alkaloids which has been called *pharmacophagy* [106]. Larvae have specialized taste receptors for the PA [107]. These and other bitter and toxic plant substances are to a large extent deposited in the insect cuticle. A potential predator (e.g., a bird) might then sense the bitter taste and learn to avoid such prey in the future [104, 108].

An unexpected "side effect" of the PA uptake was observed: the size of the male organ (as also the amount of the pheromone produced) depends quantitatively upon the amount of alkaloid ingested by the larva [101, 109 − 111]. Is this a case of parsimony? Since, without a precursor, the corema would be useless or even disadvantageous, why then should it be made at all? Differently sized coremata were also found in field-caught specimens but the ecophysiological significance of this phenomenon is not understood [109]. Incidentally, males with tiny coremata and without pheromone are fertile and sexually successful in captivity [109]. Recently, the PA uptake from the gut was found to be mediated by a carrier protein which might also serve the proper PA distribution within the larval body [112]. Although PA storage occurs primarily in the integument, alkaloid is later found in the developing corema, the spermatophores, and eventually the eggs which may gain protection by this paternal endowment [103 − 106].

Final Comments

Research into chemical communication developed exponentially, as in many other biological disciplines from early observations of curious naturalists, into a broad interdisciplinary field (Table 1). This field centers on fascinating behavioral phenomena, the

Table 1. Pheromones of Lepidoptera, general rules regarding biological meaning, production, dissipation, and processing

	♀ Pheromones	♂ Pheromones
Meaning	reproduction and reproductive isolation	seduction, attraction, competition
Glands at	abdominal tip	different body parts
Chemistry	mainly noncyclic hydrocarbons	often hetrocyclic compounds
Components	seldom 1, mostly mixtures of 2 to many	seldom 1, mostly mixtures
Amount/gland	$< 1\ \mu g$	$> 1\ \mu g$
Production	before and when luring	before use (always?)
Bio-synthesis	*de novo* from fatty acid metabolism	*de novo* or from dietary precursors
Time of exposure	long or short until mating	mostly short, rarely long
Receptor cells	on male antennae only	on antennae of both sexes
Receptor cell types specific for	each identified pheromone component	major and minor (?) components
Central nervous processing	via macroglomerulus	(?)

mechanism and meaning of which we would like to understand more. A prediction of the probable progress for even a few years is much more difficult than to look back over the last 100 years. What can we expect in the future? Perhaps, 1) an answer to the "nobel" question of how and why the different pheromonal systems evolved [22]. In this context, a clarification of genetic relatedness ("molecular evolution") in view of the similarities and the striking differences in pheromone biology can be expected. 2) Better understanding of the biosynthesis, transport, storage, and transduction of the pheromones. 3) Progress in the analysis of the neural control of pheromone-induced behavior.

1. Rabelais, F.: Songes Drolatiques de Pantagruel (1565); deutsch: Gargantua und Pantagruel, 1. Bd., 3. Buch, 22. Kap. München: Hanser 1964
2. Karlson, P., Lüscher, M.: Naturwissenschaften 46, 63 (1959); Nature 183, 55 (1959)
3. Fabre, J. H.: Souvenirs Entomologiques. Paris: Delagrave 1879
4. Forel, A.: Das Sinnesleben der Insekten. München: Reinhard 1910
5. Bertkau, P.: Arch. Naturgesch. 17, 223 (1882); Barrett, C. G.: Entomol. Mon. Magaz. 19, 90 (1882); Robson, J. E.: ibid. 23, 186 (1887)
6. Hauser, G.: Z. wiss. Zool. 34, 368 (1880)
7. Kafka, G.: Einführung in die Tierpsychologie, Bd. I. Die Sinne der Wirbellosen. Leipzig: Barth 1914
8. Frisch, K. v.: Zool. Jhb. Allg. Zool. Physiol. 37, 1 (1919)
9. Schedl, K.: Monogr. Z. angew. Entomol. 12, (1936)
10. Götz, K.: Experientia 7, 406 (1951)
11. Butenandt, A., Beckmann, R., Stamm, D., Hecker, E.: Z. Naturforsch. 14b, 283 (1959); Butenandt, A., Beckmann, P., Hecker, E.: Hoppe-Seyler's Z. physiol. Chem. 324, 71 (1961)
12. Tamaki, Y., in: Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 9, p. 145 (G. A. Kerkut, L. I. Gilbert, eds.). Oxford: Pergamon 1985
13. Arn, H., Tóth, M., Priesner, E.: List of Sex Pheromones of Lepidoptera and Related Attractants. Wädenswil/Switzerland: Internatl. Organisation Biol. Control 1986/87
14. Cardé, R. T., Charlton, R. E.: Symp. R. Ent. Soc. London 12, 241 (1984)
15. Schneider, D., in: Foundations of Sensory Science, p. 381 (W. W. Dawson, J. M. Enoch, eds.). Berlin: Springer 1984
16. Boppré, M.: Symp. R. Entomol. Soc. London 11, 259 (1984)
17. Payne, T. L., Birch, M. C., Kennedy, C. E. J. (eds.): Mechanisms in Insect Olfaction. Oxford: Univ. Press 1986
18. Kaissling, K.-E.: R. H. Wright Lectures on Insect Olfaction (K. Colbow, ed.). Burnaby, B. C.: S. Fraser Univ. 1987
19. Prestwich, G. D., Blomquist, G. J. (eds.): Pheromone Biochemistry. Orlando, FL.: Academic Press 1987
20. Baker, T. C.: Experientia 45, 248 (1989)
21. Masson, C., Mustaparta, H.: Physiol. Rev. 70, 199 (1990)
22. Löfstedt, C., in: Proc. Conf. Insect Chem. Ecol. Tábor, p. 57. Academia Prague and SPB Acad. Publ. The Hague 1990
23. Mallet, J.: Zool. J. Linn. Soc. 79, 67 (1984)
24. Carson, R.: Silent Spring. New York: Houghton Mifflin 1962; Crest Books 1964
25. Mitchell, E. R. (ed.): Managment of Insect Pests with Semiochemicals. New York: Plenum 1981
26. Kydonieus, A. F., Beroza, M.: Insect Suppression with Controlled Release Pheromone Systems. Boca Raton, FL: CRC Press 1982
27. Campion, D. G.: Crop Protect. 2, 3 (1983)
28. Ridgway, R., Silverstein, R. M., Inscoe, M.: Practical Application of Insect Pheromones and other Attractants. New York: Dekker 1988
29. Cardé, R. T.: Environ. Health Perspect. 14, 133, 144 (1976)
30. Neumann, U., Kalter, K., Charmillot, P. J., Mani, E., Blommers, L., Blanc, M., Höbaus, E., Sterk, G.: Med. Fac. Landbouww. Rijksuniv. Gent 55, 379 (1990)
31. Levinson, H. Z., Levinson, A. R., in: Chemical Ecology, p. 327 (F. J. Ritter, ed.). Amsterdam: Elsevier 1979
32. Priesner, E., in: [17], p. 225
33. Steinbrecht, R. A.: Z. Zellforsch. 64, 227 (1964)
34. Urbahn, E.: Jenaische Z. Naturwiss. 50, 277 (1913)
35. Noirot, C., Quennedy A.: Ann. Rev. Entomol. 19, 61 (1974)
36. Percy-Cunningham, J. E., MacDonald, J. A., in: [19], p. 27
37. Conner, W. E., Eisner, T., Van der Meer, R. K., Guerrero, A., Ghiringelli, D., Meinwald, J.: Behav. Ecol. Sociobiol. 7, 55 (1980)
38. Krasnoff, S. B., Roelofs, W. L.: Nature 333, 263 (1988)
39. Bjöstad, L. B., Wolf, W. A., Roelofs, W. L., in: [19], p. 77
40. Morse, D., Meighen, E., in: [19], p. 121
41. Tumlinson, J. H., Teal, P. E. A., in: [19], p. 3
42. Brand, J. M., Young, Chr., Silverstein, R. M.: Insect Pheromones. Progr. Chem. Org. Natural Products 37. Wien: Springer 1979
43. Bierl, B. A., Beroza, M., Collier, C. W.: Science 170, 87, 1970

44. Schneider, D., Kaissling, K.-E.: Zool. Jhb. Anat. *76*, 223 (1957)
45. Altner, H., Prillinger, L.: Int. Rev. Cytol. *67*, 69 (1980)
46. Keil, T. A., Steinbrecht, R. A., in: Insect Ultrastructure, Vol. 2, p. 477 (R. C. King, H. Akai, eds.). New York: Plenum 1984
47. Steinbrecht, R. A., in: [19], p. 353
48. Boeckh, J., Ernst, K. D., Sass, H., Waldow, U.: J. Ins. Physiol. *30*, 15, (1984)
49. Schneider, D.: Z. vergl. Physiol. *40*, 8 (1957)
50. Autrum, H.: ibid. *32*, 176 (1950)
51. Boeckh, J., Kaissling, K.-E., Schneider, D.: Cold Spring Harb. Symp. Quant. Biol. *30*, 263 (1965)
52. Mustaparta, H., in: Chemical Ecology of Insects, p. 37 (W. J. Bell, T. R. Cardé, eds.). London: Chapman and Hall 1984
53. Breer, H., Boekhoff, I., Tareilus, E.: Nature *345*, 65 (1990)
54. Kasang, G., in: Gustation and Olfaction, p. 245 (G. Ohloff, A. F., Thomas, eds.). London: Academic Press 1971
55. Kaissling, K.-E.: Ann. Rev. Neurosci. *9*, 121 (1986); Kaissling, K.-E., in: Sensory Transduction, p. 81 (A. Borsellino, ed.). New York: Plenum 1990
56. Vogt, R. G., in: [19], p. 385; Vogt, R. G., Prestwitch, G. D., Lerner, M. R.: J. Neurobiol. *22*, 74 (1991)
57. Steinbrecht, R. A., in: Principles of Design and Function in Nervous Systems (R. N. Singh, ed.). Goa (India): Int. Conf. Neurobiol. (in press)
58. Nakamura, T., Gold, G. H.: Nature *325*, 442 (1987); Lancet, D.: ibid. *351*, 275 (1991); Firestein, S.: TINS *14*, 270 (1991)
59. Zufall, F., Hatt, H.: Proc. Nat. Acad. Sci. USA *88*, 8520 (1991)
60. Kaissling, K.-E., Thorson, J., in: Receptors for Neurotransmitters, Hormones and Pheromones in Insects, p. 261 (D. B. Sattelle, L. M. Hall, J. G. Hildebrand, eds.). Amsterdam: Elsevier 1980
61. Buck, L., Axel, R.: Cell *65*, 175 (1991)
62. Ziegelberger, G., Maida, R.: Comm. at the Europ. Symp. Insect Taste and Olfaction II 1991 and pers. comm.
63. Kaissling, K.-E., Priesner, E.: Naturwissenschaften *57*, 23 (1970)
64. Kaissling, K.-E., in: Handb. of Sensory Physiol., Vol IV/1, p. 351 (L. M. Beidler, ed.). Berlin: Springer 1971
65. Kramer, E., in: [17], p. 59
66. Preiss, R., Kramer, E.: Naturwissenschaften *73*, 555 (1986)
67. Priesner, E., Witzgall, P., Voerman, S.: J. Appl. Entomol. *102*, 195 (1986)
68. Kasang, G., Kaissling, K.-E., Vostrowsky, O., Bestmann, H. J.: Angew. Chem. *90*, 74 (1978)
69. Cardé, R. T., Cardé, A. M., Hill, A. S., Roelofs, W. L.: J. Chem. Ecol. *3*, 71 (1977)
70. Bierl, B. A., Beroza, M., Adler, V. E., Kasang, G., Schröter, G., Schneider, D.: Z. Naturforsch. *30c*, 672 (1975)
71. Schneider, D., Lange, R., Schwarz, F., Beroza, M., Bierl, B. A.: Oecologia (Berl.) *14*, 19 (1974)
72. Hansen, K.: Physiol. Entomol. *9*, 9 (1984)
73. Boeckh, J., Boeckh, V.: J. Comp. Physiol. *132*, 235 (1979)
74. Homberg, U., Christensen, T. A., Hildebrand, J.: Ann. Rev. Entomol. *34*, 477 (1989)
75. Hansson, B. S., Ljungberg, H., Hallberg, E., Löfstedt, C.: Science (in press)
76. Kanzaki, R., Arbas, E. A., Strausfeld, N. J., Hildebrand J.: J. Comp. Physiol. *165*, 427 (1989)
77. Christensen, T. A., Mustaparta, H., Hildebrand J.: Chem. Senses *14*, 463 (1989)
78. Olberg, R. M.: J. Comp. Physiol. *152*, 297 (1983)
79. Kanzaki, R., Shibuya, T.: Brain Res. *377*, 378 (1986)
80. Roelofs, W. L., Glover, T., Tang, X.-H., Sreng, I., Robbins, P., Eckenrode, C., Löfstedt, C., Hansson, B. S., Bengtson, B. O.: Proc. Nat. Acad. Sci. USA *84*, 7585 (1987); Hansson, B. S., Löfstedt, C., Roelofs, W. L.: Naturwissenschaften *74*, 497 (1987)
81. Birch, M. C. (ed.): Pheromones. Amsterdam: North-Holland 1974
82. Müller, F.: Jenaische Z. Naturwiss. *5*, 99 (1877)
83. Clearwater, J. R.: J. Morph. *146*, 129 (1975)
84. Pliske, T. E., Salpeter, M. M.: ibid. *134*, 215 (1971)
85. Wunderer, H., Hansen K., Bell, T. W., Schneider, D., Meinwald, J.: Exp. Biol. *46*, 11 (1986)
86. Deegener, P.: Z. wiss. Zool. *71*, 276 (1901)
87. Illig, K. G.: Zoologica (Stuttgart) *38*, 1 (1902)
88. Turner, J. R. G.: Anim. Behav. *24*, 188 (1976); Turner, J. R. G.: Entomologist *102*, 90 (1988)
89. Schulz, S., Francke, W., König, W. A., Schurig, V., Mori, K., Kittmann, R., Schneider, D.: J. Chem. Ecol. *16*, 3511 (1990)
90. Kittmann, R., Schneider, D.: unpubl.
91. Pliske, T. E., Eisner, T.: Science *164*, 1170 (1969)
92. Schneider, D., Boppré, M., Schneider, H., Thompson, W. R., Boriack, C. J., Petty, R. L., Meinwald, J.: J. Comp. Physiol. *97*, 245 (1975)
93. Bogner, F., Boppré, M.: Entomol. Exp. Appl. *50*, 171 (1989)
94. Boppré, M., Petty, R. L., Schneider, D., Meinwald, J.: J. Comp. Physiol. *126*, 97 (1978)
95. Meinwald, J., Meinwald, Y. C., Mazzocchi, P. H.: Science 164, 1174 (1969)
96. Brower, L. P., Van Zandt Brower, J., Cranston, F. P.: Zoologica (New York) *50*, 1 (1965)
97. Boppré M., Vane-Wright, R. I.: Zool. J. Linn. Soc. *97*, 101 (1989)
98. Schulz, S.: Dissertation Univ. Hamburg 1987
99. Schulz, S., Francke, W., Edgar, J. A., Schneider, D.: Z. Naturforsch. *43 c*, 99 (1988)
100. Schneider, D., in: Biology and Conservation of the Monarch Butterfly (S. B. Malcolm, M. P. Zalucci, eds.). Los Angeles: Natural History Museum (in press)
101. Schneider, D., Boppré, M., Zweig, J., Horsley, S. B., Bell, T. W., Meinwald, J., Hansen, K., Diehl. E. W.: Science *215*, 1264 (1982)
102. Boppré, M., Schneider, D.: Zool. J. Linn. Soc. *96*, 339 (1989)
103. Wink, M., Nickisch-Rosenegk, E. von, Schneider, D.: Symp. Biol. Hung. (Budapest) *39*, 53 (1990)
104. Nickisch-Rosenegk, E. von, Schneider, D., Wink, M.: Z. Naturforsch. *45 c*, 881 (1990)
105. Eisner, T., Meinwald, J., in: [19], p. 251; Dussourd, D. E., Harvis, C. A., Meinwald, J., Eisner, T.: Proc. Nat. Acad. Sci. USA *88*, 9224 (1991)
106. Boppré, M.: Naturwissenschften *73*, 17 (1986)
107. Sichelschmidt, D.: Dissertation Univ. Freiburg 1991
108. Egelhaaf, A., Cölln, K., Schmitz, B., Buck, M., Wink, M., Schneider, D.: Z. Naturforsch. *45 c*, 115 (1990)
109. Boppré, M., Schneider, D.: J. Comp. Physiol. *157*, 569 (1985)
110. Schmitz, B., Buck, M., Egelhaaf, A., Schneider, D.: Roux's Arch. Dev. Biol. *198*, 1 (1989)
111. Egelhaaf, A., Rick-Wagner, S., Schneider, D.: Zoomorphology *111*, 125 (1992)
112. Wink, M., Schneider, D.: Naturwissenschaften *75*, 524 (1988)