CHEMICAL COMMUNICATION IN INSECTS, FROM PHEROMONE IDENTIFICATION TO PROTEOMICS OF OLFACCTION

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1870: Jean Henry Fabre shows that females of the Giant Peacock Moth attract males through volatile substances.
1959 Bomkybol, the sexual pheromone produced by Bombyx mori females is identified by Adolf Butenand after 20 year work (about 500,000 females needed)

$\text{(E,Z)}-10,12$-esadecadien-1-olo

In the same year Karlson e Lüscher introduce the term pheromone

$\text{(E,Z)}-10,12$-esadecadienale

Manduca sexta
Analysis of Volatiles Pheromones through Gas Chromatography coupled to Mass Spectrometry (GC-MS)

ACQUISITION OF MASS SPECTRA
IDENTIFICATION

SEPARATION

Gas: He, N₂, H₂

Sample injector

T regulated oven

Column: packed or open tubular (capillary)

Mass spectrometer detector

abundance

m/z-->

131.0
103.1 162.1
77.1
51.1
Pheromones in Terrestrial Insects

IN MOST CASES NON POLAR HYDROPHOBIC COMPOUNDS

MOLECULAR WEIGHT of volatile pheromones up to 300 Da

MOLECULAR WEIGHT of contact pheromones such as cuticular lipids up to 280-600 Da
Chemical Communication has a pivotal Role in Colony Organization and Homeostasis in Social Insects

75 exocrine glands in ants, Billen, 2009
• they prevent dehydration
• they act as recognition pheromones
• they are made of tens of compounds, mostly hydrocarbons (linear alkanes, alkenes, methyl branched alkanes)
Epicuticular Hydrocarbons are recognition Pheromones in Social Insects

Ricerca in Web of Science per
"Cuticular Hydrocarbons"
AND
"insects"
Epicuticular Hydrocarbons in Social Parasites

*Polistes dominulus*

*Polistes sulcifer*
Chemical Mimicry of the Social Parasite \textit{P. sulcifer}

After 3 days

Turillazzi et al., 1999. \textit{Naturwissenschaften}
OLFACTORY SENSILLA

Shanbhag et al., 1999.
Proteins involved in Perireceptor Events

- Highly concentrated in the sensillar lymph (10 mM for OBPs)
- Soluble proteins
- Capable of reversibly bind small molecules as odorants and pheromones
- Low molecular weight (12-35 kDa)

### ODORANT BINDING PROTEINS (OBPs)

- *Apis mellifera*: 21 OBP genes, 6 CPSs
- *Anopheles gambiae*: 57 OBP genes, 7 CSP genes
- *Bombyx mori*: 44 OBP genes, 20 CSPs

### CHEMOSENSORY PROTEINS (CSPs)

- Anopheles gambiae SAP3
Proteins involved in Perireceptor Events
Protein Identification through MASS SPECTROMETRY (MS)

Sample Preparation
→ Gel Electrophoresis

Cut spots
→ Tryptic digestion (cleaves protein at R and K residues)

Reversed phase Liquid Chromatography separation

MS Analysis
→ Peptide Mass Fingerprinting (PMF) spectrum
→ MS/MS analysis (peptides are fragmented in mass spectrometer)
→ MS/MS Peptide Sequencing Spectra
Mapping the Expression of Soluble Olfactory Proteins in Honeybees

Dani et al. 2010. J. Proteome Research

Digestion of 2D gel spots and micro LC-ESI ORBITRAP analyses

21 OBP and 6 CSP genes found in the genome

250 antennae; 10 larvae; 89 Spots digested

64 IDENTIFIED PROTEINS

LARVAE
3 OBPs and 1 CSP

WORKER ANTENNAE
12 OBPs and 2 CSPs
Mapping the Expression of Soluble Olfactory Proteins in the Mandibular Gland of Honeybee

Iovinella et al., 2011. J. Proteome Research
OBPs AFFINITY FOR LIGANDS

Iovinella et al., 2011. *J. Proteome Research*
Comparative analysis of epicuticular lipid profiles of sympatric and allopatric field populations of Anopheles gambiae s.s. molecular forms and An. arabiensis from Burkina Faso (West Africa)

B. Caputo\textsuperscript{a}, F.R. Dani\textsuperscript{b}, G.L. Horne\textsuperscript{c}, S. N'Fale\textsuperscript{d}, A. Diabate\textsuperscript{e}, S. Turillazzi\textsuperscript{b}, M. Coluzzi\textsuperscript{a}, C. Costantini\textsuperscript{e}, A.A. Priestman\textsuperscript{c}, V. Petrarca\textsuperscript{f}, A. della Torre\textsuperscript{a,*}
Mapping the expression of soluble olfactory proteins in *Anopheles gambiae* through shotgun proteomics (nano HPLC-ESI ORBITRAP)

Shotgun proteomics is a method of identifying proteins in complex mixtures using a combination of high performance liquid chromatography combined with mass spectrometry.
Mapping the expression of soluble olfactory proteins in *Anopheles gambiae* through shotgun proteomics (nano HPLC-ESI ORBITRAP)

- Antenne from 600 males and from 600 females

males are strictly phytophagous; host search by females is mainly based on olfaction

57 OBP and 7 CSP genes found in the genome

1333 GROUP PROTEINS IDENTIFIED; 28 OLFACTORY SOLUBLE PROTEINS
Mapping the expression of Soluble Olfactory Proteins in *Anopheles gambiae* through Shotgun Proteomics (nano HPLC-ESI ORBITRAP)