

**Olfactory receptor neurones specified for the same odorants in three related heliothine species (*Helicoverpa armigera*, *Helicoverpa assulta* and *Heliothis virescens*).**

**Electrophysiology linked to gas chromatography and mass spectrometry.**

Doctorata scientiarum thesis of Marit Stranden

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## PAPERS INCLUDED IN THE THESIS

This thesis is based on the following papers, which will be referred to by their Roman numerals:

- I. Stranden M., Borg-Karlson A.-K. and Mustaparta H. (2002), Receptor neuron discrimination of the germacrene D enantiomers in the moth *Helicoverpa armigera*. *Chem. Senses*, 27: 143-152
- II. Stranden M., Liblikas I., König W.A., Almaas T.J., Borg-Karlson, A.-K. and Mustaparta, H. (2003) (-)-Germacrene D receptor neurones in three species of heliothine moths: structure-activity relationships. *J. Comp. Physiol. A*, 189: 563-577
- III. Stranden M., Røstelién T., Liblikas I., Almaas T. J., Borg-Karlson, A.-K. and Mustaparta, H. (2003) Receptor neurones in three heliothine moths responding to floral and inducible plant volatiles. *Chemoecology*, 13: 143-154
- IV. Mozuraitis R., Stranden M., Ramirez M.I., Borg-Karlson A.-K. and Mustaparta H. (2002), (-)-Germacrene D increases attraction and oviposition by the tobacco budworm moth *Heliothis virescens*. *Chem. Senses*, 27: 505-509

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## ABBREVIATIONS

cAMP –adenosine 3'5'-monophosphate  
CNS – central nervous system  
DMNT – *E*-4,8-dimethyl-1,3,7-nonatriene  
EAG – electro-antennogram  
EOG – electro-olfactogram  
GC – gas chromatograph  
GC-MS – linked gas chromatography mass spectrometry  
GC-SCR – linked gas chromatography single cell recording  
IP<sub>3</sub> – inositol 1,4,5-trisphosphate  
MGC – macroglomerular complex  
MPLC – medium pressure liquid chromatography  
OBP – odour-binding protein  
PBP – pheromone-binding protein  
PE – pedunculus extrinsic neurone  
PER – proboscis extension reflex  
QSAR – quantitative structure-activity relationships  
RN – receptor neurone  
TMTT - *E,E*-4,8,12-trimethyl-1,3,7,11-tridecatetraene  
VUM – ventral unpaired median

## INTRODUCTION

The chemical senses are considered as the phylogenetically oldest of the senses. Unicellular organisms can orient along gradients of toxic and nutritional chemicals. More complex organisms have evolved senses based on advanced neural systems for detection and processing of information about the external chemical stimuli; olfaction for airborne molecules and taste for molecules dissolved in liquid. In addition, a sense for noxious molecules in air and liquid is present. Separate sensory organs contain the receptors that detect the stimuli and mediate the information further to different brain areas via interneurons. In spite of the interest and fascination about chemical stimuli throughout the history of human cultures, little knowledge, particularly about the olfactory mechanisms, has existed until recent years. Whereas visual and auditory stimuli are well defined by one feature (the wavelength), a chemical stimulus has several interactive features that make it difficult to screen for a continuum of important molecular characteristics. Thus, the problem is to find the biologically relevant odorants among the hundreds of known and unknown compounds surrounding the organisms. The identification of relevant odorants for olfactory receptor neurones (RNs) has not become less important in the recent years of olfactory research. The impressive development in molecular biological studies has resulted in the identification of numerous genes in various species coding for olfactory receptor proteins. At present, one key question is which odorants the receptor proteins are specified for. This question can only be answered by physiological studies.

### **The olfactory system**

The anatomy of the olfactory system is well known in many species. Although the location of the olfactory epithelia in vertebrates (upper nasal cavity) is very different from the location in insects (in sensilla on the antennae), the principal anatomy of the two systems is strikingly similar (reviewed by Steinbrecht 1992, Hildebrand and Shepherd 1997). The ciliar parts of the primary sensory neurones, containing the receptor proteins, are embedded in a protein-rich lymph. The axons of the RNs project directly to the primary olfactory centre of the brain, the olfactory lobe in vertebrates and

the antennal lobe in insects. In these centres, the primary axon terminals synapse with the second order of neurones in the typical spherical glomeruli (Hanström 1928, Boeckh et al. 1990, Leise 1990, Boeckh and Tolbert 1993) that seem to be conserved in number and location between individuals of the same species (Allison 1952, Meisami 1991, Rospars 1988). There are two separate olfactory systems, one for species-specific chemical signals, e.g. pheromones, and one for other odorants, e.g. food odours. The easily accessible olfactory system in insects and its similarity with the vertebrate olfactory system makes insects suitable model organisms for studying the mechanisms of olfaction.

### **Olfactory sensilla, perireceptor and transduction events**

The olfactory organs, olfactory sensilla, in insects are usually located on the antenna. Many lepidopteran species have sexually dimorphic sensilla. For instance, the *sensilla trichodea* type I in heliothine moths, containing the pheromone RNs, are only present in male antennae, whereas *s. trichodea* type II and *s. basiconica* are present in both sexes and mediate plant odour information (Almaas and Mustaparta 1990, Almaas and Mustaparta 1991, Koh et al. 1995). In addition, *s. trichodea* type II also mediates pheromone information in males (Almaas and Mustaparta 1991). The different sensilla types are classified according to internal and external anatomy (Schneider 1964, Ernst 1969, Schneider and Steinbrecht 1968, Keil 1984, Steinbrecht 1992, Keil et al. 2001). The outer structures are often hair formed, with cuticle walls perforated by pores allowing hydrophobic volatiles to enter the sensilla (Ernst 1972). The internal cavity of the sensilla is filled with the receptor lymph, which constitutes a hydrophilic barrier for the odorants. The lymph surrounds the dendrites of the olfactory neurones, which possess the membrane spanned receptor proteins. Odorant-binding proteins (OBPs), present in the receptor lymph, show selective binding to some odorants and are suggested to function both as a filter and as a carrier, transporting the odorants through the lymph to the receptor proteins (Prestwich et al. 1995, Steinbrecht 1998). These suggested functions are supported and further enlightened by recent experiments which show that pheromone-binding proteins (PBPs) in moths bind to several molecules, but conformational changes only occur in interaction with the specific pheromone (Campanacci et al. 2001, Bette et al. 2002, Mohl et al. 2002). The OBPs might also

contribute to the inactivation of the odorant, as discussed by Kaissling (reviewed in 1998). The identification of OBPs in insects started out from antennal homogenates of the giant silkworm *Antheraea polyphemus* (Vogt and Riddiford 1981). Afterwards OBPs, mainly PBPs, have been identified in several insect species (reviewed by Vogt et al. 1999). The number of OBP types identified in each species has remained small until recent studies of the fruitfly *Drosophila melanogaster* genome revealed 39 genes coding for putative OBPs (Shanbhag et al. 2001). This indicates the presence of several non-pheromonal OBPs binding host odorants.

The olfactory neurones are activated by the binding of the odorants or the complex odorant-OBPs to the membrane receptors. By activation, an intracellular G-protein coupled cascade reaction is induced, which produces the second messenger inositol 1,4,5-trisphosphate (IP<sub>3</sub>) or adenosine 3',5'-monophosphate (cAMP) (reviewed by Breer et al. 1990b, Paysan and Breer 2001, Krieger and Breer 1999). Evidence for the IP<sub>3</sub> pathway in insect olfactory RNs is based on biochemical and electrophysiological experiments. The rapid increase of the concentration of IP<sub>3</sub> upon stimulation with pheromones occurs within the time of the receptor potential (Breer et al. 1990a). Furthermore, the transduction depends on the presence of G-proteins, which are identified in insect antennae (Breer et al. 1990a, Laue et al. 1997), and on the opening of cation channels by IP<sub>3</sub>, which is demonstrated in insect olfactory neurones (Stengl 1994, Wegener et al. 1997). The cAMP pathway may also be important in insects (Krieger and Breer 1999). This is indicated by the expression of cAMP sensitive channels in the antennae (Baumann et al. 1994, Krieger et al. 1999). It is suggested that this pathway is required for information about a subset of odorants (Dubin et al. 1998).

### **Expression of receptor proteins in single neurones and the principle of convergence in the antennal lobe**

The first identification of a multigene family that might encode odorant receptor proteins was made in the rat by Buck and Axel (1991). This extremely large and divergent gene family was expressed exclusively in the olfactory tissue. The genes were further characterised by a central domain encoding seven transmembrane regions, known to be a typical feature of G-protein coupled receptors. Since then, putative genes for receptor proteins have been identified in many species of vertebrates and



invertebrates, e.g. 1000 types in the mouse and 61 in the fruitfly (reviewed by Mombaerts 1999, Keller and Vosshall 2003). Functional evidence is obtained for four olfactory receptor genes, one in the human (Wetzel et al. 1999), one in the rat (Zhao et al. 1998), one in the mouse (Touhara et al. 1999) and one in the fruitfly (Störtkuhl and Kettler 2001, Wetzel et al. 2001). The total number of gene types tested for expression is small, but so far, little co-expression of different receptor types has appeared in the neurones. Two exceptions are found; one type present in all olfactory RNs of the fruitfly is believed to possess a non-olfactory function (Keller and Vosshall 2003), and co-expression of the two olfactory receptor mRNA is found in a subset of olfactory RNs in rats (Rawson et al. 2000). Thus, the current held belief is that a single olfactory receptor gene type is expressed in each olfactory neurone. An exception is in the soil nematode *Caenorhabditis elegans*, which on average express 20 gene types coding for receptor proteins in each of the 32 chemosensory neurones (Troemel et al. 1995, 1997, reviewed by Bargmann 1998).

Olfactory RNs expressing one type of receptor proteins are in most insect species distributed over the whole antennae (i.a. Almaas and Mustaparta 1990), and in vertebrates over one of the four zones of the olfactory epithelium (Ma and Shepherd 2000, reviewed by Mombaerts 1999). Still the widely distributed neurones of the same type converge onto one or a few glomeruli, as shown both in vertebrates and invertebrates (Ressler et al. 1994, Vassar et al. 1994, Mombaerts et al. 1996, Gao et al. 2000, Vosshall et al. 2000). The ratio of convergence in insects is about 100-1000:1 and in vertebrates 25.000:1 (in rabbits) (Rospars 1988, Allison 1952). Staining of active insect pheromone RNs of specific types, supports the convergence principle (Berg et al. 1998, Berg 1998). However, exceptions from this rule have also been reported (Hansson et al. 1988, Anton and Hansson 1999). The organised convergence in insects starts before the axons grow into the antennal lobe, as an interaction between the single fibres and the glia cells, which is shown at the late metamorphic stage in the development of the antennal lobe (Rössler et al. 1999).

### **Encoding of odour information in receptor neurones**

A rough overview of odorants eliciting electrical responses across the olfactory epithelia can be obtained by recordings of electro-antennogram (EAG) in insects and electro-

olfactogram in vertebrates (EOG) (Schneider 1955, Ottoson 1956, 1971). To determine which of the odorants activate a single olfactory RN (the molecular receptive range of the neurone), responses to the odorants are recorded from single cells by placing a glass capillary or tungsten microelectrode into the base of one sensillum hair (Schneider 1957a,b, Boeckh 1962), or a glass capillary microelectrode over a cut hair (“tip-recording”) (Kaissling 1974). Trying to characterise the RNs in respect to specificity, one faces two major problems. The naturally produced odorants exist in blends of volatiles, and commercially available compounds are rarely 100% pure. It is important to use highly pure compounds for stimulation; otherwise the response of the RNs might be caused by the impurities in the samples tested. Obviously, testing naturally produced volatiles by direct stimulation does not show which of the constituents are active. A fruitful method is the linkage of high resolution gas chromatography to single cell recordings (GC-SCR), first carried out in pheromone analysis by Wadhams (1982), and later for analysing plant odorants (Tømmerås and Mustaparta 1989, Blight et al. 1995, Wibe and Mustaparta 1996). With this method, constituents of a mixture of volatiles can be presented to the RNs separately.

Early studies of olfactory RNs, carried out by testing commercially available compounds, has resulted in different principles on how odour information is encoded. Based on these studies, Vareschi (1971) defined two gross categories of RNs. One category was called “odour specialists”, where neurones are highly specialised for one compound and show the same specificity, i.e. the same dose-response relationships. The other category was termed “odour generalists”, i.e. RNs responding to many compounds and showing significantly different and overlapping “reaction spectra” (molecular receptive range). The pheromone RNs fulfil the requirement of “odour specialists” (i.a. reviewed by Schneider 1969, 1992, Hansson 1995, Mustaparta 1996), also according to another definition that demands a “biological importance (relevance)” of the odorant (Schneider and Steinbrecht 1968, Kaissling 1974). In the early studies, the plant odour RNs were categorised as “odour generalists”, but showed different sub-divisions in various species, including some narrowly tuned neurones (reviewed by Masson and Mustaparta 1990). The question about biological relevance of the plant odorants came more into focus by testing naturally produced odours and trying to isolate the active compounds. The possibility that key compounds exist for different food types was ruled

out in studies of the cockroach *Periplaneta americana* (Sass 1978, Selzer 1981). Here, stimulation with fractions of lemon oil revealed seven RN types responding to different fractions. The major component limonene, did not elicit responses in any of the RNs. Recent evidence for plant odour RNs being as sensitive and selective as pheromone RNs as well as RNs responding to several compound classes, is taken from studies using direct stimulation (Dickens 1990, Hansson et al. 1999, Jönsson and Anderson 1999, Larsson et al. 2001, Shields and Hildebrand 2001) and particularly from studies employing GC-SCR (Wibe and Mustaparta 1996, Røsteliën et al. 2000a,b, Stensmyr et al. 2001, 2003, Barata et al. 2002, Bichão et al. 2003). The latter studies exclude responses to the numerous inactive plant constituents and frequently show strongest responses to minor components. This stresses the importance of using GC-SCR for identifying plant odorants.

Based on the specificity of the studied RNs, two principles have emerged on how the odour information is mediated from the periphery to the central nervous system (CNS). One principle called “labelled line”, is used in chemical senses to express that information about one compound is mediated to the brain by one type of RNs, i.e. it involves no overlap of molecular receptive ranges between different RN types (Shepherd 1984). The other extreme principle called “across-fibre pattern mechanism”, means that information about one compound is mediated by different RN types, i.e. with overlap of molecular receptive ranges and different sensitivity to the compound (Gesteland et al. 1965). The mediation of pheromone information from the periphery to the antennal lobe in insects seems to follow the principle of “labelled line”. Furthermore, the RNs belonging to each type project into one of the male specific glomeruli (Berg et al. 1998, Berg 1998, Galizia et al. 2000b), constituting the macroglomerular complex (MGC) that receives information about insect produced signals (Boeckh and Boeckh 1979, reviewed by Boeckh and Tolbert 1993). Information about food and host odours is commonly believed to be mediated by the more complex mechanism of “across-fibre pattern” (reviewed by Lemon and Getz 1999), in spite of the fact that narrowly tuned RNs are found. Projections by functional types of RNs in the numerous “ordinary” glomeruli remain to be determined. However, optical recordings have shown glomerular maps of odour qualities that are conserved between individuals (reviewed by Galizia and Menzel 2001).

### **Olfactory coding and olfactory learning**

The antennal lobe has two major groups of neurones, the local inter-neurones and the projection neurones forming a network with the synaptic terminals of the RNs (reviewed by Chapman 1998, Anton and Homberg 1999). The GABAergic local inter-neurones innervating many glomeruli, are thought to mediate important inhibition in the antennal lobe, e.g. to increase the contrasts between activated and non-activated glomeruli (Homberg et al. 1989, Joerges et al. 1997, Galizia et al. 2000a, Sachse and Galizia 2002) and inhibit activation between odour pulses of pheromones (Christensen et al. 1993). The projection neurones conduct the information to higher brain centres, the mushroom bodies and the lateral protocerebrum. The mushroom bodies are shown to play a significant role in learning and memory of odours (reviewed by Menzel 1999, De Belle and Kanzaki 1999, Heisenberg 2003), while the lateral protocerebrum is a premotoric area, from which descending neurones make synapses with motor neurones in the suboesophageal ganglion and the thoracic ganglia (Strausfeld 1976).

A challenging question in odour information processing concerns how olfactory information is coded spatially and temporally, leading to odour discrimination. Since each glomerulus seems to receive information from one type of RNs, the next question is whether there is a correspondence between the input and the output of the glomeruli in the antennal lobe. This is found for the MGC of *H. virescens*, by intracellular recordings of antennal lobe neurones (Christensen et al. 1995, Vickers et al. 1998, Berg et al. 1998, Berg 1998). It means that projection neurones responding to antennal stimulation with an odorant have at least one dendrite branch in the glomerular unit receiving the information. However, in another species a mismatch between input and output information is shown in the antennal lobe (Hansson et al. 1994, Anton and Hansson 1999). More data on different species are needed in order to draw general conclusions about this principle. Intra- and extracellular recordings and multi-unit recordings of projection neurones have shown that different blends of plant volatiles activate different sub-populations of projection neurones, indicating a spatial representation of odour qualities (Laurent and Davidowitz 1994, Laurent et al. 1996, Christensen et al. 2000). In the locust and the honeybee (*Apis mellifera*), each projection neurone shows an odorant specific slow temporal pattern (Laurent et al. 1996). In

addition, a fast temporal pattern is recorded as local field potential oscillation cycles, ascribed to activation of different projection neurone populations. The oscillations, caused by the GABAergic local interneurons, are shown to be important for discrimination of structural similar odorants in the honeybee (Stopfer et al. 1997). It is speculated whether the observed oscillatory synchronisations may provide coding strategies for odour concentration and odour quality (reviewed by Stopfer et al. 1999, Laurent 1999, 2002, Galizia and Menzel 2000). Another study of projection neurones in a moth species, using stimulation with brief pheromone pulses, revealed a temporal pattern of synchronous activity that was dependent on odour intensity and intermittency (Christensen et al. 2000). However, the temporal pattern was not found to be oscillatory or odour specific. Whether these differences can be ascribed to different coding principles in different insect species or in different olfactory systems (general odorants versus pheromones) remains to be clarified.

An important aspect of olfaction is learning of odours. In order to recognise an odour, both neuronal detection and memory of the odour are necessary. An interesting question is - What are the neural mechanisms underlying learning and memory of odours? The importance of the mushroom bodies in olfactory learning and memory is well described in the honeybee by classical conditioning experiments using appetitive learning (reviewed by Menzel 1999, Müller 2002). The conditioned stimulus (odour) is associated with the unconditioned stimulus (sucrose stimuli and reward) that elicits the proboscis extension reflex (PER). One neurone, an octopaminergic ventral unpaired median neurone (VUMmx1), is shown to mediate information between the olfactory and the taste pathway in the learning process (Hammer 1993). Another neurone projecting from the mushroom bodies to the lateral protocerebrum, the pedunculus extrinsic neurone (PE1), is shown to change the responses after learning experiments (Mauelshagen 1993). The cellular and molecular mechanisms are enlightened by biochemical studies in the honeybee and by molecular biological studies of the fruitfly (reviewed by Menzel 1999, Heisenberg 2003). Appetitive learning of olfactory information is also shown in lepidopteran species, including heliothine moths (Hartlieb 1996, Fan et al. 1997, Daly and Smith 2000, Daly et al. 2001a,b).

## **The nature of plant odorants**

The large number of diverse compounds produced by plant species is well known from research in natural product chemistry as well as in chemical ecology. Plant compounds are built from products of the photosynthesis in diverse biosynthetic routes catalyzed by various enzymes. The compounds essential for growth and development of the plant are called primary metabolites, and others secondary metabolites (Hartmann 1996). The same compound may have both a primary and a secondary role. Secondary metabolites were first described as by-products of the plant metabolism by Sachs in 1873, and were traditionally considered as metabolic wastes and detoxification products (Haslam 1986, Luckner 1990). Today these compounds are classified as an essential part of the plant biochemical equipment to cope with the environment (Fraenkel 1959, Hartmann 1996, Pickett et al. 1999). Ehrlich and Raven (1964) were among the first to suggest that secondary metabolites evolved in a co-evolutionary arms race of plant defences against herbivory and herbivore animal responses. The plants produce and accumulate toxins, which is followed by a reciprocal response in the insect to detoxify or excrete the toxins. Being toxic also to the plant themselves, the secondary metabolites are often produced as pro-toxins (i.e. enzymatically ignited upon attack), constitutively accumulated in special organs (e.g. vacuoles, glandular trichomes) or induced (i.e. produced in response to attack) (Hartmann 1985).

The blend of volatiles produced by a plant is a complex mixture, often consisting of hundreds of compounds. The blends can be collected by various methods of distillation, headspace collection and extraction (reviewed by Silverstein and Rodin 1966, Agelopoulos and Pickett 1998, Millar and Sir 1998). The trapped volatiles are analysed by separation in a gas chromatograph, and the compounds are identified by mass spectrometry, followed by confirmation with authentic materials. Continually more plant constituents present in nature are identified, due to more sensitive analytical methods employed. Plant volatiles belong to many different chemical groups like short chain alcohols, aldehydes, ketones, esters, aromatic phenoles and lactones as well as mono- and sesquiterpenes (reviewed by Gibbs 1974, Smith 1976, Bernays and Chapman 1994, Ohloff 1994, Schoonhoven et al. 1998). Some compounds are mainly found in restricted plant taxa, like the isothiocyanates in Brassicacea (reviewed by Kjær

1976, Fahey et al. 2001). Others are commonly occurring, like the “green leaf volatiles” (mainly six-carbon alcohols and aldehydes) that are products of the lipid metabolism in green leaves (Visser and Avé 1978, Hatanaka 1993). Flower fragrances, constituting hundreds of compounds, may include different molecules than those of the vegetative plant parts (reviewed by Dudareva and Pichersky 2000). The quantity and quality of the volatile blends released by plants are affected by both biotic and abiotic factors (i.a. Paré and Tumlinson 1999, Gouinguéné and Turlings 2002).

Most of the plant constituents are produced via a few basic biogenetic routes, leading to one or a few key-metabolites, from which numerous derivatives are formed, usually by simple enzymatic transformations (Hartmann 1996). For instance, geranyl diphosphate is the unique precursor of all monoterpenes and farnesyl diphosphate of all sesquiterpenes (reviewed by Dewick 1999, Rohmer 1999). The production of compounds is often temporally and spatially restricted in the plants by the expression of genes coding for the enzymes, as shown for *de novo* biosynthesis of floral volatiles (Dudareva and Pichersky 2000). Furthermore, the enzymes can be enantiospecific, producing only one of the two enantiomers of a molecule (Schmidt et al. 1998, 1999, reviewed by Ohloff 1994).

### **Heliothine moths**

Plant produced volatiles are important cues for the herbivorous insects, including heliothine moths, in the location of their hosts for feeding and oviposition (Rembold and Tober 1985, Tingle et al. 1990, Mitchell et al. 1991, Tingle and Mitchell 1992). The emitted plant volatiles are transported by turbulent air that breaks the signal up into discrete packages with increasing distance from the source (Murlis 1986). Females searching for host-plants fly upwind by detection of attractants present in the air plume, i.e. showing positive anemotaxis toward the odour source (Tingle et al. 1990, Mitchell et al. 1991, Tingle and Mitchell 1992). After making contact with a plant, acceptance or rejection of the plant is made also on the basis of information mediated by other senses; taste and mechano-sensation (reviewed by Bernays and Chapman 1994, Schoonhoven et al. 1998). Experience of a host plant visit seems to affect later choices of host plants in

female heliothine moths (Firempong and Zalucki 1991). It was shown that experience with a host plant significantly increased the probability of selecting that plant species for subsequent oviposition and feeding (Cunningham et al. 1998a,b). Thus, it is speculated whether learning is involved in the mechanisms of host plant selection (Cunningham et al. 1999).

The subfamily Heliothinae (Insecta; Lepidoptera; Noctuidae) consists of more than 80 different species, of which several are considered as world-wide major pests on crops, like the American tobacco budworm moth *Heliothis virescens* (Fabricius) and the cotton bollworm moth *Helicoverpa armigera* (Hübner) (Todd 1978). *H. virescens*, is distributed over the American continent and uses as principal hosts, i.a. plants of tobacco, cotton, tomato, sunflower and soybean (Fitt 1989, Matthews 1991). *H. armigera*, geographically separated from *H. virescens*, is distributed over South Europe, Asia, Australia, Africa and Eastern Pacific. As a principal host, this species uses plants of i.a. maize, sorghum, sunflower, cotton, tobacco, soybean, pulses (chick pea, pigeon pea), safflower, rapeseed and groundnuts (Fitt 1989, Matthews 1991). *H. armigera* has been found on at least 60 cultivated and 67 wild host plants in 39 families (reviewed by Reed and Pawar 1982, Zalucki et al. 1986). The major pest status of the two heliothine species are explained by their high polyphagy, mobility, fecundity and the ability to have facultative diapause, in addition to the ability to resist different pesticides (Fitt 1989). The Oriental tobacco budworm *Helicoverpa assulta* (Guenée), partly sympatric with *H. armigera*, is distributed over Asia. This species, considered oligophagous, has a relatively narrow host plant range, mainly feeding on plant species of Solanacea such as tobacco, tomato, hot pepper and several *Physalis* species (Hill 1983, Matthews 1991, Park 1991).

Olfaction in heliothine moths has been studied in several species, particularly as it concerns pheromone communication. Chemical analyses combined with behavioural studies have revealed which compounds and ratios of them act in intra- as well as interspecific communication (i.a. Kehat et al. 1980, Vetter and Baker 1983, Kehat and Dunkelblum 1990, Cork et al. 1992, Boo et al. 1995, Vickers and Baker 1997). The detection of these chemical signals by the RN as well as the processing of the information in the antennal lobe has been studied (reviewed by Mustaparta 2002). However, the knowledge of encoding and processing of other odorants have been scarce



in heliothine moths as well as in other organisms. Until recent GC-SCR studies by Røstelién et al. (2000a,b), little knowledge has existed about plant odorants in heliothine moths. The objectives of the present thesis were to identify important plant odorants detected by females of the three species, *H. virescens*, *H. armigera* and *H. assulta*, and to determine how the information is encoded in the RNs.

## AIMS OF THE THESIS

The aims of the thesis were:

1. To identify naturally produced plant volatiles that are detected by receptor neurones in females of the heliothine moths *Heliothis virescens*, *Helicoverpa armigera* and *Helicoverpa assulta* (Paper I, II, III).
2. To determine the molecular receptive range and sensitivity (specificity) of the plant odour receptor neurones (Paper I, II, III).
3. To compare the specificity for functional classification of the plant odour receptor neurones within and between the three related heliothine species (Paper II, III).
4. To explore the possibility of staining plant odour receptor neurones for determining the projection pattern in glomeruli in the antennal lobe (Paper II, III).
5. To assess the biological importance of one odorant activating a major type of plant odour receptor neurones in females of one species (*Heliothis virescens*) (Paper IV).

## SURVEY OF THE INDIVIDUAL PAPERS

In the papers forming the basis of this thesis, efforts have been made to elucidate the five aims given above. Five RN types have been studied in three related heliothine species, concerning specificity. Preliminary data on the projection of plant odour receptor neurones is obtained. For one important odorant, the behavioural significance is revealed.

## Paper I

Paper I was based on the findings by Røsteliën et al. (2000a) that *H. virescens* females have one major type of plant odour RNs responding to the sesquiterpene germacrene D. By electrophysiological recordings linked to gas chromatography, single RNs in females of the related species *H. armigera* were found to respond with high sensitivity and selectivity to germacrene D. Germacrene D was present as a constituent in headspace or in extracts of different plant species, in fractions isolated from essential oil by medium pressure liquid chromatography (MPLC) or in reference samples (provided by Prof. W. A. König). Since germacrene D consists of two enantiomers, the aim was to determine whether these neurones responded selectively to one enantiomer or to both. Optical enantiomers in general and those of germacrene D in particular are difficult to obtain as 100% optically pure compounds. Therefore, this study was performed by the use of a chiral GC-column (provided by Prof. W. A. König), which could separate the germacrene D enantiomers. By installing this column in parallel with a non-chiral column in the GC, the RNs could be tested for mixtures and fraction of plant volatiles via both columns. When germacrene D was injected into the chiral column, the two enantiomers were well separated; (+)-germacrene D eluted before (-)-germacrene D. The results obtained indicated that all germacrene D RNs belonged to the same type; i.e. all responded to both enantiomers, but (-)-germacrene D had about 10 times stronger effect than the (+)-enantiomer. Parallel dose-response curves for the two enantiomers were obtained by direct stimulations. The enantiomeric composition of germacrene D, determined on the basis of the neurone responses, was found to differ in six plant species and in different individuals of one species. The results, showing the presence of only one neurone type for receiving the information about germacrene D in the various plants, suggest that the two enantiomers mediate the same kind of information to the moth brain, but with different intensity.

## Paper II

The aim of the study in paper II was to find out whether RNs responding to germacrene D might be present in more of the heliothine species, and whether RNs responding to

the same compounds exploit the same specificity, i.e. whether they show similar structure-activity relationships. Females of the three related species *H. virescens*, *H. armigera* and *H. assulta* were studied. The presence of germacrene D RNs was shown in *H. virescens* by Røstelien et al. (2000a) and in *H. armigera* in paper I. In paper II, germacrene D RNs were also identified in *H. assulta*. In addition, structure-activity data of the germacrene D RN type was revealed in the three species. Altogether 69 recorded RNs were classified, based on 436 GC-SCR using five different column types, and included up to 37 recordings from the single neurones.

The RNs in the three species showed similar ranking of responses to the nine active sesquiterpenes. (-)-Germacrene D was about 10 times more effective than the (+)-enantiomer, being again 10 times more effective than (-)- $\alpha$ -ylangene. Weaker effects were obtained for (+)- $\alpha$ -copaene, one unidentified sesquiterpene (no. 3), (+)- $\beta$ -ylangene,  $\beta$ -copaene and both enantiomers of one unidentified sesquiterpene (no. 6). All active compounds were structurally similar to (-)-germacrene D. Comparing the structures of the active molecules, shows that the important properties of (-)-germacrene D are the flexibility of the ten-membered ring system and the three double bonds acting as electron rich centres, as well as the direction of the isopropyl group. The narrowly tuning of the RNs was demonstrated by the lack of activity of related sesquiterpenes, like (+)- $\alpha$ -ylangene and (-)- $\alpha$ -copaene.

Paper II also shows the presence of germacrene D in various plant species, i.e. in about half of the tested samples of 30 plant species (hosts and non-hosts) presented as headspace samples, essential oils and extracts. The “secondary” active compounds were only present in a few plant samples. The active compounds were isolated from essential oils by MPLC for stimulation of the RNs and for identification by GC-MS.

In trying to explore the projection of the germacrene D RNs in the antennal lobe, stain was applied to the base of the sensillum from which the recordings of the germacrene D RNs were made. Out of seven experiments, one successful staining of a RN in *H. assulta* was obtained, showing four selectively stained axon terminals in the antennal lobe. Three of them were located close to the antennal lobe (one filling a larger volume than the others), and the fourth was located in the ventro-medial part of the lobe.

### Paper III

The presence of the functionally similar germacrene D RNs in all three heliothine species raised the question whether these species also possess other types of functionally similar plant odour RN types. In *H. virescens* females, Røstelien et al. (2000b) identified four RN types that were co-located in the same sensilla, three of them responding to the inducible compounds *E*- $\beta$ -ocimene, *E,E*- $\alpha$ -farnesene and *E,E*-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), respectively. In the study reported in paper III, the three RN types were also found in females of *H. armigera* and *H. assulta*. Additional data on the molecular receptive ranges of the three RN types in all three species were provided. The compounds activating the fourth co-located RN type were also identified in the three species, of which the flower volatile geraniol was found to be most effective. Structure-activity relationships of all four co-located RN types were worked out by the identification of the active compounds in the plant material of 19 plant species tested. The identifications were proved by retesting the authentic compounds that were commercially available. Fifty-two RNs were recorded, and the classification was based on 135 GC-SCR, which included up to 38 recordings from the single neurones.

The compounds activating each RN type were structurally similar. The RN type I was activated by the monoterpenes, *E*- $\beta$ -ocimene,  $\beta$ -myrcene, *Z*- $\beta$ -ocimene, *E*-4,8-dimethyl-1,3,7-nonatriene [DMNT] and dihydromyrcene, the RN type II was activated by the sesquiterpenes, *E,E*- $\alpha$ -, *Z,E*- $\alpha$ - and *E*- $\beta$ -farnesene, the RN type III was activated by the *homo*-sesquiterpene TMTT and the RN type IV was activated by the monoterpene alcohols, geraniol, citronellol, (*S*)-(+)- and (*R*)-(-)-linalool and one unidentified compound. The narrowly tuning of the RNs to the few compounds was demonstrated by the presence in the plant samples of many structurally similar compounds that were inactive. The four co-located RN types in the three insect species showed the same relative response strength to the active odorants.

In trying to explore the projection of the four co-located RNs in the antennal lobe, stain was applied to the base of the sensillum from which the recordings of the RNs were made. Out of three experiments, one successful staining of the four co-

located RNs in *H. assulta* was obtained, showing four selectively stained axons in the antennae and four axon terminals in the antennal lobe. Three of them were located in different areas close to the antennal nerve, and the fourth was located in the ventro-medial part of the lobe.

## **Paper IV**

The presence of the numerous germacrene D RNs on the antennae of females of *H. virescens* (Røsteliën et al. 2000a, paper II), raised the question about the biological significance of this plant odorant. A two-choice wind-tunnel was designed to study the preference of mated *H. virescens* females for host plants with (-)-germacrene D dispensers versus plants with control dispensers. (-)-Germacrene D (purity > 99.2%, enantiomeric purity > 99.9%), isolated from the essential oil of ylang-ylang (*Cananga odorata*) by MPLC, was used in the behavioural tests. The tobacco plants used in these experiments belonged to a strain that contained no germacrene D, as shown by electrophysiological experiments. Plants with dispensers having low release rates of (-)-germacrene D had a greater attractiveness to the moths than tobacco plants with control dispensers. Oviposition was observed on the plants with (-)-germacrene D, and a significantly larger number of eggs was found on these plants.

## **DISCUSSION**

### **Encoding of plant odour information in receptor neurones**

The results obtained in paper I-III contribute to the understanding of the encoding of plant odour information in herbivorous insects. By the use of GC-SCR with the different GC-columns, it has been possible to identify which of the numerous plant volatiles are detected by the RNs in heliothine moths and to compare the responses within and between species. The results have shown a clear classification of the RNs in different types, each type showing a strong response to one compound and weaker responses to a few others of related structures. Since the identification is made on the basis of screening naturally produced volatiles, in principle of whole plant blends, the

active compounds are probably biologically relevant, i.e. odorants for which the RNs are evolved. This view is further strengthened by the consistent responses to the same components present in many of the plant species, and by the responses to the same compounds in the three related heliothine moths. The five types of RNs presented here constitute 91% of all olfactory RNs that were recorded. The high probability of obtaining the five RN types in stable recordings made us focus on identifying all odorants activating these RNs for determining the structure-activity relationships. In respect to the principles on how olfactory information is encoded in the RNs, the following data are important. The five RN types have narrow molecular ranges with no overlap between them, and they are as sensitive to one of the compounds as the pheromone RNs are for their key stimuli. This indicates that the information about these plant odorants is mediated to the brain according to the principle of “labelled-lines” rather than of “across fibre patterns”. It means that the code to the brain of a blend quality of these odorants is the ratio of activity in the five RN types.

### **Narrowly tuned receptor neurones**

The present results of narrowly tuned RNs in heliothine moths (Paper I-III) are in agreement with results from studies of other insect species obtained by the use of GC-SCR (Wibe and Mustaparta 1996, Stensmyr et al. 2001, 2003, Barata et al. 2002, Bichão et al. 2003). The large number of plant olfactory RNs recorded from various insect species have shown narrow tuning with only limited overlap of molecular receptive ranges within chemical groups, and no overlap between different chemical groups. These data, based on GC-SCR, are more reliable than testing synthetic chemicals, since the pre-selection of the test compounds has already ignored a number of possible active odorants. Furthermore, at high concentration, one may obtain responses of the neurones without knowing what the key stimulus is. Many narrowly tuned neurones, e.g. pheromone receptor neurones, respond additionally to high concentrations of other compounds (Hansson et al. 1988). The narrow tuning of plant olfactory RNs found by GC-SCR (Paper I-III), correlates well with the results obtained in molecular biological studies, showing that one neurone expresses a single type of the identified olfactory receptor proteins (Keller and Vosshall 2003). Interaction with a

single type of receptor proteins seems more likely in neurones responding to a narrow set of related chemicals than in neurons responding to many compounds of different chemical groups. The possibility still exists in other organisms that some RNs are broadly tuned, and may perhaps have receptors interfering with certain parts of very different odour molecules (reviewed by Korsching 2002). However, data in the present study, showing reduced or no activity by small changes of the key molecule indicates the importance of the whole molecular structure for providing interaction of certain groups with the receptor protein. Since we do not yet know the molecular features of the receptor proteins, the binding characteristics between the odorant and the receptor protein can not be proposed yet. However, the identification of genes coding for olfactory receptor proteins on the antennae of *H. virescens*, is in progress (Krieger et al. 2002). With more olfactory genes identified in heliothine moths and olfactory receptor proteins sequenced and characterised by crystallography, it will be interesting to study the binding that might take place between the receptor protein and the odorants identified and presented in this thesis. Furthermore, the recent identification and cloning of an OBP expressed in male (*sensilla basiconica*) and female (*s. basiconica* and *s. trichodea*) *H. armigera* (Wang et al. 2003), is promising for studying the binding of the odorants to the OBPs.

In physiological studies of vertebrate olfactory RNs, direct stimulation with a few selected commercial compounds is still common. The traditional view has been that RNs are activated by several structurally divergent molecules (i.a. Malnic et al. 1999, Duchamp-Viret et al. 1999, 2000, reviewed by Buck 2000). One may ask whether the biological key stimulus of the olfactory RNs is present among the odorants, and whether the stimuli are presented in physiological concentrations. Despite the pre-selection of stimuli, recent studies have revealed more narrowly tuned olfactory receptors. One example is the rat olfactory receptor (OR-I7), tested for 74 related and unrelated volatile compounds, which responded best to octanal, and weaker to heptanal and nonanal (Zhao et al. 1998). It is further shown that a difference in only one amino acid between a rat and a mouse olfactory receptor protein, change the highest affinity from octanal to heptanal, respectively (Krautwurst et al. 1998). Another study has revealed olfactory RNs with narrow tuning in general, but with overlapping molecular receptive range (Kajiya et al. 2001). The general view in vertebrates is still that the olfactory system

uses a combinatorial receptor coding scheme to encode odour identities (reviewed by Buck 2000, Korsching 2002). Obviously one expects to find broadly tuned human olfactory RNs when comparing the number of chemical stimuli humans are able to discriminate (>10 000) with the smaller number (347) of candidate functional olfactory receptor genes for the detection of odorants (Glusman et al. 2001, Zozulya et al. 2001).

### **Molecular features of the odorants**

Although the receptor proteins have only recently been known, the interest for important molecular features of odorants has been shown in many studies of structure-activity relationships (i.a. Klopping 1971, Beets 1978, Ohloff 1986, Turin 1996). The structures of the odorants identified in this thesis show some significant features for stimulation of the five RN types. One important feature is the enantiomeric configuration, here shown for germacrene D (Paper I, II). The interest for optical isomers has a long tradition in olfaction, not least since Wright (1977) proposed the hypothesis of Dyson (1938) that the interaction between the odorant and the receptors were dependent on intra-molecular vibrations (IR-spectra) of the odorant. The olfactory discrimination of optical isomers can not be explained by this hypothesis. Optical isomers, existing as mirror image molecules, have otherwise identical chemical properties, including IR-spectra. The discrimination of (+)- and (-)-carvone in humans is well known (Friedman and Miller 1971). However, we do not yet know how these enantiomers are discriminated by the receptors. The first studies, showing how information about enantiomers was received by olfactory RNs, were in bark beetles (Mustaparta et al. 1980) and in two moth species (Hansen et al. 1983), showing that a selective type of RNs had evolved for each of the enantiomers of pheromone compounds. Since then, enantioselective RNs for pheromones have been demonstrated in other insect species (reviewed by Masson and Mustaparta 1990, Leal 2001). Obviously, since each enantiomer mediates a particular message to the insect, e.g. attraction and disruption of attraction, separate RN types have evolved for mediating the different messages of these signals. For plant odorants, it is less obvious whether the organism needs enantioselective RNs. One might expect that insects have evolved different RN types for enantiomers produced by separate enantioselective enzymes



resulting in the presence of exclusively one or both isomers in various plants. Therefore, it was interesting to find that heliothine moths have only one RN type among the large number of germacrene D RNs that respond to both enantiomers but consistently with 10 times higher sensitivity for (-)-germacrene D (Paper I, II). From this, one might assume that the moths do not need to distinguish well between the two enantiomers, otherwise two RN types would have evolved. The results on the germacrene D RN type (Paper I) are the first to show that two pure plant enantiomers activate the same RNs, but with different stimulatory effect. Another example is given in paper III, where both enantiomers of linalool elicited “secondary” responses in the geraniol RN type, (*R*)-(-)-linalool being more effective than the (*S*)-(+)-enantiomer. The fact that chiral recognition of molecules is important in biological activity (Ohloff 1994) stresses the importance of testing optically pure samples of odorants when studying the molecular receptive ranges of olfactory RNs. Obviously it is important to use chiral GC-columns for separating optical isomers, or using synthesised enantiomers of high purity in studies of molecular receptive ranges of the RNs.

In addition to chirality of the molecules, the structure-activity relationships of the five RN types (Paper I-III) also demonstrate the importance of other molecular features of the odorant in stimulating the RNs. The significance of electron dense parts and the flexibility of the molecules, reflected by the positions of double-bonds as well as the position and type of the functional groups for activating the RNs, have been shown in many previous studies (i.a. Priesner 1959, 1977, Kafka 1974, Schneider et al. 1977, Bengtsson et al. 1990, reviewed by Ohloff 1986, Masson and Mustaparta 1990, Mustaparta 2002). However, since each odorant-receptor interaction is unique, it is important to determine the molecular features in each case. In future experiments, when pure compounds of the odorants identified in the present thesis are available, more detailed quantitative structure-activity relationships (QSAR) of the five RN types can be determined in more details. This will enable the use of computational methods to point out the optimal configuration of the molecules that might be important for the interaction with the receptor proteins.

### **Functionally similar receptor neurone types in related species**

Detection of the same odorants by closely related species has raised the question of whether the functional properties of the receptors are similar or different with respect to specificity. In heliothine moths, the same compounds are used as pheromones and interspecific signals, but in different compositions (reviewed by Mustaparta 1996, 1997). The previous findings of pheromone RNs with similar specificity was interesting, but perhaps not surprising considering the significance of the genetical components in the evolution of pheromone communication and the few compounds involved as signals. In contrast to this is the vast variation in volatiles released by plants. Many insect species have evolved polyphagy whereas a few others have evolved oligophagy. Thus, one might expect diverse functional properties (specificity) of the RNs detecting plant odours. From this point of view, it was surprising to find that the identified RNs in the three heliothine species exhibit similar specificities (Paper I-III). They both detect the same key compound, and show similar sensitivity to the secondary active compounds of structural similarity. This suggests that the identified compounds play a significant role in the interaction with the plants. It is particularly interesting that the one oligophagous species, *H. assulta*, has evolved the same RN types as the polyphagous *H. virescens* and *H. armigera*. The results suggest that the five RN types have evolved from a common ancestor and have been unchanged in spite of employing different host plant ranges.

The conservation of the plant odour RN types in the three heliothine species is also unexpected in considering that changes of one or a few amino acids in the sequence of a protein can result in a different compound produced in plant biosynthesis (Wang and Pichersky 1999) as well as in an olfactory receptor protein changing the affinity to a different odorant (Krautwurst et al. 1998). In the evolutionary context, the five RN types presented in this thesis seem to have the same degree of stability as most of the pheromone RN types in the heliothine moths (Mustaparta 1996, 1997), suggesting similar selection pressure for maintaining the gene types. However, different RN specificity, as found for some RNs detecting interspecific signals, is pointed out to have evolved as a consequence of other important pheromone compounds used by the species (Mustaparta 1996, 1997). It will be interesting to find out whether the remaining plant

odour RN types in the heliothine moths show identical or different specificity. This will elucidate whether the use of different host plants is reflected in the RN specificity, in the CNS processing of the plant odour information or if other mechanisms are responsible for the different host range.

### **The number of RN types responding to plant odorants**

The identification of numerous genes coding for different types of olfactory receptor proteins in all organisms studied (Mombaerts 1999, Keller and Vosshall 2003), indicates that a large number of plant odour RN types should also be present in heliothine moths. Among the RNs recorded so far in heliothine moths, 20 different types have been identified on the basis of GC-SCR, each type responding to different compounds. In addition to the five RN types responding to compounds within the chemical groups of acyclic monoterpenes, monoterpene alcohols, sesquiterpenes and *homo*-terpenes (Røstelien et al. 2000a,b, Paper I-III), RN types responding to compounds of monocyclic monoterpenes, oxygenated bicyclic alcohols, aromatic hydrocarbons and “green leaf volatiles” are also recorded (Røstelien et al. unpublished results). More RN types are expected to be found in continued GC-SCR studies of heliothine moths. This expectation is based on the finding that each RN type projects in one or a few glomeruli of the antennal lobe. In the fruitfly, 36 RN types project to the 43 glomeruli, each glomeruli receiving information from only one type (Vosshall 2001, Keller and Vosshall 2003). The number of “ordinary” glomeruli (62) in the antennal lobe of heliothine moths is assumed to be involved in plant odour information processing, which indicates a higher number than the 20 plant odour RN types identified so far (Berg et al. 2002, Skiri et al. unpublished results). In future studies it will be possible to compare the number of RN types to be identified by GC-SCR and the number of receptor protein types to be identified in molecular biological studies of heliothine moths.

The fact that the five RN types presented here constitute 91% of all of the recorded olfactory neurones responding to plant odours needs to be considered. It may reflect the relative number of each neurone type present on the antennae, assuming that all neurones are equally accessible to the electrophysiological recordings. This suggests

that the five RN types are of particular importance for the heliothine species. Alternatively, the other neurones may belong to sensilla that are not frequently penetrated because of the localisation. Although we have never found indications of RN types with particular localisation, we can not exclude this possibility.

The next question is in which glomeruli the different RN types, project. A functional organisation of glomeruli has been shown for the MGC in heliothine moths (Berg et al. 1998, Berg 1998). Since the plant odour RNs are localised in the shorter *s. trichodea* type II and *s. basiconica*, it has not yet been possible to carry out recordings from cut hairs with the “tip-recordings” technique. Therefore, we explored the possibility of staining the RNs selectively by applying the stain to the recording site of the tungsten microelectrode, after making sure that the neurone was penetrated by the electrode (observed by the rise in spike activity followed by silence). The preliminary results showing selective stained axons terminating in 4 glomeruli (Paper II and III), are promising for further studies of the functional organisation of the 62 “ordinary” glomeruli. Interestingly, optical imaging experiments reveal calcium responses in corresponding areas of the antennal lobe of *H. virescens* when stimulating with the same plant odorants (Skiri et al. 2002, unpublished results). Of particular interest is the projection of the (-)-germacrene D RNs that is easily obtained in heliothine antennae. The projections of the RNs in heliothine moths seem to have no branching in more glomeruli (Paper II and III) in contrast to single primary olfactory axons of the locust projecting in several glomeruli (Laurent and Naraghi 1994, Anton and Hansson 1996).

### **Behavioural responses to plant odorants**

In the identification of plant odorants that are detected by the oligophagous *H. assulta* and the polyphagous *H. armigera* and *H. virescens*, the interesting question is which odorants and mechanisms make these moths select different host plants. If all RN types are functionally similar, the different behavioural response to the compounds must be based on different processing of the odour information in the CNS. This is shown for the insect produced signals in some heliothine species, being detected by functionally similar RNs, but eliciting different behaviours (Mustaparta 1996, 1997). Alternatively, the different heliothine moths may have species specific RN types for some plant

odours, which are not yet identified. In order to resolve these questions, more behavioural studies are needed. In the present thesis, only behavioural responses to (-)-germacrene D were examined (Paper IV). Since attraction to plants is thought to be elicited by blends rather than single compounds, (-)-germacrene D was added to host plants containing no germacrene D. Dispensers with a low release rate of (-)-germacrene D placed on the host plant, caused increased attraction and oviposition of mated *H. virescens* females to the plant compared with the control plant. Whether the increased number of eggs laid was an indirect result from the increased attraction or (-)-germacrene D also had an effect on oviposition was not elucidated. Another interesting question is whether the related sesquiterpenes activating the (-)-germacrene D RNs elicit the same behavioural response at higher doses. This would be expected if the response is only dependent on increased activation in these RNs, and the compounds are ineffective on other RNs. Of particular interest is to test the behavioural effect of (+)-germacrene D, for comparing with the response to (-)-germacrene D. In field studies in Australia, attraction to (-)-germacrene D by *H. armigera* females is under investigation (Gregg and Del Socorro, unpublished results).

Among the other plant odorants identified in the present thesis (Paper I-III), myrcene, *Z*- $\beta$ -ocimene and racemic linalool are in previous studies found attractive for *H. armigera* females when presented as constituents of synthetic blends (Rembold et al. 1991, Bruce and Cork 2001). We can only speculate about the behavioural role of the other identified odorants (Paper I-III). Geraniol is a typical floral scent. A plant in the flowering phase provides the food source, and in the floral and fruit phases the plants are in the most attractive phases for ovipositing heliothine moths (reviewed by Fitt 1989). The feeding of *H. virescens* caterpillars is shown to induce certain volatiles in host plants, which repel conspecific, mated females (De Moraes et al. 2001). Thus, the odorants activating the three other co-located RN types may elicit avoidance reaction, since these compounds are induced in several host plants upon attack by feeding larvae (reviewed by Turlings and Benrey 1998, Paré and Tumlinson 1999, Dicke and Van Loon 2000).

## Learning of plant odorants

The studies of this thesis have focused on which odorants of host and non-host plants are detected by the heliothine moths. The extremely sensitive RNs, particularly for the key stimuli (nanograms for (-)-germacrene D), indicate that the odorants may be detected at some distance from the plant, making the insect able to decide whether to fly toward the plant or not. In this situation, one may assume that the quality and the ratio of the odorants are important for the attraction to the host plant. When arriving on the plant, the concentration of the odorants might be quite high, and also the odorants with weak effect might be detected. After landing, taste and mechano-sensation are more important for the further evaluation of the plant as a host for nutrition or egg-laying (Ramaswamy 1988, Ramaswamy et al. 1987, Jackson et al. 1984, Li et al. 2001). When exposed to the relatively high concentration of the whole blend of the flower during nectar feeding, the insects learn to associate the odour with the nutritional reward, which is also reflected by increased probability of selecting the same host species after experience (Firempong and Zalucki 1991, Cunningham et al. 1998a,b). In an ongoing project, the ability of heliothine moths to learn the plant odorants identified (Paper I-III) is studied by the use of the PER (Skiri et al., unpublished results). Interesting questions about olfactory discrimination can be answered by these time consuming but rather simple experiments; do the heliothine moths show different ability of learning plant odorants used in different contexts, and do they manage to discriminate information about odorants that are encoded in the same RNs? In our laboratory, neuronal connections between the olfactory and taste pathways in *H. virescens* females are also studied. Projections in the suboesophageal ganglion of taste RNs on the antennae and proboscis have been described (Jørgensen 2003, Kvellø 2003), and intracellular recordings combined with stainings have shown one neurone with similar morphology to the VUM neurone in the honeybee (Rø et al. unpublished results). The results in the present thesis contribute to these projects on olfactory coding and learning, by providing knowledge about biologically relevant plant odorants. Altogether the aim is to elucidate the neuronal mechanisms underlying olfactory coding and olfactory learning involved in nectar feeding as well as in host plant selection by heliothine moths.

## CONCLUSIONS AND PROSPECTS

The results of this study show that plant odour information in females of the three heliothine moths *H. virescens*, *H. armigera* and *H. assulta*, is received by narrowly tuned RNs with non-overlapping molecular receptive ranges. The five RNs described are all strongly activated by one key odorant and show weaker response to a few related molecules. The RN types are functionally similar within and between the three heliothine species. A new staining method is tested which show projections of the RNs in the antennal lobe. One odorant, being the key stimulus for the major RN type, was found to be attractive when added to host plants that did not contain this odorant.

The identified biologically relevant odorants provide a basis for further studies of olfaction in heliothine moths. This knowledge is presently used in studies of olfactory coding and olfactory learning in addition to behavioural experiments. In future experiments combining physiology, molecular biology and chemistry, the identified odorants may be utilised to study the molecular and cellular function of olfactory RNs.

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## REFERENCES

- Agelopoulos, N. G. and J. A. Pickett. 1998. Headspace analysis in chemical ecology: effects of different sampling methods on ratios of volatile compounds present in headspace samples. *J Chem Ecol* 24:1161-1172.
- Allison, A. C. 1952. The morphology of the olfactory system in the vertebrates. *Biol Rev* 28:195-244.
- Almaas, T. J. and H. Mustaparta. 1990. Pheromone reception in tobacco budworm moth, *Heliothis virescens*. *J Chem Ecol* 16:1331-1347.
- Almaas, T. J. and H. Mustaparta. 1991. *Heliothis virescens*: response characteristics of receptor neurons in sensilla trichodea type 1 and type 2. *J Chem Ecol* 17:953-972.
- Anton, S. and B. S. Hansson. 1996. Antennal lobe interneurons in the desert locust *Schistocerca gregaria* (Forsk.) : Processing of aggregation pheromones in adult males and females. *J Comp Neur* 370:85-96.
- Anton, S. and B. S. Hansson. 1999. Physiological mismatching between neurons innervating olfactory glomeruli in a moth. *P Roy Soc B* 266:1813-1820.
- Anton, S. and U. Homberg. 1999. Antennal lobe structure. Pages 97-124 in B. S. Hansson, ed. *Insect olfaction*. Springer-Verlag, Berlin Heidelberg.
- Barata, E. N., H. Mustaparta, J. A. Pickett, L. J. Wadhams, and J. Araujo. 2002. Encoding of host and non-host plant odours by receptor neurones in the eucalyptus woodborer, *Phoracantha semipunctata* (Coleoptera: Cerambycidae). *J Comp Physiol A* 188:121-133.
- Bargmann, C. I. 1998. Neurobiology of the *Chaenorhabdites elegans* genome. *Science* 282:2028-2033.
- Baumann, A., S. Frings, M. Godde, R. Seifert, and U. B. Kaupp. 1994. Primary structure and functional expression of a *Drosophila* cyclic nucleotide-gated channel present in eyes and antennae. *EMBO J* 13:5040-5050.
- Beets, M. G. J. 1978. Structure-activity relationships in human chemoreception. Applied Science Publishers, London.
- Bengtsson, M., T. Liljefors, B. S. Hansson, C. Löfstedt, and S. V. Copaja. 1990. Structure-activity relationships for chain-shortened analogs of (Z)-5-decenyl acetate, a pheromone component of the turnip moth, *Agrotis segetum*. *J Chem Ecol* 16:667-684.
- Berg, B. G. 1998. Encoding of pheromone information in two related moth species. (Ph.D. Thesis). NTNU.
- Berg, B. G., T. J. Almaas, J. G. Bjaalie, and H. Mustaparta. 1998. The macroglomerular complex of the antennal lobe in the tobacco budworm moth *Heliothis virescens*: specified subdivision in four compartments according to information about biologically significant compounds. *J Comp Physiol A* 183:669-682.
- Berg, B. G., C. G. Galizia, R. Brandt, and H. Mustaparta. 2002. Digital atlases of the antennal lobe in two species of tobacco budworm moths, the oriental *Helicoverpa assulta* (male) and the American *Heliothis virescens* (male and female). *J Comp Neur* 446:123-134.
- Bernays, E. A. and R. F. Chapman. Host-plant selection by phytophagous insects. 1994. New York, Chapman & Hall.
- Bette, S., H. Breer, and J. Krieger. 2002. Probing a pheromone binding protein of the silkmoth *Antheraea polyphemus* by endogenous tryptophan fluorescence. *Ins Bioch Mol Biol* 32:241-246.
- Bichão, H., A.-K. Borg-Karlson, J. Araújo, and H. Mustaparta. 2003. Identification of plant odours activating receptor neurones in the weevil *Pissodes notatus* F. (Coleoptera, Curculionidae). *J Comp Physiol A* 189:203-212.

- Blight, M. M., J. A. Pickett, L. J. Wadhams, and C. M. Woodcock. 1995. Antennal perception of oilseed rape, *Brassica napus* (Brassicaceae), volatiles by the cabbage seed weevil *Ceutorhynchus assimilis* (Coleoptera, Curculionidae). *J Chem Ecol* 21:1649-1664.
- Boeckh, J. 1962. Electrophysiologische Untersuchungen an einzelnen Geruchsrezeptoren auf den Antennen des Totengräbers (*Necrophorus*, Coleoptera). *Z Vgl Physiol* 46:212-248.
- Boeckh, J. and V. Boeckh. 1979. Threshold and odor specificity of pheromone-sensitive neurons in the deutocerebrum of *Antheraea pernyi* and *A. polyphemus* (Saturniidae). *J Comp Physiol* 132:235-242.
- Boeckh, J., P. Distler, K. D. Ernst, Hösl.M, and D. Malun. 1990. Olfactory bulb and antennal lobe. Pages 201-227 in D. Schild, ed. *Chemosensory information processing*. Springer Verlag, Berlin.
- Boeckh, J. and L. P. Tolbert. 1993. Synaptic organization and development of the antennal lobe in insects. *Microsc Res Tech* 24:260-280.
- Boo, K. S., K. C. Park, D. R. Hall, A. Cork, B. G. Berg, and H. Mustaparta. 1995. (Z)-9-tetradecenal: a potent inhibitor of pheromone-mediated communication in the oriental tobacco budworm moth; *Helicoverpa assulta*. *J Comp Physiol A* 177:695-699.
- Breer, H., I. Boekhoff, and E. Tareilus. 1990a. Rapid kinetics of second messenger formation in olfactory transduction. *Nature* 345:65-68.
- Breer, H., J. Krieger, and K. Raming. 1990b. A novel class of binding proteins in the antennae of the silk moth *Antheraea pernyi*. *Insect Biochem* 20:735-740.
- Bruce, T. J. and A. Cork. 2001. Electrophysiological and behavioral responses of female *Helicoverpa armigera* to compounds identified in flowers of African marigold, *Tagetes erecta*. *J Chem Ecol* 27:1119-1131.
- Buck, L. and R. Axel. 1991. A novel multigene family may encode odorant receptors: a molecular basis for odour recognition. *Cell* 65:175-187.
- Buck, L. B. 2000. The molecular architecture of odor and pheromone sensing in mammals. *Cell* 100:611-618.
- Campanacci, V., J. Krieger, S. Bette, J. N. Sturgis, A. Lartigue, C. Cambillau, H. Breer, and M. Tegoni. 2001. Revisiting the specificity of *Mamestra brassicae* and *Antheraea polyphemus* pheromone-binding proteins with a fluorescence binding assay. *J Biol Chem* 276:20078-20084.
- Chapman, R. F. 1998. *The insects. Structure and function*. Cambridge University Press, Cambridge.
- Christensen, T. A., H. Mustaparta, and J. G. Hildebrand. 1991. Chemical communication in heliothine moths. II. Central processing of intra- and interspecific olfactory messages in the male corn earworm moth *Helicoverpa zea*. *J Comp Physiol A* 169:259-274.
- Christensen, T. A., V. M. Pawlowski, H. Lei, and J. G. Hildebrand. 2000. Multi-unit recordings reveal context-dependent modulation of synchrony in odor-specific neural ensembles. *Nature Neurosci* 3:927-931.
- Christensen, T. A., B. R. Waldrop, I. D. Harrow, and J. G. Hildebrand. 1993. Local interneurons and information processing in the olfactory glomeruli of the moth *Manduca sexta*. *J Comp Physiol A* 173:385-399.
- Cork, A., K. S. Boo, E. Dunkelblum, D. R. Hall, K. Jee-Rajunga, M. Kehat, E. Kong Jie, K. C. Park, P. Tepgidagarn, and Xun Liu. 1992. Female sex pheromone of oriental tobacco budworm, *Helicoverpa assulta* (Guenee) (Lepidoptera: Noctuidae): identification and field testing. *J Chem Ecol* 18:403-418.
- Cunningham, J. P., M. F. A. Jallow, D. J. Wright, and M. P. Zalucki. 1998a. Learning in host selection in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Anim Behav* 55:227-234.
- Cunningham, J. P., S. A. West, and D. J. Wright. 1998b. Learning in the nectar foraging behaviour of *Helicoverpa armigera*. *Ecol Entomol* 23:363-369.
- Cunningham, J. P., M. P. Zalucki, and S. A. West. 1999. Learning in *Helicoverpa armigera* (Lepidoptera: Noctuidae): a new look at the behaviour and control of a polyphagous pest. *Bull Ent Res* 89:201-207.
- Daly, K. C., S. Chandra, M. L. Durtschi, and B. H. Smith. 2001a. The generalization of an olfactory-based conditioned response reveals unique but overlapping odour representations in the moth *Manduca sexta*. *J Exp Biol* 204:3085-3095.
- Daly, K. C., M. L. Durtschi, and B. H. Smith. 2001b. Olfactory-based discrimination learning in the moth, *Manduca sexta*. *J Insect Physiol* 47:375-384.
- Daly, K. C. and B. H. Smith. 2000. Associative olfactory learning in the moth *Manduca sexta*. *J Exp Biol* 203:2025-2038.

- De Belle, J. S. and R. Kanzaki. 1999. Protocerebral olfactory processing. Pages 243-281 in B. S. Hansson, ed. *Insect olfaction*. Springer-Verlag, Berlin Heidelberg.
- De Moraes, C. M., M. C. Mescher, and J. H. Tumlinson. 2001. Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature* 410:577-580.
- Dewick, P. M. 1999. The biosynthesis of C5-C25 terpenoid compounds. *Nat Prod Rep* 16:97-130.
- Dicke, M. and J. J. A. Van Loon. 2000. Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomol Exp Appl* 97:237-249.
- Dickens, J. C. 1990. Specialized receptor neurons for pheromones and host plant odors in the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae). *Chem Senses* 15:311-331.
- Dubin, A. E., M. M. Liles, and G. L. Harris. 1998. The K<sup>+</sup> channel gene ether a go-go is required for the transduction of a subset of odorants in adult *Drosophila melanogaster*. *J Neurosci* 18:5603-5613.
- Duchamp-Viret, P., M. A. Chaput, and A. Duchamp. 1999. Odor response properties of rat olfactory receptor neurons. *Science* 284:2171-2174.
- Duchamp-Viret, P., A. Duchamp, and M. A. Chaput. 2000. Peripheral odor coding in the rat and frog: quality and intensity specification. *J Neurosci* 20:2383-2390.
- Dudareva, N. and E. Pichersky. 2000. Biochemical and molecular genetic aspects of floral scents. *Plant Physiol* 122:627-633.
- Dyson, G. M. 1938. The scientific basis of odour. *Chem Ind* 57:647-651.
- Ehrlich, P. R. and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18:586-608.
- Ernst, K. D. 1969. Die Feinstruktur von Riechsensillen auf der Antenne des Aaskäfers *Necrophorus* (Coleoptera). *Z Zellforsch* 94:72-102.
- Ernst, K. D. 1972. Die Ontogenie der basicischen Riechsensillen auf der Antenne von *Necrophorus*. *Z Zellforsch* 132:95-106.
- Fahey, J. W., A. T. Zalcmann, and P. Talalay. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56:5-51.
- Fan, R.-J., P. Anderson, and B. S. Hansson. 1997. Behavioural analysis of olfactory conditioning in the moth *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *J Exp Biol* 200:2969-2976.
- Firepong, S. and M. P. Zalucki. 1991. Host plant selection by *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae); the role of certain plant attributes. *Aust J Zool* 39:343-350.
- Fitt, G. P. 1989. The ecology of *Heliothis* species in relation to agroecosystems. *Ann Rev Entomol* 34:17-52.
- Fraenkel, G. S. 1959. The raison d'être of secondary plant substances. *Science* 129:1466-1470.
- Friedman, L. and J. G. Miller. 1971. Odor incongruity and chirality. *Science* 172:1044-1046.
- Galizia, C. G., A. Küttner, J. Joerges, and R. Menzel. 2000a. Odour representation in honeybee olfactory glomeruli shows slow temporal dynamics: an optical recording study using a voltage-sensitive dye. *J Insect Physiol* 46:877-886.
- Galizia, C. G. and R. Menzel. 2000. Probing the olfactory code. *Nature Neurosci* 3:853-854.
- Galizia, C. G. and R. Menzel. 2001. The role of glomeruli in the neural representation of odours: results from optical recording studies. *J Insect Physiol* 47:115-130.
- Galizia, C. G., S. Sachse, and H. Mustaparta. 2000b. Calcium responses to pheromones and plant odours in the antennal lobe of the male and female moth *Heliothis virescens*. *J Comp Physiol A* 186:1049-1063.
- Gao, Q., B. Yuan, and A. Chess. 2000. Convergent projections of *Drosophila* olfactory neurons to specific glomeruli in the antennal lobe. *Nature Neuroscience* 3:780-785. <http://bioinfo.weizmann.ac.il/HORDE>.
- Gesteland, R. C., J. Y. Lettvin, and Pitts. 1965. Chemical transmission in the nose of the frog. *J Physiol London* 181:525-529.
- Gibbs, R. D. 1974. *Chemotaxonomy of flowering plants*. McGill-Queen's University Press, Cambridge.
- Glusman, G., I. Yanai, I. Rubin, and D. Lancet. 2001. The complete human olfactory subgenome. *Genome Res* 11:685-702.
- Gouinguéné, S. and T. C. J. Turlings. 2002. The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiol* 129:1296-1307.
- Hammer, M. 1993. An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. *Nature* 366:59-63.
- Hansen, K., D. Schneider, and M. Boppré. 1983. Chiral pheromone and reproductive isolation between the gypsy- and nun moth. *Naturwissenschaften* 70:466-467.

- Hansson, B. S. 1995. Olfaction in Lepidoptera. *Experientia* 51:1003-1027.
- Hansson, B. S., S. Anton, and T. A. Christensen. 1994. Structure and function of antennal lobe neurons in the male turnip moth, *Agrotis segetum* (Lepidoptera: Noctuidae). *J Comp Physiol A* 175:547-562.
- Hansson, B. S., M. C. Larsson, and W. S. Leal. 1999. Green leaf volatile-detecting olfactory receptor neurones display very high sensitivity and specificity in a scarab beetle. *Phys Ent* 24:121-126.
- Hansson, B. S., J. N. C. van der Peers, and J. Löfqvist. 1988. Comparison of male and female olfactory cell response to pheromone compounds and plant volatiles in the turnip moth, *Agrotis segetum*. *Physiol Ent* 14:147-155.
- Hanström, B. *Vergleichende Anatomie des Nervensystems der wirbellosen Tiere*. 1928. Berlin, Springer, 628 pp.
- Hartlieb, E. 1996. Olfactory conditioning in the moth *Heliothis virescens*. *Naturwissenschaften* 83:87-88.
- Hartmann, T. 1985. Prinzipien des pflanzlichen Sekundär-stoffwechsels. *Plant Systematics and Evolution* 150:15-34.
- Hartmann, T. 1996. Diversity and variability of plant secondary metabolism: a mechanistic view. *Entomol Exp Appl* 80:177-188.
- Haslam, E. 1986. Secondary metabolism - facts and fiction. *Natural Product Reports* 3:217-249.
- Hatanaka, T. 1993. The biogenesis of green odour by green leaves. *Phytochemistry* 34:1201-1218.
- Heisenberg, M. 2003. Mushroom body memoir: from maps to models. *Nature Rev Neurosci* 4:266-275.
- Hildebrand, J. G. and G. M. Shepherd. 1997. Mechanisms of olfactory discrimination: Converging evidence for common principles across phyla. *Annu Rev Neurosci* 20:595-631.
- Hill, D. S. 1983. *Agricultural insect pest of tropics and their control*. Cambridge Press.
- Homberg, U., T. A. Christensen, and J. G. Hildebrand. 1989. Structure and function of the deutocerebrum in insects. *Ann Rev Entomol* 34:477-501.
- Jackson, D. M., R. F. Severson, A. W. Johnson, J. F. Chaplin, and M. G. Stephenson. 1984. Ovipositional response of tobacco budworm moths (Lepidoptera: Noctuidae) to cuticular chemical isolates from green tobacco leaves. *Environ Entomol* 13:1023-1030.
- Joerges, J., A. Küttner, C. G. Galizia, and R. Menzel. 1997. Representations of odours and odour mixtures visualized in the honeybee brain. *Nature* 387:285-288.
- Jørgensen, K. 2003. Resepsjon av smaksinformatjon og prjeksjon av smaksreseptorer i suboesophagealgangliet hos nattflyarten *Heliothis virescens*. (Master Thesis). NTNU.
- Jönsson, M. and P. Anderson. 1999. Electrophysiological response to herbivore-induced host plant volatiles in the moth *Spodoptera littoralis*. *Physiol Entomol* 24:377-385.
- Kafka, W. A. 1974. A formalism on selective molecular interactions. Pages 275-278 in L. Jaenicke, ed. *Mosbacher Colloquium der Gesellschaft für Biologische Chemie*. Springer-Verlag, Berlin-Heidelberg-New York.
- Kaissling, K.-E. 1998. Olfactory transduction in moths: II. Extracellular transport, deactivation and degradation of stimulus molecules. *Series on Biophysics and Biocybernetics* 5:113-137.
- Kaissling, K. E. 1974. Sensory transduction in insect olfactory receptors. Pages 243-273 in L. Jaenicke, ed. *Biochemistry of sensory functions*. Springer-Verlag, Berlin.
- Kajjiya, K., K. Inaki, M. Tanaka, T. Haga, H. Kataoka, and K. Touhara. 2001. Molecular bases of odor discrimination: reconstitution of olfactory receptors that recognize overlapping sets of odorants. *J Neurosci* 21:6018-6025.
- Kehat, M. and E. Dunkelblum. 1990. Behavioral responses of male *Heliothis armigera* (Lepidoptera: Noctuidae) moths in a flight tunnel to combinations of components identified from female sex pheromone glands. *J Insect Behaviour* 3:75-83.
- Kehat, M., S. Gothilf, E. Dunkelblum, and S. Greenberg. 1980. Field evaluation of female sex pheromone components of the Cotton bollworm, *Heliothis armigera* (Hübner). *Entomol Exp Appl* 27:188-193.
- Keil, T. A. 1984. Reconstruction and morphometry of silkmoth olfactory hairs: A comparative study of sensilla trichodea on the antennae of male *Antheraea polyphemus* and *Antheraea pernyi* (Insecta, Lepidoptera). *Zoomorphology* 104:147-156.
- Keil, T. A., E. Hartlieb, C. Bock, and R. A. Steinbrecht. 2001. Die Mikrowelt der Insektantennasen. *Naturwiss Rdsch* 54:519-525.
- Keller, A. and L. B. Vosshall. 2003. Decoding olfaction in *Drosophila*. *Curr Opin Neurobiol* 13:103-110.
- Kjær, A. 1976. Glucosinolates in the Cruciferae. Pages 207-219 in J. G. Vaughan, A. J. MacLeod, and B. M. G. Jones, eds. *The biology and chemistry of Cruciferae*. Academic Press, London.

- Klopping, H. L. 1971. Olfactory theories and the odors of small molecules. *J Agri Food Chem* 19:999-1004.
- Koh, Y. H., K. C. Park, and K. S. Boo. 1995. Antennal sensilla in adult *Helicoverpa assulta* (Lepidoptera: Noctuidae): morphology, distribution, and ultrastructure. *Annu Entomol Soc Am* 88:519-530.
- Korsching, S. 2002. Olfactory maps and odor images. *Curr Opin Neurobiol* 13:387-392.
- Krautwurst, D., K.-W. Yau, and R. R. Reed. 1998. Identification of ligands for olfactory receptors by functional expression of a receptor library. *Cell* 95:917-926.
- Krieger, J. and H. Breer. 1999. Olfactory reception in invertebrates. *Science* 286:720-723.
- Krieger, J., K. Raming, Y. M. E. Dewer, S. Bette, S. Conzelmann, and H. Breer. 2002. A divergent gene family encoding candidate olfactory receptors of the moth *Heliothis virescens*. *Europ J of Neurosci* 16:619-628.
- Krieger, J., J. Strobel, A. Vogl, W. Hanke, and H. Breer. 1999. Identification of a cyclic nucleotide- and voltage-activated ion channel from insect antennae. *Ins Biochem Mol Biol* 29:255-267.
- Kvello, P. 2003. Munddelene med sanseorganer og de assosierte reseptornevronenes projeksjoner i sentralnervesystemet hos nattflyarten *Heliothis virescens*. (Master Thesis). NTNU.
- Larsson, M. C., W. S. Leal, and B. S. Hansson. 2001. Olfactory receptor neurons detecting plant odours and male volatiles in *Anomala cuprea* beetles (Coleoptera: Scarabaeidae). *J Insect Physiol* 47:1065-1076.
- Laue, M., R. Maida, and A. Redkozubov. 1997. G-protein activation, identification and immunolocalization in pheromone sensitive sensilla trichodea of moths. *Cell Tissue Res* 288:149-158.
- Laurent, G. 1999. A systems perspective on early olfactory coding. *Science* 286:723-728.
- Laurent, G. 2002. Olfactory network dynamics and the coding of multidimensional signals. *Nature Rev* 3:884-895.
- Laurent, G. and H. Davidowitz. 1994. Encoding of olfactory information with oscillating neural assemblies. *Science* 265:1872-1875.
- Laurent, G. and M. Naraghi. 1994. Odorant-induced oscillations in the mushroom bodies of the locust. *Neuroscience* 14:2993-3004.
- Laurent, G., M. Wehr, and H. Davidowitz. 1996. Temporal representations of odors in an olfactory network. *Neuroscience* 16:3837-3847.
- Leal, W. S. 2001. Molecules and macromolecules involved in chemical communication of scarab beetles. *Pure Appl Chem* 73:613-616.
- Leise, E. M. 1990. Modular construction of nervous systems: a basic principle of design for invertebrates and vertebrates. *Brain Res Rev* 15:1-23.
- Lemon, W. C. and W. M. Getz. 1999. Neural coding of general odors in insects. *Ann Entomol Soc Am* 92:861-872.
- Li, G., Z. Han, L. Mu, X. Qin, C. Chen, and Y. Wang. 2001. Natural oviposition-detering chemicals in female cotton bollworm, *Helicoverpa armigera* (Hubner). *J Insect Physiol* 47:951-956.
- Luckner, M. 1990. Secondary metabolites in microorganisms, plants, and animals. G. Fischer, Jena.
- Ma, M. and G. M. Shepherd. 2000. Functional mosaic organization of mouse olfactory receptor neurons. *Proc Natl Acad Sci USA* 97:12869-12874.
- Malnic, B., J. Hirono, T. Sato, and L. B. Buck. 1999. Combinatorial receptor codes for odors. *Cell* 96:713-723.
- Masson, C. and H. Mustaparta. 1990. Chemical information processing in the olfactory system of insects. Part 1. Periphery. (H. Mustaparta). *Physiol Rev* 70:199-245.
- Matthews, M. 1991. Classification of the Heliiothinae. *Natural Resources Institute Bulletin* 40:1-198.
- Mauelshagen, J. 1993. Neural correlates of olfactory learning paradigms in an identified neuron in the honeybee brain. *J Neurophysiol* 69:609-625.
- Meisami, E. 1991. Chemoreception. Pages 335-434 in C. L. Prosser, ed. *Neural and integrative animal physiology*. Wiley-Liss, New York.
- Menzel, R. 1999. Memory dynamics in the honeybee. *J Comp Physiol A* 185:323-340.
- Millar, J. G. and J. J. Sir. 1998. Preparation, cleanup and preliminary fractionation of extracts. Pages 1-31 in J. G. Millar and K. F. Haynes, eds. *Methods in chemical ecology*. Chemical methods. Kluwer Academic Publishers, Boston.
- Mitchell, E. R., F. C. Tingle, and R. R. Heath. 1991. Flight activity of *Heliothis virescens* (F.) females (Lepidoptera: Noctuidae) with reference to host-plant volatiles. *J Chem Ecol* 17:259-266.

- Mohl, C., H. Breer, and J. Krieger. 2002. Species-specific pheromonal compounds induce distinct conformational changes of pheromone binding protein subtypes from *Antheraea polyphemus*. *Invert Neurosci* 4:165-174.
- Mombaerts, P. 1999. Molecular biology of odorant receptors in vertebrates. *Annu Rev Neurosci* 22:487-509.
- Mombaerts, P., F. Wang, C. Dulac, S. K. Chao, A. Nemes, M. Mendelsohn, J. Edmondson, and R. Axel. 1996. Visualizing an olfactory sensory map. *Cell* 87:675-686.
- Murlis, J. 1986. The structure of odour plumes. Pages 27-38 in T. L. Payne, M. C. Birch, and C. E. J. Kennedy, eds. *Mechanisms in insect olfaction*. Clarendon Press, Oxford.
- Mustaparta, H. 1996. Central mechanisms of pheromone information processing. *Chem Senses* 21:269-275.
- Mustaparta, H. 1997. Olfactory coding mechanisms for pheromone and interspecific signal information in related moth species. Pages 144-163 in R. T. Cardé and A. K. Minks, eds. *Insect pheromone research: New directions*. Chapman & Hall, New York.
- Mustaparta, H. 2002. Encoding of plant odour information in insects: peripheral and central mechanisms. *Entomol Exp Appl* 104:1-13.
- Mustaparta, H., M. E. Angst, and G. N. Lanier. 1980. Receptor discrimination of enantiomers of the aggregation pheromone ipsdienol, in two species of *Ips*. *J Chem Ecol* 6:689-701.
- Müller, U. 2002. Learning in honeybees: From molecules to behaviour. *Zoology* 105:313-320.
- Ohloff, G. 1986. Chemistry of odor stimuli. *Experientia* 42:271-279.
- Ohloff, G. 1994. Scent and Fragrances. The fascination of odors and their chemical perspectives. Springer Verlag, Berlin Heidelberg.
- Ottoson, D. 1956. Analysis on the electrical activity of the olfactory epithelium. *Acta Physiol Scand (Suppl)* 35:1-83.
- Ottoson, D. 1971. The electro-olfactogram. Pages 95-131 in L. M. Beidler, ed. *Handbook of sensory physiology*, vol. IV. Chemical Senses part I. Springer, Berlin.
- Paré, P. W. and J. H. Tumlinson. 1999. Plant volatiles as a defense against insect herbivores. *Plant Physiol* 121:325-331.
- Park, K. C. Composition and activity of female sex pheromone in the Oriental tobacco budworm moth, *Helicoverpa assulta* (Guenee). (Ph. D. Thesis). 1-85. 1991. Seoul National University.
- Paysan, J. and H. Breer. 2001. Molecular physiology of odor detection: current views. *Pflügers Arch-Eur J Physiol* 441:579-586.
- Pickett, J. A., D. W. M. Smiley, and C. M. Woodcock. 1999. Secondary metabolites in plant-insect interactions: dynamic systems of induced and adaptive responses. *Adv Bot Res* 30:91-115.
- Prestwich, G. D., G. Du, and S. LaForest. 1995. How is Pheromone Specificity Encoded in Proteins? *Chem Senses* 20:461-469.
- Priesner, E. 1959. Specificity studies in pheromone receptors of noctuid and tortricid lepidoptera. Pages 1-15 in F. J. Ritter, ed. *Chemical Ecology: Odour Communication in animals*.
- Priesner, E. 1977. Sensory efficacy of alkyl-branched pheromone analogues in noctuid and tortricid Lepidoptera. *Z.Naturforsch* 32c:979-991.
- Ramaswamy, S. B. 1988. Host finding by moths: Sensory modalities and behaviours. *J Insect Physiol* 34:235-249.
- Ramaswamy, S. B., W. Keung, and G. T. Baker. 1987. Sensory cues and receptors for oviposition by *Heliothis virescens*. *Entomol Exp Appl* 43:159-168.
- Rawson, N. E., J. Eberwine, R. Dotson, J. Jackson, P. Ulrich, and D. Restrepo. 2000. Expression of mRNAs encoding for two different olfactory receptors in a subset of olfactory receptor neurons. *J Neurochem* 75:185-195.
- Reed, W. and C. S. Pawar. *Heliothis*: a global problem. International Crops Research Institute for the Semi-Arid Tropics. 9-14. 1982. Patancheru - India. Proceedings of the International Workshop on *Heliothis* Management.
- Rembold, H., A. C. Köhne, and A. Schroth. 1991. Behavioral response of *Heliothis armigera* Hb. (Lep., Noctuidae) moths on a synthetic chickpea (*Cicer arietinum* L.) kairomone. *J Appl Ent* 112:254-262.
- Rembold, H. and H. Tober. 1985. Kairomones as pigeonpea resistance factors against *Heliothis armigera*. *Insect Sci Applic* 6:249-252.
- Ressler, K. J., S. L. Sullivan, and L. B. Buck. 1994. Information coding in the olfactory system: Evidence for a stereotyped and highly organized epitope map in the olfactory bulb. *Cell* 79:1245-1255.

- Rohmer, M. 1999. The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. *Natural Product Reports* 16:574.
- Rospars, J. P. 1988. Structure and development of the insect antennodeutocerebral system. *Int J Insect Morphol & Embryol* 17:243-294.
- Røstelien, T., A.-K. Borg-Karlson, J. Fäldt, U. Jacobsson, and H. Mustaparta. 2000a. The plant sesquiterpene germacrene D specifically activates a major type of antennal receptor neuron of the tobacco budworm moth *Heliothis virescens*. *Chem Senses* 25:141-148.
- Røstelien, T., A.-K. Borg-Karlson, and H. Mustaparta. 2000b. Selective receptor neurone responses to  $E$ - $\beta$ -ocimene,  $\beta$ -myrcene,  $E,E$ - $\alpha$ -farnesene and *homo*-farnesene in the moth *Heliothis virescens*, identified by gas chromatography linked to electrophysiology. *J Comp Physiol A* 186:833-847.
- Rössler, W., L. A. Oland, M. R. Higgins, J. G. Hildebrand, and L. P. Tolbert. 1999. Development of a glia-rich axon-sorting zone in the olfactory pathway of the moth *Manduca sexta*. *J Neurosci* 19:9865-9877.
- Sachs, J. 1873. *Lehrbuch der Botanik*. W. Engelmann, Leipzig.
- Sachse, S. and C. G. Galizia. 2002. Role of inhibition for temporal and spatial odor representation in olfactory output neurons: A calcium imaging study. *J Neurophysiol* 87:1106-1117.
- Sass, H. 1978. Olfactory receptors on the antenna of *Periplanteta*: response constellations that encode food odors. *J Comp Physiol* 128:227-233.
- Schmidt, C. O., H. J. Bouwmeester, J.-W. de Kraker, and W. A. König. 1998. Biosynthesis of (+)- and (-)-germacrene D in *Solidago canadensis*: isolation and characterization of two enantioselective germacrene D synthases. *Angew Chem Int Ed* 37:1400-1402.
- Schmidt, C. O., H. J. Bouwmeester, S. Franke, and W. A. König. 1999. Mechanisms of the biosynthesis of sesquiterpene enantiomers (+)- and (-)-germacrene D in *Solidago canadensis*. *Chirality* 11:353-362.
- Schneider, D. 1955. Micro-Electroden registrieren die elektrischen Impulse einzelner Sinnesnervenzellen der Schmetterlingsantenne. *Industrie-Electronik (Hamburg)* 3:3-7.
- Schneider, D. 1957a. Electrophysiological investigation on the antennal receptors of the moth during chemical and mechanical stimulation. *Experientia* 13:89-91.
- Schneider, D. 1957b. Electrophysiologische untersuchungen von Chemo- und mechanorezeptorender antennae des seidenspinners *Bombix mori* L. *Z Vgl Physiol* 40:8-41.
- Schneider, D. 1964. Insect antennae. *Ann Rev Entomol* 9:103-122.
- Schneider, D. 1969. Insect olfaction: Deciphering system for chemical messages. *Science* 163:1031-1037.
- Schneider, D. 1992. 100 years of pheromone research. An essay on lepidoptera. *Naturwissenschaften* 79:241-250.
- Schneider, D., W. A. Kafka, M. Beroza, and B. A. Bierl. 1977. Odor receptor responses of male gypsy and nun moths (Lepidoptera, Lymantriidae) to disparlure and its analogues. *J Comp Physiol* 113:1-15.
- Schneider, D. and R. A. Steinbrecht. 1968. Checklist of insect olfactory sensilla. *Symp Zool Soc Lond* 23:279-297.
- Schoonhoven, L. M., T. Jermy, and J. J. A. Van Loon. 1998. *Insect-Plant Biology. From physiology to evolution*. Chapman & Hall, London.
- Selzer, R. 1981. The processing of a complex food odor by antennal olfactory receptors of *Periplanta americana*. *J Comp Physiol* 144:509-233.
- Shanbhag, S. R., D. Hekmat-Scafe, M.-S. Kim, S.-K. Park, J. R. Carlson, C. Pikielny, D. P. Smith, and R. A. Steinbrecht. 2001. Expression mosaic of odorant-binding proteins in *Drosophila* olfactory organs. *Microsc Res Tech* 55:297-306.
- Shepherd, G. M. 1984. Are there labeled lines in the olfactory pathway? Pages 307-321 in D. W. Pfaff, ed. *Taste, Olfaction, and the central nervous system. A festschrift in Honor of Carl Pfaffmann*. The Rockefeller University Press, New York.
- Shields, V. D. C. and J. G. Hildebrand. 2001. Responses of a population of antennal olfactory receptor cells in the female moth *Manduca sexta* to plant-associated volatile organic compounds. *J Comp Physiol A* 186:1135-1151.
- Silverstein, R. M. and J. O. Rodin. 1966. Insect pheromone collection with absorption columns. I. Studies on model organic compounds. *J Econ Entomol* 59:1152-1154.
- Skiri, H. T., C. G. Galizia, S. Sachse, and H. Mustaparta. 2002. Olfaction in Heliothine moths: III. Optical imaging of glomerular activity in the antennal lobe elicited by insect and plant produced odorants. *FENS abstr* 1:470.

- Smith, P. M. 1976. The chemotaxonomy of plants. Edward Arnold, Bristol.
- Steinbrecht, R. A. 1992. Experimental morphology of insect olfaction: Tracer studies, x-ray microanalysis, autoradiography and immunocytochemistry with silkworm antennae. *Microsc Res Tech* 22:336-350.
- Steinbrecht, R. A. 1998. Odorant-binding proteins: Expression and function. *Ann NY Acad Sci* 855:323-332.
- Stengl, M. 1994. Inositol-trisphosphate-dependent calcium currents precede cation currents in insect olfactory receptor neurons in vitro. *J Comp Physiol A* 174:187-194.
- Stensmyr, M. C., E. Giordano, A. Ballio, A. M. Angioy, and B. S. Hansson. 2003. Novel natural ligands for *Drosophila* olfactory receptor neurones. *J Exp Biol* 206:715-724.
- Stensmyr, M. C., M. C. Larsson, S. Bice, and B. S. Hansson. 2001. Detection of fruit- and flower-emitted volatiles by olfactory receptor neurons in the polyphagous fruit chafer *Pachnoda marginata* (Coleoptera: Cetoniinae). *J Comp Physiol A* 187:509-519.
- Stopfer, M., S. Bhagavan, B. H. Smith, and G. Laurent. 1997. Impaired odour discrimination on desynchronization of odour-encoding neural assemblies. *Nature* 390:70-74.
- Stopfer, M., M. Wehr, K. MacLeod, and G. Laurent. 1999. Neural dynamics, oscillatory synchronisation, and odour codes. Pages 163-180 in B. S. Hansson, ed. *Insect olfaction*. Springer-Verlag, Berlin Heidelberg.
- Strausfeld, N. J. 1976. *Atlas of an Insect Brain*. Springer-Verlag, Berlin, Heidelberg, New York.
- Störtkuhl, K. F. and R. Kettler. 2001. Functional analysis of an olfactory receptor in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 98:9381-9385.
- Tingle, F. C. and E. R. Mitchell. 1992. Attraction of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) to volatiles from extracts of cotton flowers. *J Chem Ecol* 18:907-914.
- Tingle, F. C., E. R. Mitchell, and R. R. Heath. 1990. Preferences of mated *Heliothis virescens* and *H. subflexa* females for host and nonhost volatiles in a flight tunnel. *J Chem Ecol* 16:2889-2898.
- Todd, E. L. 1978. A checklist of species of *Heliothis* Oschenheimer (Lepidoptera: Noctuidae). *Proc Entomol Soc Wash* 80:1-14.
- Touhara, K., S. Sengoku, K. Inaki, A. Tsuboi, J. Hirono, T. Sato, H. Sakano, and T. Haga. 1999. Functional identification and reconstitution of an odorant receptor in single olfactory neurons. *Proc Natl Acad Sci USA* 96:4040-4045.
- Troemel, E. R., J. H. Chou, N. D. Dwyer, H. A. Colbert, and C. I. Bargmann. 1995. Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. *Cell* 83:207-218.
- Troemel, E. R., B. E. Kimmel, and C. I. Bargmann. 1997. Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in *C. elegans*. *Cell* 91:161-169.
- Turin, L. 1996. A spectroscopic mechanism for primary olfactory reception. *Chem Senses* 21:773-791.
- Turlings, T. C. J. and B. Benrey. 1998. Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience* 5:321-333.
- Tømmerås, B. Å. and H. Mustaparta. 1989. Single cell responses to pheromones, host and non-host volatiles in the ambrosia beetle *Typodendron lineatum*. *Entomol Exp Appl* 52:141-148.
- Vareschi, E. 1971. Duftunterscheidung bei der Honigbiene - Einzelzell-Ableitungen und Verhaltensreaktionen. *Z Vergl Physiol* 75:143-173.
- Vassar, R., S. K. Chao, R. Sitcheran, J. M. Nuñez, L. B. Vosshall, and R. Axel. 1994. Topographic organization of sensory projections to the olfactory bulb. *Cell* 79:981-991.
- Vetter, R. S. and T. C. Baker. 1983. Behavioral responses of male *Heliothis virescens* in a sustained-flight tunnel to combinations of seven compounds identified from female sex pheromone glands. *J Chem Ecology* 9:747-759.
- Vickers, N. J. and T. C. Baker. 1997. Flight of *Heliothis virescens* males in the field in response to sex pheromone. *Phys Ent* 22:277-285.
- Vickers, N. J., T. A. Christensen, and J. G. Hildebrand. 1998. Combinatorial odor discrimination in the brain: Attractive and antagonist odor blends are represented in distinct combinations of uniquely identifiable glomeruli. *J Comp Neur* 400:35-56.
- Visser, J. H. and D. A. Avé. 1978. General green leaf volatiles in the olfactory orientation of the Colorado potato beetle, *Leptinotarsa decemlineata*. *Entomol Exp Appl* 24:738-749.
- Vogt, R. G., F. E. Callahan, M. E. Rogers, and J. C. Dickens. 1999. Odorant binding protein diversity and distribution among the insect orders, as indicated by LAP, an OBP-related protein of the true bug *Lygus lineolaris* (Hemiptera, Heteroptera). *Chem Senses* 24:481-495.



- Vogt, R. G. and L. M. Riddiford. 1981. Pheromone binding and inactivation by moth antennae. *Nature* 293:161-163.
- Vosshall, L. B. 2001. The molecular logic of olfaction in *Drosophila*. *Chem Senses* 26:207-213.
- Vosshall, L. B., A. M. Wong, and R. Axel. 2000. An olfactory sensory map in the fly brain. *Cell* 102:147-159.
- Wadhams, L. J. 1982. Coupled gas chromatography - Single cell recording: a new Technique for use in the analysis of insect pheromones. *Z.Naturforsch* 37c:947-952.
- Wang, G.-R., K.-M. Wu, and Y.-Y. Guo. 2003. Cloning, expression and immunocytochemical localization of a general odorant-binding protein gene from *Helicoverpa armigera* (Hübner). *Insect Biochem Molc Biol* 33:115-124.
- Wang, J. and E. Pichersky. 1999. Identification of specific residues involved in substrate discrimination in two plant *O*-methyltransferases. *Arch Biochem Biophys* 368:172-180.
- Wegener, J. W., W. Hanke, and H. Breer. 1996. Second messenger-controlled membrane conductance in locust (*Locusta migratoria*) olfactory neurons. *J Insect Physiol* 43:595-605.
- Wetzel, C. H., H.-J. Behrendt, G. Gisselmann, K. F. Störtkuhl, B. Hovemann, and H. Hatt. 2001. Functional expression and characterization of a *Drosophila* odorant receptor in a heterologous cell system. *Proc Natl Acad Sci USA* 98:9377-9380.
- Wetzel, C. H., M. Oles, C. Wellerdieck, M. Kuczkowiak, G. Gisselmann, and H. Hatt. 1999. Specificity and sensitivity of a human olfactory receptor functionally expressed in human embryonic kidney 293 cells and *Xenopus laevis* oocytes. *J Neurosci* 19:7426-7433.
- Wibe, A. and H. Mustaparta. 1996. Encoding of plant odours by receptor neurons in the pine weevil (*Hylobius abietis*) studied by linked gas chromatography-electrophysiology. *J Comp Physiol A* 179:331-344.
- Wright, R. H. 1977. Odor and molecular vibration: neural coding of olfactory information. *J Theor Biol* 64:473-502.
- Zalucki, M. P., G. Darglish, S. Firempong, and P. Twine. 1986. The biology and ecology of *Heliothis armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae) in Australia: What do we know? *Aust J Zool* 34:779-814.
- Zhao, H., L. Ivic, J. M. Otaki, M. Hashimoto, K. Mikoshiba, and S. Firestein. 1998. Functional expression of a mammalian odorant receptor. *Science* 279:237-242.
- Zozulya, S., F. Echeverri, and T. Nguyen. 2001. The human olfactory receptor repertoire. *Genome Biology* 2:research0018.1-0018.12, <http://genomebiology.com/2001-2/6/research/0018>.

## **INDIVIDUAL PAPERS**

- I. Strandén M., Borg-Karlson A.-K. and Mustaparta H. (2002), Receptor neuron discrimination of the germacrene D enantiomers in the moth *Helicoverpa armigera*. *Chem. Senses*, 27: 143-152
- II. Strandén M., Liblikas I., König W.A., Almaas T.J., Borg-Karlson, A.-K. and Mustaparta, H. (2003) (-)-Germacrene D receptor neurones in three species of heliothine moths: structure-activity relationships. *J. Comp. Physiol. A*, in press  
DOI: 10.1007/s00359-003-0434-y
- III. Strandén M., Røstelién T., Liblikas I., Almaas T. J., Borg-Karlson, A.-K. and Mustaparta, H. (2003) Receptor neurones in three heliothine moths responding to floral and inducible plant volatiles. *Chemoecology*, in press
- IV. Mozuraitis R., Strandén M., Ramirez M.I., Borg-Karlson A.-K. and Mustaparta H. (2002), (-)-Germacrene D increases attraction and oviposition by the tobacco budworm moth *Heliothis virescens*. *Chem. Senses*, 27: 505-509

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**Doctoral theses in Biology**  
**Norwegian University of Science and Technology**

<b>Year</b>	<b>Name</b>	<b>Degree</b>	<b>Title</b>
08.04.74	Tor-Henning Iversen	Dr. philos Botany	The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism
1978	Tore Slagsvold	Dr. philos. Zoology	Breeding events of birds in relation to spring temperature and environmental phenology.
1980	Helge Reinertsen	Dr. philos Botany	The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton
1980	Arnfinn Langeland	Dr. philos. Zoology	Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake.
17.11.82.	Gunn Mari Olsen	Dr. scient Botany	Gravitropism in roots of <i>Pisum sativum</i> and <i>Arabidopsis thaliana</i>
03.12.82.	Dag Dolmen	Dr. philos. Zoology	Life aspects of two sympatric species of newts ( <i>Triturus</i> , <i>Amphibia</i> ) in Norway, with special emphasis on their ecological niche segregation.
17.02.84.	Eivin Røskaft	Dr. philos. Zoology	Sociobiological studies of the rook <i>Corvus frugilegus</i> .
28.05.84.	Anne Margrethe Cameron	Dr. scient Botany	Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinizing hormone in male mature rats
13.12.84.	Asbjørn Magne Nilsen	Dr. scient Botany	Alveolar macrophages from expectorates – Biological monitoring of workers exosed to occupational air pollution. An evaluation of the AM-test
18.10.85.	Jarle Mork	Dr. philos. Zoology	Biochemical genetic studies in fish.
19.11.85.	John Solem	Dr. philos. Zoology	Taxonomy, distribution and ecology of caddisflies ( <i>Trichoptera</i> ) in the Dovrefjell mountains.
06.12.85.	Randi E. Reinertsen	Dr. philos. Zoology	Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds.
31.10.86.	Bernt-Erik Sæther	Dr. philos. Zoology	Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach.
10.12.86.	Torleif Holthe	Dr. philos. Zoology	Evolution, systematics, nomenclature, and zoogeography in the polychaete orders <i>Oweniimorpha</i> and <i>Terebellomorpha</i> , with special reference to the Arctic and Scandinavian fauna.
17.02.87.	Helene Lampe	Dr. scient. Zoology	The function of bird song in mate attraction and territorial defence, and the importance of song repertoires.
08.04.87.	Olav Hogstad	Dr. philos. Zoology	Winter survival strategies of the Willow tit <i>Parus montanus</i> .
04.06.87.	Jarle Inge Holten	Dr. philos Bothany	Autecological investigations along a coast-inland transect at Nord-Møre, Central Norway

05.08.87. Rita Kumar	Dr. scient Botany	Somaclonal variation in plants regenerated from cell cultures of <i>Nicotiana sanderae</i> and <i>Chrysanthemum morifolium</i>
18.09.87. Bjørn Åge Tømmerås	Dr. scient. Zoology	Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction.
04.03.88. Hans Christian Pedersen	Dr. philos. Zoology	Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care.
25.03.88. Tor G. Heggberget	Dr. philos. Zoology	Reproduction in Atlantic Salmon ( <i>Salmo salar</i> ): Aspects of spawning, incubation, early life history and population structure.
29.09.88. Marianne V. Nielsen	Dr. scient. Zoology	The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels ( <i>Mytilus edulis</i> ).
21.10.88. Ole Kristian Berg	Dr. scient. Zoology	The formation of landlocked Atlantic salmon ( <i>Salmo salar</i> L.).
20.04.89. John W. Jensen	Dr. philos. Zoology	Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth.
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07.12.89. Reidar Andersen	Dr. scient. Zoology	Interactions between a generalist herbivore, the moose <i>Alces alces</i> , and its winter food resources: a study of behavioural variation.
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15.03.91. Asbjørn Moen	Dr. philos Botany	The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands

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19.08.94. Morten Bakken	Dr. scient. Zoology	Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, <i>Vulpes vulpes</i> .
09.12.94. Arne Moksnes	Dr. philos. Zoology	Host adaptations towards brood parasitism by the Cuckoo.
16.12.94. Solveig Bakken	Dr. scient Bothany	Growth and nitrogen status in the moss <i>Dicranum majus</i> Sm. as influenced by nitrogen supply
09.02.95 Olav Vadstein	Dr. philos Botany	The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions.
22.03.95. Hanne Christensen	Dr. scient. Zoology	Determinants of Otter <i>Lutra lutra</i> distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink <i>Mustela vison</i> .
27.04.95. Svein Håkon Lorentsen	Dr. scient. Zoology	Reproductive effort in the Antarctic Petrel <i>Thalassoica antarctica</i> ; the effect of parental body size and condition.
06.06.95. Chris Jørgen Jensen	Dr. scient. Zoology	The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity
09.06.95. Martha Kold Bakkevig	Dr. scient. Zoology	The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport.
13.12.95. Vidar Moen	Dr. scient. Zoology	Distribution patterns and adaptations to light in newly introduced populations of <i>Mysis relicta</i> and constraints on Cladoceran and Char populations.
13.12.95 Hans Haavardsholm Blom	Dr. philos Bothany	A revision of the <i>Schistidium apocarpum</i> complex in Norway and Sweden.
08.03.96 Jorun Skjærmo	Dr. scient Botany	Microbial ecology of early stages of cultivated marine fish; impact fish-bacterial interactions on growth and survival of larvae.
31.05.96. Ola Ugedal	Dr. scient. Zoology	Radiocesium turnover in freshwater fishes
14.06.96. Ingibjörg Einarsdóttir	Dr. scient. Zoology	Production of Atlantic salmon ( <i>Salmo salar</i> ) and Arctic charr ( <i>Salvelinus alpinus</i> ): A study of some physiological and immunological responses to rearing routines.
20.06.96. Christina M. S. Pereira	Dr. scient. Zoology	Glucose metabolism in salmonids: Dietary effects and hormonal regulation.
07.11.96. Jan Fredrik Børseth	Dr. scient. Zoology	The sodium energy gradients in muscle cells of <i>Mytilus edulis</i> and the effects of organic xenobiotics.

22.11.96. Gunnar Henriksen	Dr. scient. Zoology	Status of Grey seal <i>Halichoerus grypus</i> and Harbour seal <i>Phoca vitulina</i> in the Barents sea region.
07.02.97 Gunvor Øie	Dr. scient Bothany	Eevaluation of rotifer <i>Brachionus plicatilis</i> quality in early first feeding of turbot <i>Scophthalmus maximus</i> L. larvae.
14.02.97 Håkon Holien	Dr. scient Botany	Studies of lichens in spruce forest of Central Norway. Diversity, old growth species and the relationship to site and stand parameters.
28.02.97. Ole Reitan	Dr. scient. Zoology	Responses of birds to habitat disturbance due to damming.
04.03.97. Jon Arne Grøttum	Dr. scient. Zoology	Physiological effects of reduced water quality on fish in aquaculture.
07.03.97. Per Gustav Thingstad	Dr. scient. Zoology	Birds as indicators for studying natural and human-induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher.
07.03.97. Torgeir Nygård	Dr. scient. Zoology	Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as Biomonitors.
14.03.97. Signe Nybø	Dr. scient. Zoology	Impacts of long-range transported air pollution on birds with particular reference to the dipper <i>Cinclus cinclus</i> in southern Norway.
24.03.97. Atle Wibe	Dr. scient. Zoology	Identification of conifer volatiles detected by receptor neurons in the pine weevil ( <i>Hylobius abietis</i> ), analysed by gas chromatography linked to electrophysiology and to mass spectrometry.
11.04.97. Rolv Lundheim	Dr. scient. Zoology	Adaptive and incidental biological ice nucleators.
29.08.97. Arild Magne Landa	Dr. scient. Zoology	Wolverines in Scandinavia: ecology, sheep depredation and conservation.
10.09.97 Kåre Magne Nielsen	Dr. scient Botany	An evolution of possible horizontal gene transfer from plants to soil bacteria by studies of natural transformation in <i>Acinetobacter calcoaceticus</i> .
05.11.97. Jarle Tufto	Dr. scient. Zoology	Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models
07.11.97. Trygve Hesthagen	Dr. philos. Zoology	Population responses of Arctic charr ( <i>Salvelinus alpinus</i> (L.)) and brown trout ( <i>Salmo trutta</i> L.) to acidification in Norwegian inland waters
14.11.97. Trygve Sigholt	Dr. philos. Zoology	Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon ( <i>Salmo salar</i> ) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet
12.12.97. Jan Østnes	Dr. scient. Zoology	Cold sensation in adult and neonate birds
28.04.98 Seethaledsumy Visvalingam	Dr. scient Botany	Influence of environmental factors on myrosinases and myrosinase-binding proteins.
30.04.98. Thor Harald Ringsby	Dr. scient. Zoology	Variation in space and time: The biology of a House sparrow metapopulation
29.05.98. Erling Johan Solberg	Dr. scient. Zoology	Variation in population dynamics and life history in a Norwegian moose ( <i>Alces alces</i> ) population: consequences of harvesting in a variable environment



26.06.98	Sigurd Mjøen Saastad	Dr. scient Botany	Species delimitation and phylogenetic relationships between the <i>Sphagnum recurvum</i> complex (Bryophyta): genetic variation and phenotypic plasticity.
26.06.98.	Bjarte Mortensen	Dr. scient Botany	Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro.
16.10.98.	Gunnar Austrheim	Dr. scient Botany	Plant biodiversity and land use in subalpine grasslands. – A conservtaion biological approach.
11.12.98.	Bente Gunnveig Berg	Dr. scient. Zoology	Encoding of pheromone information in two related moth species
15.02.99.	Kristian Overskaug	Dr. scient. Zoology	Behavioural and morphological characteristics in Northern Tawny Owls <i>Strix aluco</i> : An intra- and interspecific comparative approach
19.02.99.	Hans Kristen Stenøien	Dr. scient Bothany	Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts)
12.03.99.	Trond Arnesen	Dr. scient Botany	Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway.
23.04.99.	Ingvar Stenberg	Dr. scient. Zoology	Habitat selection, reproduction and survival in the White-backed Woodpecker <i>Dendrocopos leucotos</i>
07.05.99.	Stein Olle Johansen	Dr. scient Botany	A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis.
31.05.99	Trina Falck Galloway	Dr. scient. Zoology	Muscle development and growth in early life stages of the Atlantic cod ( <i>Gadus morhua</i> L.) and Halibut ( <i>Hippoglossus hippoglossus</i> L.)
10.06.99.	Torbjørn Forseth	Dr. scient. Zoology	Bioenergetics in ecological and life history studies of fishes.
10.06.99	Marianne Giæver	Dr. scient. Zoology	Population genetic studies in three gadoid species: blue whiting ( <i>Micromisistius poutassou</i> ), haddock ( <i>Melanogrammus aeglefinus</i> ) and cod ( <i>Gradus morhua</i> ) in the North-East Atlantic
11.06.99	Hans Martin Hanslin	Dr. scient Botany	The impact of environmental conditions of density dependent performance in the boreal forest bryophytes <i>Dicranum majus</i> , <i>Hylocomium splendens</i> , <i>Plagiochila asplenigides</i> , <i>Ptilium crista-castrensis</i> and <i>Rhytidiadelphus lokeus</i> .
15.06.99	Ingrid Bysveen Mjølnerød	Dr. scient. Zoology	Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon ( <i>Salmo salar</i> ) revealed by molecular genetic techniques
13.07.99	Else Berit Skagen	Dr. scient Botany	The early regeneration process in protoplasts from <i>Brassica napus</i> hypocotyls cultivated under various g-forces
24.09.99	Stein-Are Sæther	Dr. philos. Zoology	Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe
02.11.99	Katrine Wangen Rustad	Dr. scient. Zoology	Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer's disease
26.11.99	Per Terje Smiseth	Dr. scient. Zoology	Social evolution in monogamous families: mate choice and conflicts over parental care in the Bluethroat ( <i>Luscinia s. svecica</i> )

27.11.99	Gunnbjørn Bremset	Dr. scient. Zoology	Young Atlantic salmon ( <i>Salmo salar</i> L.) and Brown trout ( <i>Salmo trutta</i> L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions
01.12.99	Frode Ødegaard	Dr. scient. Zoology	Host specificity as parameter in estimates of arthropod species richness
10.12.99.	Sonja Andersen	Dr. scient Bothany	Expressional and functional analyses of human, secretory phospholipase A2
28.01.00	Salvesen, Ingrid	Dr. scient Botany	Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture
14.04.00.	Ingar Jostein Øien	Dr. scient. Zoology	The Cuckoo ( <i>Cuculus canorus</i> ) and its host: adaptations and counteradaptions in a coevolutionary arms race
01.09.00	Makridis, Pavlos	Dr. scient Botany	Methods for the microbial econtrol of live food used for the rearing of marine fish larvae
02.11.00.	Sigbjørn Stokke	Dr. scient. Zoology	Sexual segregation in the African elephant ( <i>Loxodonta africana</i> )
10.11.00.	Odd A. Gulseth	Dr. philos. Zoology	Seawater tolerance, migratory behaviour and growth of Charr, ( <i>Salvelinus alpinus</i> ), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard
23.11.00.	Pål A. Olsvik	Dr. scient. Zoology	Biochemical impacts of Cd, Cu and Zn on brown trout ( <i>Salmo trutta</i> ) in two mining-contaminated rivers in Central Norway
12.12.00.	Sigurd Einum	Dr. scient. Zoology	Maternal effects in fish: Implications for the evolution of breeding time and egg size
27.04.01.	Jan Ove Evjemo	Dr. scient. Zoology	Production and nutritional adaptation of the brine shrimp <i>Artemia</i> sp. as live food organism for larvae of marine cold water fish species
04.05.01	Hilmo, Olga	Dr. scient Botany	Lichen response to environmental changes in the managed boreal forest systems
23.11.01.	Ingebrigt Uglem	Dr. scient. Zoology	Male dimorphism and reproductive biology in corkwing wrasse ( <i>Symphodus melops</i> L.)
30.11.01.	Bård Gunnar Stokke	Dr. scient. Zoology	Coevolutionary adaptations in avian brood parasites and their hosts
18.01.02.	Ronny Aanes	Dr. scient	Spatio-temporal dynamics in Svalbard reindeer ( <i>Rangifer tarandus platyrhynchus</i> )
25.01.02.	Mariann Sandsund	Dr. scient. Zoology	Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses
22.03.02.	Dag-Inge Øien	Dr. scient Botany	Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway
26.04.02.	Frank Rosell	Dr. scient. Zoology	The function of scent marking in beaver ( <i>Castor fiber</i> )
05.04.02.	Janne Østvang	Dr. scient Botany	The Role and Regulation of Phospholipase A <sub>2</sub> in Monocytes During Atherosclerosis Development
11.10.02.	Terje Thun	Dr. philos Biology	Dendrochronological constructions of Norwegian conifer chronologies providing dating of historical material
15.11.02.	Birgit Hafjeld Borgen	Dr. scient Biology	Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth

06.12.02. Bård Øyvind Solberg	Dr. scient Biology	Effects of climatic change on the growth of dominating tree species along major environmental gradients
18.12.02. Per Winge	Dr. scient Biology	The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in <i>Arabidopsis thaliana</i> and
19.12.02. Henrik Jensen	Dr. scient Biology	Causes and consequences of individual variation in fitness-related traits in house sparrows
11.04.03. Jens Rohloff	Dr. philos Biology	Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control
11.04.03. Åsa Maria O. Espmark Wibe	Dr. scient Biology	Behavioural effects of environmental pollution in threespine stickleback <i>Gasterosteus aculeatus</i> L.
09.05.03. Dagmar Hagen	Dr. scient Biology	Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach
13.06.03. Bjørn Dahle	Dr. scient Biology	Reproductive strategies in Scandinavian brown bears
20.06.03. Cyril Lebogang Taolo	Dr. scient Biology	Population ecology, seasonal movement and habitat use of the African buffalo ( <i>Syncerus caffer</i> ) in Chobe National Park, Botswana