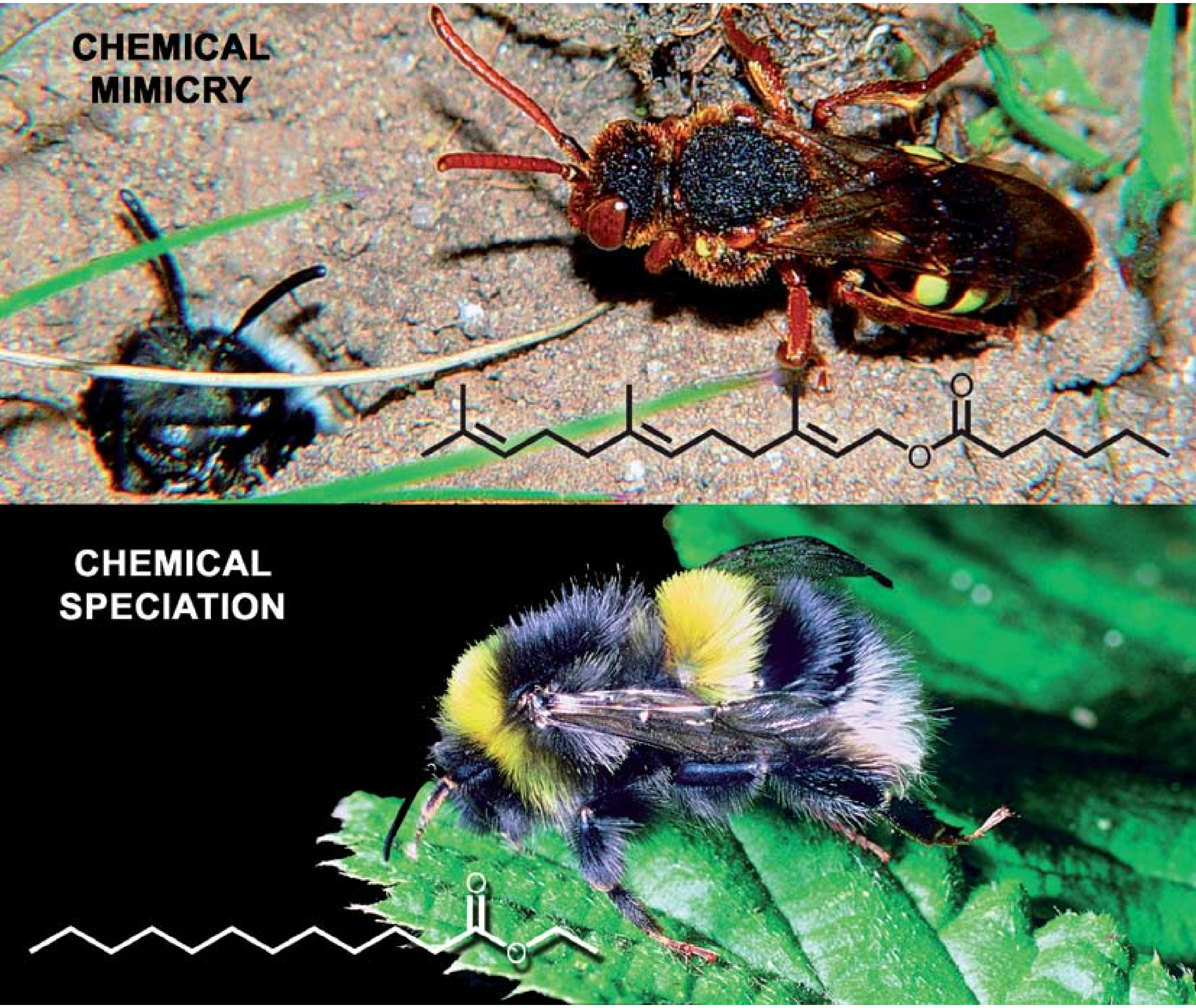
ChemComm

Chemical Communications

www.rsc.org/chemcomm

Number 34 | 14 September 2008 | Pages 3941–4080



ISSN 1359-7345

RSC Publishing

1359-7345(2008)34;1-#

Chemical communication by behaviour-guiding olfactory signals

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Received (in Cambridge, UK) 16th August 2007, Accepted 17th March 2008
First published as an Advance Article on the web 19th May 2008
DOI: 10.1039/b712681f

Chemical Ecology is a new interdisciplinary research area with close collaborations between chemists and biologists of different descriptions. It has developed during the last 40 years because of an interest in the structure, function and evolution of chemical signalling among organisms and also because of the hope to be able to use the ubiquitous phenomenon to control organisms, like pest insects. This *feature article* highlights the growth of the discipline and the progress made, through examples from the author's own work on chemical communication in insects and flowering plants. The research deals with olfactory signals, *i.e.* volatile chemical compounds perceived by the sense of smell. Analytical techniques and methods are an important part of the work.

Introduction

All multicellular living organisms employ olfactory signals to guide their behaviours. That is a rule with few, if any, exceptions. The scents are mainly perceived through the sense of smell and they are linked to all vital needs such as development and feeding, recognition and nesting, mating, alarm and defence. The chemical signals frequently consist of more than a single compound, most often either two or three, or complex blends with many components, often members of homologous series of compounds. They are the products of the acetogenic (fatty acid derivatives), the mevalogenic (isoprenoids), the benzenoid (aromatic) and other biosynthetic pathways. They are often species-specific, like *pheromones*, defined as signals between individuals of the same species that could serve recognition and sexual selection and be involved in speciation. When they represent chemical communication between different organisms, like between plants and insects, we use the more general term *semiochemicals*. Chemical signals show a large *chemodiversity* and contribute to establishing and maintaining the enormous *biodiversity* found among living organisms.

Most of the work in this field, which now goes under the name of *Chemical Ecology*, has so far been done on insects and plants, which are the topics of this article, but chemical communication is increasingly being studied in micro-organisms, aquatic organisms, and mammalians—including Man. From the chemical point of view there are many similarities between them. The very same compounds can turn up in very diverse types of organisms. Applied aspects of olfactory signals include the selective and non-toxic control of organisms, such as pest insects of importance in agriculture, forestry and medicine.

The famous entomologist Jean-Henri Fabre (1823–1915), a school teacher from Avignon, France, studied, among many other phenomena in the world of insects, the distant attraction of males of the great peacock moth (*Saturnia pyri*), Fig. 1, to the females, and wrote poetically about this phenomenon at the beginning of the last century:[1]

"*Like light, odour has its X-rays. Should science one day, instructed by the insect, endow us with a radiograph of smells, this artificial nose will open out to us a world of marvels.*"

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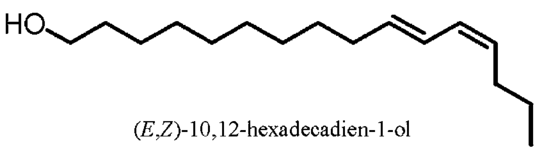
Fig. 1 Male *Saturnia pyri*, the great peacock moth. Observe the large, feather-like antenna—the female has more thread-like ones and at the abdominal tip the sex pheromone gland.

How right he was—even if he thought that the sense of smell was based on electromagnetic radiation! Collaborative, interdisciplinary research efforts on natural chemical signals have now been going on for more than 40 years. So – Where are we now? What do we know? And what are the future prospects of this branch of Science?

Much is known today about the structures and compositions of the chemical signals, their behavioural effects, the reception of odours, their practical use, and the optimal methods and techniques of analysing them; a certain amount is also known about their biosynthesis, their genetics, their ecological and evolutionary roles, for example. But much remains to be done, many fascinating problems and phenomena are left to be studied. This overview should exemplify some of what we know today, some of the fundamental facts and phenomena of olfactory signals.

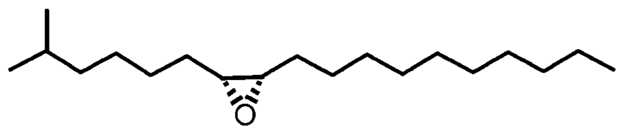
The pioneering work of Adolf Butenandt in Munich (Nobel laureate in 1939 for his studies on hormones) on the female sex attractant of the silk moth, *Bombyx mori*, led to its identification as a doubly unsaturated straight chain alcohol called *bombycol*: (*E*,*Z*)-10,12-hexadecadien-1-ol, published in 1959.



(*E*,*Z*)-10,12-hexadecadien-1-ol

The report[2] starts with a sigh: "After more than 20 years of experimental effort, we have now succeeded in identifying the female sexual attractant of the silk moth". In the same year the term *pheromone* was coined by Karlson and Luscher.[3] These are the grounds for counting that year as the starting point for Chemical Ecology, although the term was introduced 10 years later, in 1969, when some scientists had begun studies on olfactory signals. Pioneering work on the electrophysiology of insect olfactory reception was done by D. Schneider and his group. This type of study has been further developed.

There are some good reasons why work in this area commenced in the 1960s and 1970s. A major one, really a prerequisite, was the development of sensitive and informative analytical chemical techniques, especially isolation/enrichment techniques and separation/identification through gas chromatography and mass spectrometry, by which small amounts of volatile compounds could be identified. This enabled us, in many cases, to achieve the goal of analysing volatiles from single individuals and thereby being able to compare them. You could, for instance, see the chemical variation between individuals or compare secretions during different stages in their development and find biological correlations to it. At the same time there were crucial improvements in the techniques of measuring and recording olfactory-elicited behaviour, both in the field and in the laboratory, and in making electrophysiological recordings from insect antenna. Other reasons or motivations were the curiosity concerning the role played by chemical signals in guiding behaviour (ethology) and forming liaisons between organisms (ecology), and the applied aspect, the possibility of using selective signals for the control of pest insects. Some pioneering work had been done since the 1930s at USDA (United States Department of Agriculture) laboratories, *e.g.* on the sex pheromone of the gypsy moth, *Lymantria dispar*, but its correct structure, (7*R*,8*S*)-7,8-epoxy-2-methyloctadecane ("*disparlure*"), was not determined until 40 years later.[4] This illustrates the importance of the development of sensitive analytical techniques.



(7*R*,8*S*)-7,8-epoxy-2-methyloctadecane

The chemical identities of the two moth sex pheromones, "bombycol" and "disparlure", illustrate the common structures of long chain fatty acid derivatives, so many identified since then as moth pheromones, for instance in the laboratory of W. Roelofs, attached to Cornell University. There, T. Eisner and J. Meinwald carried out pioneering studies, especially on defensive mechanisms of arthropods.

Important years in the further development of the interdisciplinary field are 1975: the first publication of the *Journal of Chemical Ecology* (JCE) under the dedicated editorship of the late R. M. Silverstein and J. Simeone, and 1984: the formation of the *International Society of Chemical Ecology* (ISCE), with annual meetings in different countries. From the job point of view, research laboratories, environmental agencies and pharmaceutical and other chemical companies developed a need for people experienced in working with small amounts of biologically active compounds. Recently they have proved to be major employers, together with universities, for people trained in Chemical Ecology.

Since the number of potential odour components, an estimate based on the number of known organic chemicals of enough volatility and other suitable characteristics, exceeds 1 million, and since more than 1.5 million species of various multicellular organisms are known, named and described—about 800 000 species of insects and 250 000 species of higher plants—it is certainly correct to talk about both a rich chemodiversity and biodiversity. Chemical signals must have been a fundamental aspect in the origin of life, even in an early phase characterized by chemical interactions, and as chemotactical agents for avoiding toxic chemicals and attraction to nutrients. The geneticist Theodor Dobzhansky stated[5] that "*nothing in biology makes sense except in the light of evolution*". This statement includes of course the molecular level of biology, and can therefore serve as a guiding principle for chemical ecology.

Analytical micro-techniques

The chemist working together with biologists trying to elucidate the chemical identity of volatile signals, which guide behaviours, should ideally have at his disposal a highly potent instrument, preferably field-borne, which could answer, with high sensitivity and precision, these questions: which compounds and what amounts of them are emitted? The biologist concurrently asks the questions: what are the active components? what behaviour do they elicit? and with what developmental phase or state do they correlate? A single super-instrument does not yet exist but we are coming quite close today by applying a combination of various potent techniques in an optimal way and in an interdisciplinary fashion.

The normal analytical sequence is: isolation/concentration, separation, identification, with behavioural observations and experiments at the start and at the end of this sequence, sometimes also as an integral part during steps in the analytical procedure.[6–8] The first step should ideally be carried out in such a way that the material to be collected is not chemically altered, either qualitatively or quantitatively. Methods used for this step are solvent extraction (such as from glands or other body parts), sorption (adsorption/desorption) of volatiles—many different excellent sorbents are now available—and direct pre-column injection. All the techniques find use, sometimes in combination.[9,10] The gas chromatograph, especially directly coupled to a mass spectrometer (GC–MS), has proved an ideal tool in these kinds of studies, used in all three steps. To help out, we have often used a simple effluent splitter and a revolving fraction collector.[11,12] For the chemical identification the GC–MS is the foremost tool; often the information obtained by this technique directly identifies a compound. We have done some systematic studies of mass spectral fragmentations of some types of compounds.[13] Hydrogenation on a micro scale can be a helpful identification technique,[14] by giving additional information about the chemical structures, and sometimes thin layer chromatography (TLC) can be of use as an additional separation technique.[15] Access to well-defined reference compounds is essential both for the chemical analyses and for behavioural tests.[16,17] A special challenge arises when encountering chiral compounds, especially when the analysis is done on a micro scale, like working with single individuals of insects when obtaining material in the low-nanogram, or even the picogram range.[18] It should not be forgotten that a trained nose can be a very valuable supplement in the analyses. It can often give a hint of the presence of an odour signal, and it is sometimes a good indication of types of chemical compounds (like in wine tastings).

A somewhat idealised summary of the integrated chemical (molecular) and biological (behavioural) procedure is attempted in the scheme:

1. **Preparatory behavioural observations**, and sometimes recordings (like filming), under natural, or near-natural, conditions. Knowledge about life cycles of target organisms. General observations (scent emission, for instance).

2. **Isolation** (retrieval) of volatile material from the object by one of many possible techniques. It can be a. preparation of glandular tissue (under a microscope), followed by solvent extraction; b. driving off volatile compounds in a pre-column tube of a gas chromatograph; c. concentrating samples in a cold trap; d. sorption (adsorption/desorption) on a synthetic adsorbent (like microgranular carbon, Tenax or Porapak). The two latter methods can accumulate volatile material over time, thereby enriching the recovered sample.

A cleaning step may be needed, especially in method a.—with the risk of losing material or effecting an unwanted chemical change.

Possible behavioural tests (here mainly in the laboratory) to check activity.

Alternatively, or complementarily, GC–EAD, gas chromatography coupled to electro-antennal detection, a very potent technique.

3. **Separation** of, often complex, isolates into fractions or compounds, by gas chromatography, or in some cases other chromatographic techniques. This step is most often combined with the identification step, as in another "hyphenated" technique: gas chromatography–mass spectrometry (GC–MS).

4. **Identification** of behaviourally active compounds. The foremost tool is the GC–MS. This calls for reference material, either directly retrieved from a data bank of the instrument, or through a collection of well-defined compounds.

Other techniques, like IR and NMR, have gradually developed towards higher sensitivity, and then become valuable auxiliary methods in the analyses.

Behavioural experiments (tests) are important to ascertain activity.

5. **Behavioural experiments** should ideally be performed both in the laboratory and in the field. Laboratory methods include flight chambers and olfactometers, which are often arranged for choice tests. This phase of the analyses closes the cycle: field–laboratory–field.

Because of the usually high sensitivity of the receptor systems (the sense of olfaction), high purity is an important aspect.

The outcome of the analyses stands or falls with good methods and techniques. We have strived to attain high sensitivity and information, often working with single specimens, and with faithfulness to the natural material, as well as the highest possible coupling between the steps in the chemical analysis and experiments/observations of behaviour. Improvements have been made in the gas chromatographic and mass spectral techniques as well as in micro-chemical analyses. Fig. 2 shows some technical improvements.

Among heavier instrumentation a microwave spectrometer was built and tested. With it rotational spectra of volatile compounds can be recorded. Unfortunately it is of limited use in our area because of low sensitivity and restriction to relatively rigid molecules with a permanent dipole moment—but in principle it could be "X-ray crystallography in the gas phase".

There is an "analytical window", which summarizes an optimal area of analysis defined by the volatility of a compound and by the amounts available. Problems can arise in the analyses of extremely volatile compounds (for example, by evaporation),

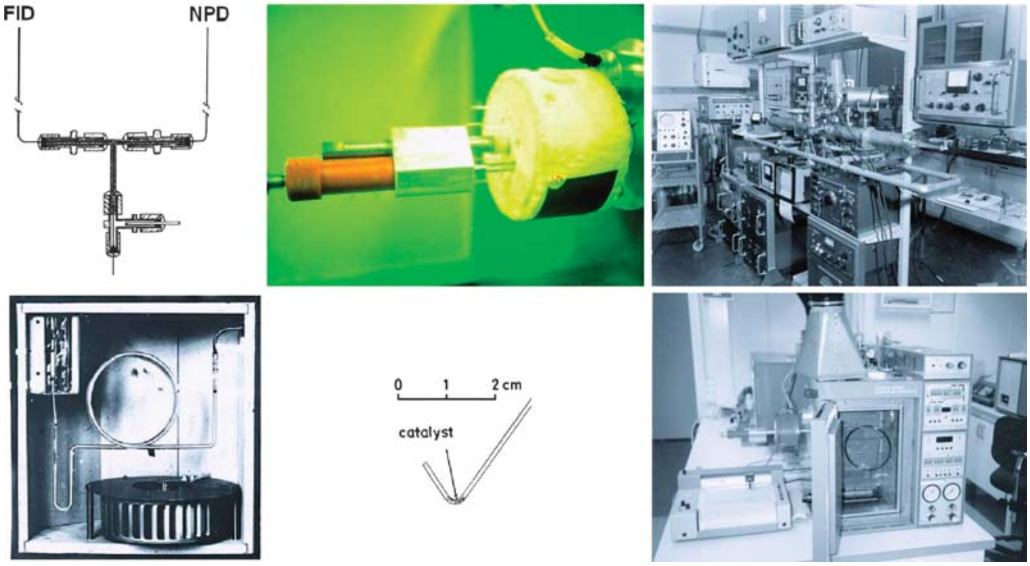


Fig. 2 Examples of equipment for chemical analyses—improvements of techniques. *Upper left*: gas chromatographic micro-split for FID and NPD detectors, or for one detector and micro-fraction collection; *upper centre*: revolving micro-collection device with six micro glass tubes in a cooling mantle attached at the outlet of a gas chromatograph (glass capillary columns); *upper right:* a microwave (1 cm region) rotational spectrometer equipped with gas cell (long tube in the centre) and a 6 Kc s^{-1} Stark electrode; *lower left*: an early gas chromatograph fitted with a precolumn (upper left corner) and a packed glass column; *lower centre*: capillary glass tube with palladium catalyst for hydrogenation on a micro scale; *lower right*: gas chromatograph fitted with glass capillary column, splitter, and fraction collector (on the left side), equipped with both FID and NPD detectors. Reproduced with permission: *Upper left*: A.-B. Wassgren and G. Bergstrom, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1984, **7**, 155. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. *Upper centre*: A.-B. Wassgren and G. Bergstrom, *J. Chem. Ecol.*, 1984, **10**, 1547, with kind permission of Springer Science and Business Media. *Lower centre*: J. Bergstrom and G. Bergstrom, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1985, **8**, 144. Copyright Wiley-VCH Verlag GmbH & Co. KGaA.

and of compounds of very low volatility. There is also a minimum amount of compounds needed for detection; analyses can be at the picogram level with the more sensitive techniques. In some cases we encounter substances sensitive to chemical change, like autoxidation. Therefore, behavioural tests are needed to ascertain biological activity. NMR has often proved to be too insensitive for the analyses of olfactory signals when very small amounts are available and sufficient purity is not achieved, but this technique is improving so that this very potent technique can be of use in some cases. A combined chemical–biological technique, which has proved to be most valuable, is GC–EAD, gas chromatography coupled with electro-antennal detection (of insect antenna, *i.e.* single sensilla or even single receptor cells). GC separation can also be combined with observation/measurement of insect behaviour in flight tunnels (such as for moths) and walking bioassays (such as with beetles).

Of fundamental importance to our understanding of the structure and function of chemical communication is the application of genetic methods, which are now increasingly being applied. The necessary "agreement" (coupling) between the sender and the receiver in a chemical signal system can be brought about by inheritance, by learning, or by a combination of these mechanisms.

Scope of the article

The aim of this article is to give examples of chemical communication between insects and between plants and insects, to specifically address the phenomena of chemical structures, biological functions, and analytical techniques. The evolutionary background for the large variety of chemical compounds will be discussed together with the links between the compositions of the chemical signals and the respective biological functions. Since we have now, in this field, studied many species, representing some major groups of the 30 orders of insects, especially in Hymenoptera (bees, bumblebees, ants and sawflies), Coleoptera (beetles), Neuroptera (antlions) and Lepidoptera (butterflies and moths), we have many good examples of the chemical and behavioural interactions of insects, and their relationships to plants. The main primary examples discussed here represent, respectively, bees (sections 1 and 2), bumblebees (section 3), antlions (section 4), bark beetles (section 5), pine sawflies (section 6), larvae of butterflies and sawflies (section 7) and flowering plants (section 8), including the chemical interactions between the plants and insects. Some related work by colleagues, which complements the examples given, is referred to briefly in connection with each example and finally some trends in the development of Chemical Ecology are mentioned, with indications of a few possible further research directions in this field. Some of the basic texts in this area of Chemical Ecology are referred to in references 19–27.

1. Chemical mimetism: discovery of the identical similarity of marking pheromones between host bees and parasitic bees

We discovered this phenomenon when analysing volatile compounds from exocrine glands of bees since we were

Fig. 3 *Andrena cineraria* female (host) at the nest opening (left) and *Nomada lathburyana* female (parasite), waiting for entrance (right).

interested in their role as pollinators and also curious about their own olfactory signals. In females of certain species of *Andrena* and *Melitta* bees (nest hosts), and in males of certain *Nomada* species (nest parasites), we found identical compounds: isoprenoid and straight chain esters of short acids.[28–30] Why it is so was at first unknown since the respective host females and parasitic males do not normally meet, and they do not have an immediate functional relationship. The nest-parasitic *Nomada* females gain entrance into the nests of host-bee females, *Andrena* or *Melitta*, in order to lay their eggs, Fig. 3. Fig. 4 compares the morphology of *Nomada* and *Andrena* females.

We found that the parasitic males (*Nomada*), in their mandibular glands (in the head) produce the very same compounds as the host females produce in their Dufour glands (in the abdomen). The male parasite transfers the mimetic compound to his female during mating, and thereby perfumes her with the marking pheromone of the host,[31] Fig. 5a and b. In this way the parasitic *Nomada* female is recognized as a conspecific female when entering the nest of the host-bee females, *Andrena* or *Melitta*, in order to lay their eggs, Fig. 3, and there is no fight between host and parasite females.

The female parasite, hidden by this deceptive camouflage, thereby avoids being attacked by the host bee. The dominating mimetic compounds were identified as either geranyl octanoate or farnesyl hexanoate depending on the species of the *Andrena–Nomada* pairs and as octadecyl butyrate for the *Melitta–Nomada* pair, Fig. 6a and b.

The mandibular gland secretions of *Andrena* bees, both males and females, include: monoterpenes, straight chain ketones and,

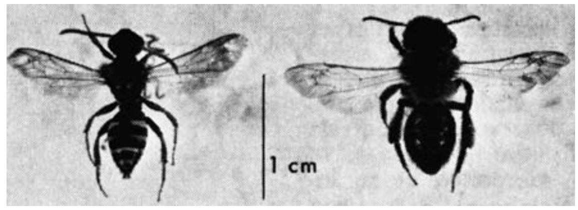


Fig. 4 Females of *Nomada* sp. (left) and *Andrena* sp. (right).

as a new class, spiroketals, but none of the mimetic compounds.[32–36] The production and emission of the mimetic substances exclusively by the parasitic males may imply sexual selection through the composition and/or the quantity of the male secretion. There has probably been a stronger selection pressure for males to produce mimetic compounds; in this way they can be said to have a nuptial gift for their females. The phenomenon ought to be investigated further by behavioural experiments and linked genetic studies. We found the same phenomenon in Sweden (about 30 species studied) and in bees of these species from North America.[37a]

A dendrogram showing possible relations among the major groups of bees was shown in Charles D. Michener's book: *The Social Behavior of the Bees*, Belknap Press/Harvard, 1974. It can be seen that the host bees and the parasites are located far apart, *Andrena*/*Melitta* and *Nomada*, respectively. One can ask how the clepto-parasitic mimetism has evolved. The knowledge of phylogenetic/systematic relationships has developed, and a recent treatment of some of the diverse opinions can be found in the second edition of Michener's book: *The Bees of the World*, Johns Hopkins University Press, 2007, section 20, pp. 88–92. The Nomadini and the Andreninae/Melittinae are placed somewhat closer together, but they are still clearly separated. The former is in the Megachilidae family, long-tongued bees, whereas the latter two belong in short-tongued families. A recent phylogeny by B. N. Danforth *et al.*[37b] is based on five genes and morphology. It contains several important references.

In another cleptoparasitic bee, *Epeolus*, females produce a cephalic secretion containing spiro-compounds and pyrazines,[38a] Fig. 7.

It has been shown by Tengo *et al.*[39] that in the solitary bee *Andrena wilkella* only the naturally occurring enantiomer of the main component, 2,8-dimethyl-1,7-[5.5]undecane, with (2*S*,6*R*,8*S*) configuration, attracted patrolling males in the

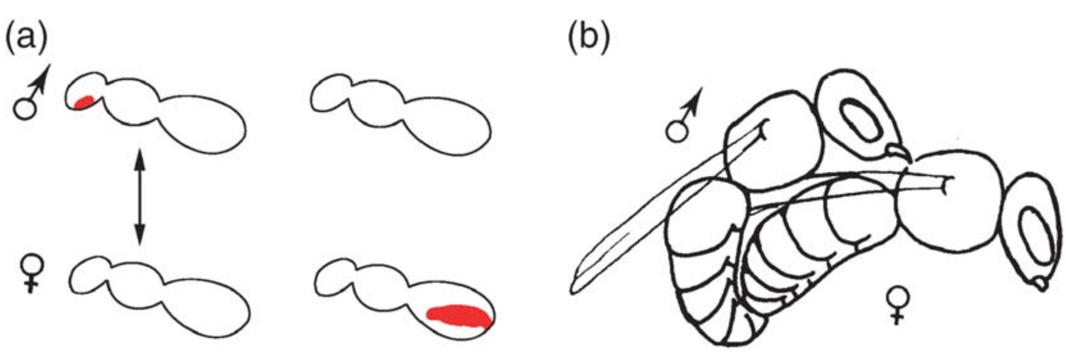


Fig. 5 (a) Left. *Nomada* and *Andrena*, females and males, respectively, showing (red) the Dufour gland of *Andrena* female, and the mandibular gland of *Nomada* male, producing the same marking compounds. (b) Right. Male and female *Nomada* bees *in copula*, showing how volatile compounds from the male mandibular gland can be transferred to the thorax of the female.

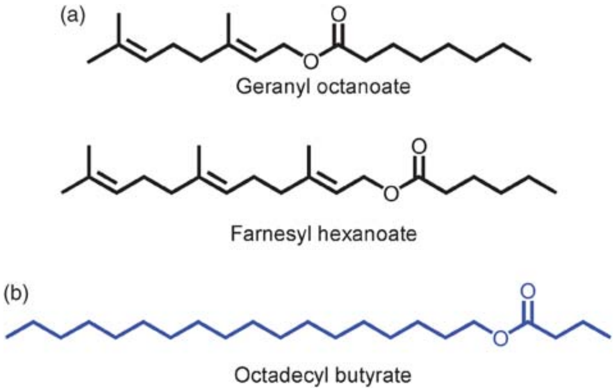


Fig. 6 (a) Marking compounds of *Andrena* females and the "corresponding" *Nomada* male: geranyl octanoate or farnesyl hexanoate in different species. (b) Marking compound from *Melitta*, another host bee genus, and their *Nomada* parasites.

field. This was corroborated by EAG (= electro-antennogram) studies, which means registration of electrical impulses from olfactory receptors. So, this is one clear case of discrimination between enantiomers.

Francke *et al.* identified interesting new sesquiterpene and nor-sesquiterpene ketones in female cephalic secretions of the cuckoo bee, *Nomada lathburiana*.[40] The main component was 2,6,10-trimethylundeca-(5*E*)-2,5,9-trien-4-one.

Host–parasite relationships have also been studied in *Sphecodes* bees towards their halictid hosts.[41] These behaviours seem to involve volatile compounds emanating from the Dufour gland.

In the stingless bee, *Trigona recursa*, Ayasse *et al.* have found[42] hexyl decanoate, produced by the labial gland (located in the head), to be the main component responsible for the trail following behaviour of foragers. This study has now been widened to two other species, *T. spinipes* and *T. corvina*. In the former, octyl octanoate is the dominant trail-following compound, whereas in the other a blend of no fewer than 12 substances (alkyl and terpene esters) seems to have this function.

Besides the discovery of chemical mimetism, this example of chemical communication in insects also shows that they emanate from specific glands, of which there can be about 10 different ones in one individual. The volatile compounds found represent the acetogenic and the isoprenoid biosynthetic routes. The volatility of the mimetic substances is quite low and this serves the marking function: the deposited compounds can remain for weeks.

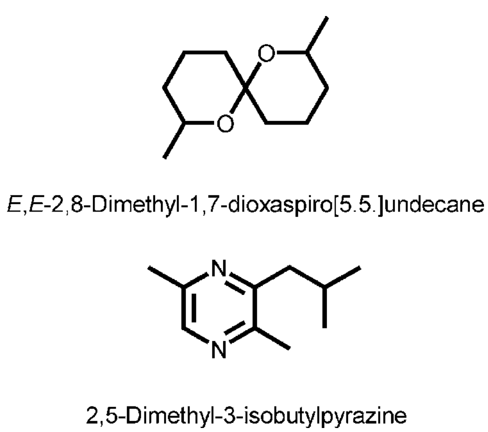


Fig. 7 Examples of spiroacetals and pyrazines found in other cleptoparasitic bee species: *Epeolus cruciger* and *E. variegatus*, respectively.

2. Dual function: musk smelling nest markings and hydrophobic wall lining in bees

Nature is economic—a specific biosynthetic route can be employed for different purposes. We found this with the secretion from the Dufour gland, located in the abdomen of females of certain bee genera: *Halictus*, *Lasioglossum*, *Colletes* and *Evylaeus* (34 species studied altogether). On the one hand straight chain C16–C24 ω-hydroxy acids, which are the primary products, can be polymerized and make up a protective hydrophobic wall lining (a bit like the cutin of apple cuticle). On the other hand, ω-hydroxy acids can also be internally cyclized to produce musk smelling macrocyclic lactones of different sizes. As stable, relatively low-volatile compounds they are ideal as species-, kin- and individual-specific combinations for long-lasting nest and territory markings.[43–48] In one study of the Dufour gland secretion in *Evylaeus malachurum* bees,[38b] which contain C16–C24 macrocyclic lactones, plus isopentenyl esters and hydrocarbons, individual blends were analysed. The difference in amalgamation distance between nestmates (sister bees) and nonnestmate (strange) bees, see Fig. 8a and b, shows that nestmates are more similar than nonnestmates. There is both a species-specific marker, 20-eicosanolide and 22-docosanolide, always being the two dominating components, and individuality. Indeed, structurally related musk smelling macrocyclic ketones are well-known marking substances from mammals, such as the musk deer (muscone) and the civet cat (civettone), and these compounds have been used in perfumery for centuries.

3. Species specificity: characteristic marking pheromones of male bumblebees and the discovery of formation of new species

Male bumblebees secrete from their labial glands, in the head, a blend of compounds, which was found to be species-specific; 38 species occurring in Scandinavia and five from North America have been analysed.[49–67] The secretion is applied during their repetitive marking flights on various objects in their way like twigs, leaves, and litter on the ground, Fig. 9 and 10.

The volatile blend attracts females, and other males of the same species, and increases the likelihood of male–female conspecific encounters. The blends are composed of straight chain fatty acid derivatives and/or sesqui- and diterpenes. Females accept as partners only males with the right scent of their own species.

In each one of two taxa (systematic units), *Bombus lucorum* and *B. lapponicus*, we discovered that the main components of the marking secretions from separate individuals were different compounds. This means that two different forms of each of the two species (populations) could be discerned. They are now classified by taxonomists as different species on the basis

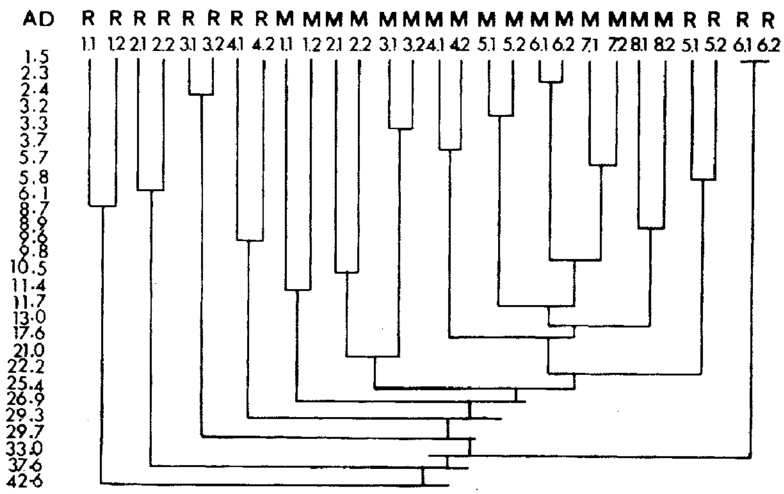
(a)

VARIATION IN COMPOSITION OF DUFOUR'S GLAND SECRETIONS OF INDIVIDUAL *E. malachurum* BEES[a]

Nest	Median Amalgamation Distance: Two samples of the same glandular exudate	Samples of glandular exudate from nestmate (sister bees)	Samples of glandular exudate from nonnestmates (strange bees)	P
1	4.99 (N = 14)	30.96 (N = 8)	54.2 (N = 6)	<0.001
2	4.78 (N = 15)	30.36 (N = 9)	52.02 (N = 6)	<0.001
3	7.68 (N = 15)	20.7 (N = 9)	52.62 (N = 6)	<0.001
4	6.10 (N = 11)	34.14 (N = 5)	52.26 (N = 6)	<0.001

[a] Samples originating from nestmates (sister bees) and nonnestmates (strange bees) were compared by cluster analysis of cases. The data of each nest are presented as median amalgamation distance calculated before the clustering of the cases.

(b)



Dendrogram representing the degree of similarity in the composition of Dufour's gland secretions of 14 *E. malachurum* bees. Eight of the bees were nestmates (marked by M), the other six were nonnestmates collected at random in the nesting area (marked by R). Each glandular exudate was injected twice. Accordingly, case R 1,2 is the second injection of extract of the first bee collected at random, while case M 3,2 is the second injection of the third bee collected from nest M. The amalgamation distances depicted to the left of the dendogram are calculated after the clustering procedure.

Fig. 8 (a) Variation in composition of Dufour's gland secretions of individual *E. malachurum* bees. (b) Dendrogram showing the degree of similarity between 14 bees. Reproduced from ref. 38(*b*) with kind permission of Springer Science and Business Media.

Fig. 9 Male *Bombus cryptarum* (formerly *Bombus lucorum* "dark") scent-marking a hazel leaf.

Fig. 10 Male *Bombus lapidarius* scent-marking a hazel twig.

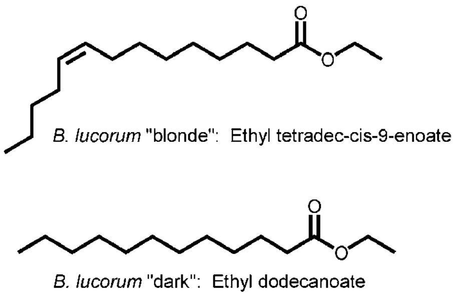


Fig. 11 Major components of the marking pheromones of *Bombus lucorum* (*B. lucorum* "blonde") and *Bombus cryptarum* (*B. lucorum* "dark"), formerly treated as a single species.

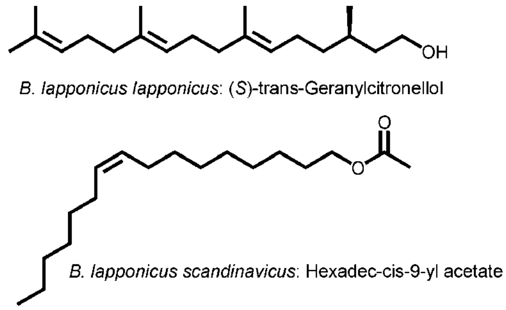


Fig. 12 Major components of the marking pheromones of *Bombus lapponicus* (*Bombus lapponicus lapponicus*), and *Bombus monticola* (*Bombus lapponicus scandinavicus*), formerly treated as a single species.

of clearly different compositions of their marking pheromones, Fig. 11 and 12. The chemical analyses, which were made with single individuals, were made possible even early on because of the relatively high amount of labial gland secretion, 0.1–1.0 mg per individual.

They represent cases of sympatric speciation since the forms/new species in many parts of their area of distribution occur at the same time and in the same space. They presumably originated as genetic divergences in populations from a single species inhabiting the same geographical region. They could then, theoretically, interbreed. Although in theory the populations could interbreed there is in fact a behavioural mating barrier between the populations based on different pheromone composition. Among some species occurring in the same area at the same time, there might also be a vertical flight separation, just like air traffic—if the terrain allows it.

Within subgenera, such as Pyrobombus, Alpinobombus, Megabombus and Psithyrus, there are some chemical similarities and concurrently distinct differences. Species in the subgenus Megabombus are differentiated, Fig. 13 by the chemical composition of their male labial gland secretion. One can even discern three subgroups: *M. hortorum* and *M. consobrinus* producing isoprenoids and fatty acid derivatives, mainly 9*Z*-nonadecene; *M. subterraneus* and *M. distinguendus* make different diterpenes; and a group of six Megabombus species which give off species-specific blends of fatty acid derivatives, mainly long chain aldehydes, alcohols, and acetates.

The semiochemistry of the bumblebee *B. hypnorum* has been studied in a comparative way, such as intra- and intercolonial variation in the Dufour gland secretion and concerning pheromonal dominance signals, by collaborative efforts.[68,69] This followed studies on individual and group specific odours[70] and on the complexity and species specificity of Dufour gland secretions,[71] and was continued by the analyses of the exocrinology of queen *B. terrestris*[72] and studies on chemical signals on eggs of *B. terrestris*.[73] Aggressive compounds in social parasitic bumblebees[74] have also been studied, including identification of the queen sex pheromone components,[75] which turned out to be a mixture of 21 compounds, including heptadecene, 2-nonanone and methyl oleate. A study has begun aimed at the chemical mimicry in relationships between *B. terrestris* nest and its social parasite *B. vestalis*.[75]

This example shows how male bumblebees can produce species-specific marking secretions by using blends of compounds produced by the fatty acid derivative route and the isoprenoid pathway. It also demonstrates how species specificity can be

		Species									
Mol. wt.	Compound	*M.(M.) hortorum*	*M.(M.) consobrinus*	*M.(T.) pascuorum*^{a}	*M.(T.) humilis*	*M.(T.) muscorum*	*M.(T.) ruderarius*^{b}	*M.(T.) sylvarum*^{b}	*M.(T.) veteranus*	*M.(S.) subterraneus*	*M.(S.) distinguendus*
222	all-*trans*-Farnesol	17									
288	Geranylgeranial	21	3								96
290	Geranylcitronellal									100	
290	Geranyllinalool										4
238	Hexadecenal			40^c	4^e						
266	Octadecenal				32^f						
294	Icosenal				3						
240	Hexadecenol			60^c	44^d	48^d	88^d	29^c	65		
268	Octadecenol	7^d	1		8^f		12^d	2	15		
296	Icosenol				6						
282	Hexadecenyl acetate				3			69^c	15		
310	Octadecenyl acetate					52^d			5		
266	Nonadecene	55^d	96^d								

^{a}Data from Ref. 7. ^{b}Data from Ref. 2. ^{c}*Z*-7. ^{d}*Z*-9. ^{e}Δ-9. ^{f}Δ-11.

Fig. 13 Major components of the marking secretions of 10 bumblebee species within the subgenus Megabombus.

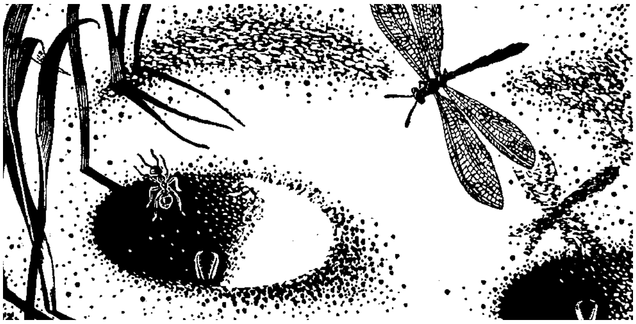


Fig. 14 Antlions. The larva (the antlion proper) lies buried at the bottom of a pit in the sand and is depicted with a possible prey, an ant, on the edge. The adult insect, in flight, to the right.

attained by the syntheses of characteristic blends of relatively few compounds, structurally related but different. The discovery of two chemically different forms (populations), in each of what was formerly considered as two species, probably represents the first cases of chemical speciation involving olfactory signals in animals.

Fig. 15 A unique photo, taken at night, of antlions (*Myrmeleon formicarius*) *in copula*; female above, male below.

4. Minimal two-component species-specific sex pheromones in antlions

Antlions proper are the three-year larvae of species of Myrmeleontidae, a family of the insect order Neuroptera. They make pits in the sand and lie in wait, ready at the bottom to catch prey, such as ants, Fig. 14. Adults live for about two weeks, mating and egg-laying. Mating takes place typically in the tops of pine trees (*Pinus silvestris*), after the antlion adults have emerged from their pupae, dried their wings and flown a short distance. Fig. 15 shows the mating position with the female holding onto a twig and the coupled male hanging down.

Male adult antlions possess a thoracic gland, which is only rudimentary in the female, Fig. 16a–c. The secretion of this gland, which has a characteristic smell, serves as a male sex pheromone. It contains species-specific blends of just two components in each of the five species analysed.[76–79] Three of the species, *Euroleon nostras*, *Grocus bore* and *Myrmeleon formicarius*, occur in Scandinavia; the other two species studied, *Synclysis baetica* and *Acanthaclisis occitanica*, were collected in Israel.

The volatile compounds emitted represent variations of two biosynthetic routes, one isoprenoid and one acetogenic, Fig. 17. In the molecular scheme, the four uppermost compounds are monoterpenes resulting from the isoprenoid pathway. The other two compounds are mono-unsaturated secondary alcohols of different chain length (11 and 13 carbons) and result from the acetogenic pathway. This pattern of just two dominating substances in male sex pheromones is uncommon.

The pyranoid monoterpenes nerol oxide and 10-homo-nerol oxide possess a characteristic sweetish-pungent scent which can be encountered also from sun-exposed lacquered wood (such as from newly lacquered wooden boats), presumably formed in that case by photo-oxidation of nerol. This hint actually helped us in the chemical analysis. The antlions have an "archaic-looking", irregular flight. *Myrmelion formicarius* is said to be the oldest species phylogenetically, and this is obviously reflected in the most basic composition of its two-component secretion. It is worth stressing that the three Scandinavian species and the two from Israel have the same type of minimal two-component scent system.

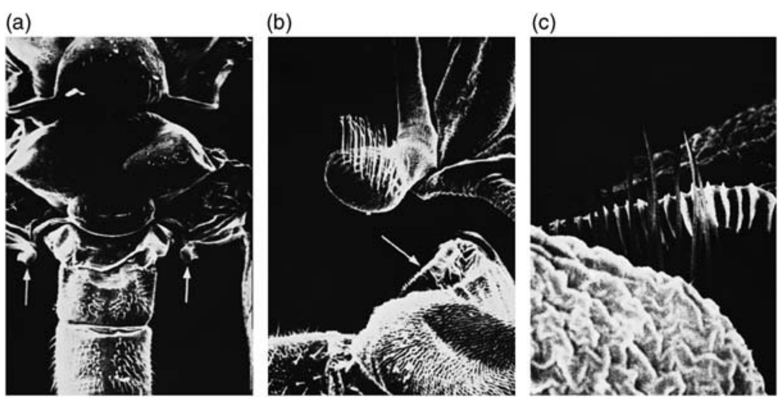


Fig. 16 (a)–(c) Collage of three sweep-electron-microscope (SEM) pictures of (a) (left) the inner back of the wings with spreading organs, small brushes, marked with arrows; (b) (centre) enlargement of a brush fitting into a cleft in the thorax where the scent gland opens; (c) (right) further enlargement showing a comb-like structure at the gland opening in which the spreading brush fits at each stroke of the wing. Reproduced with permission from: R. Elofsson and J. Lofqvist, *Zool. Scripta*, 1974, **3**, 35, published by the Royal Swedish Academy of Sciences.

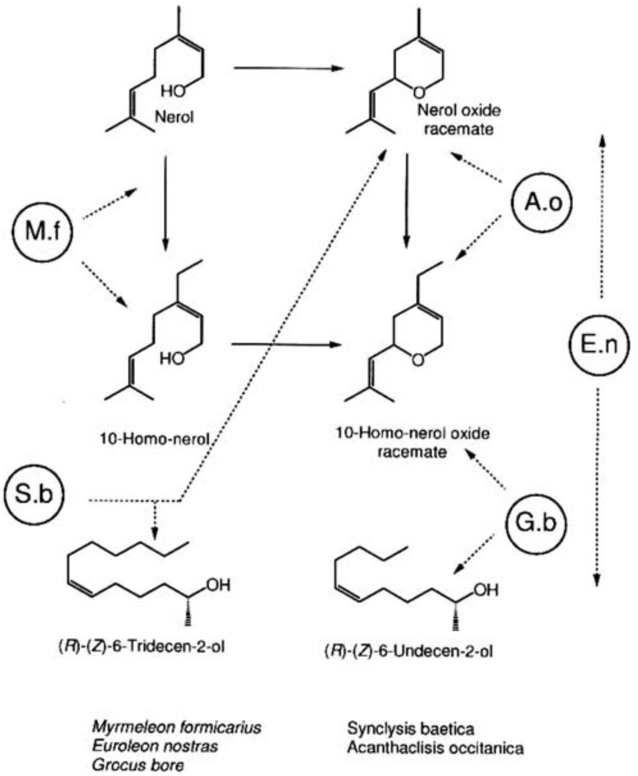


Fig. 17 Molecular formulas of the six different compounds, representing two different biosynthetic routes, which, pairwise (dotted arrows), are responsible for the species specificity of the five analysed species.

5. Olfactory signals of two bark beetle species: contrasting ways of achieving specificity of male aggregation and sex pheromones

The active sex pheromone of male *Ips typographus* was found to be a blend of 2-methyl-3-buten-2-ol and *cis*-verbenol. They are oxygenated products linked to the detoxification, by the beetle, of monoterpenes from the wood, Fig. 18–20. This communication system, and that of the related *Pityogenes chalcographus*, was studied in detail as a collaborative effort by four Swedish research groups, two chemical and two biological, during a nine-year project (part of the chemical results given in refs. 80–89). The ultimate practical goal of the project was to come up with control methods for important forest pest insects.

The involvement of yeasts (*Candida* and *Hansenula*) in the production and interconversion of *Ips typographus* monoterpenes was studied and also the quantitative variation between individuals and between attack phases (*Ips typographus*). Both yeast strains were found to convert *cis*-verbenols to verbenone, and one *Candida* strain (*C. nitratophila*) converts (1*R*)-*cis*-verbenol to *trans*-verbenol and (1*S*)-*cis*-verbenol to verbenone.

Blends of methylbutenol and *cis*-verbenol have been employed successfully as an attractant in traps, see Fig. 20, right, either for monitoring, *i.e.* relatively few traps over a larger area, which are surveyed regularly as an "early warning", or for population reduction, which calls for a large number of traps.

Fig. 18 Spruce tree (*Picea abies*) with small glass tubes fitted to bark beetle entrance holes and with a minipump (centre right) and a larger pump (at the bottom end of the red tubing) to collect emitted volatiles through adsorption.

By contrast, *Pityogenes chalcographus*, which is a closely related species, produces in the male a strongly synergistic blend of "chalcogran" (2-ethyl-1,6-dioxaspiro[4.4]nonane, identified primarily by Prof. Dr W. Francke, Hamburg, a close colleague) and methyl (*E*,*Z*)-2,4-decadienoate as pheromone,† Fig. 21 and 22.

Finding the two synergistically active compounds was challenging since extracts obtained by body washing the beetles also contained large quantities of volatiles emitted from the tree, see Fig. 23. In order to find the active compounds we had

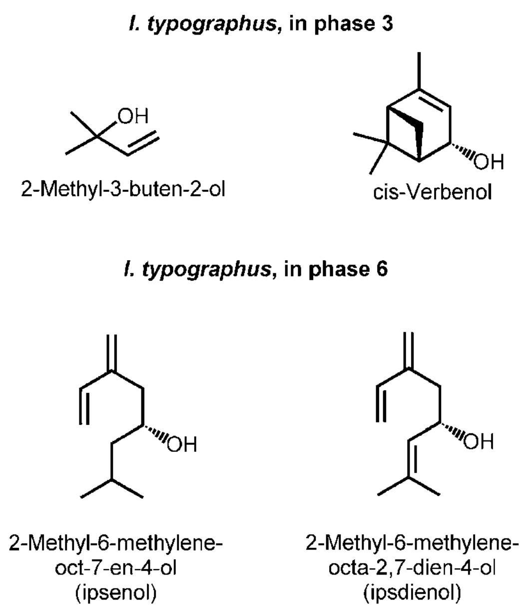


Fig. 19 Signal molecules (pheromones) of pioneer male *Ips typographus*; the two upper ones attract females, and other males, to the tree ("mass attack"), the lower ones are used as a signal (after mating and egg-laying has taken place) to leave the tree.

Fig. 20 A collage of, left: damage to the inside of the bark, caused by the bark beetle "*typographus*"!; centre: mating chambers, made by the male, with one male and three females; right: bark beetles caught in a 2 l plastic flask baited with the sex pheromone.

Fig. 21 Male *Pityogenes chalcographus*.

to perform successive gas chromatographic fractionations, recombinations of fractions and behavioural testing.

It is an interesting question as to why *Pityogenes chalcographus* has evolved such a highly specific signal, very different from other volatiles in the forest milieu, whereas the closely related *Ips typographus* uses two oxidation/detoxification products from the wood. The *Pityogenes chalcographus* components are probably not directly related to sequestered wood substances.

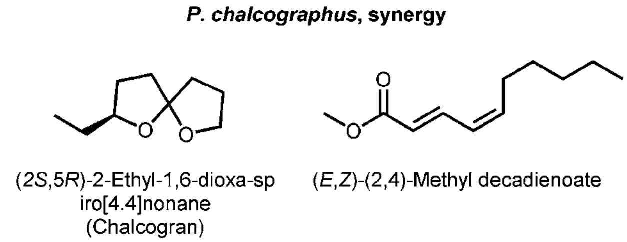


Fig. 22 Molecular formulas of the two sex pheromone compounds.

Other forest insects studied include pine shoot beetles, *Tomicus piniperda* and *T. minor*, and the pine weevil, *Hylobius abietis*.

Some further studies on the role of volatile compounds in bark beetles (Scolytidae) are referred to in one section[34] of the comprehensive review by Francke and Dettner.[90]

It is worth noting that Tolasch *et al.* found as female sex pheromones of six species of click beetles (*Agriotes*, Elateridae) geranyl and/or (*E*,*E*)-farnesyl esters of fatty acids with 2 to 8 carbon atoms,[91] quite similar to what we found earlier (see above) in *Andrena*/*Melitta* and *Nomada* (see Fig. 6a above). Major compounds in *Agriotes brevis* were geranyl and farnesyl butyrates.

6. Specificity of multi-chiral sex pheromones of pine sawflies

We have analysed the female sex attractant in ten species of Diprionid sawflies (Hymenoptera, family Diprionidae) during a four-year European collaborative research project involving seven groups. This study has since been continued both because of the challenge of the chemical analyses—small amounts of highly specific compounds—and the biological

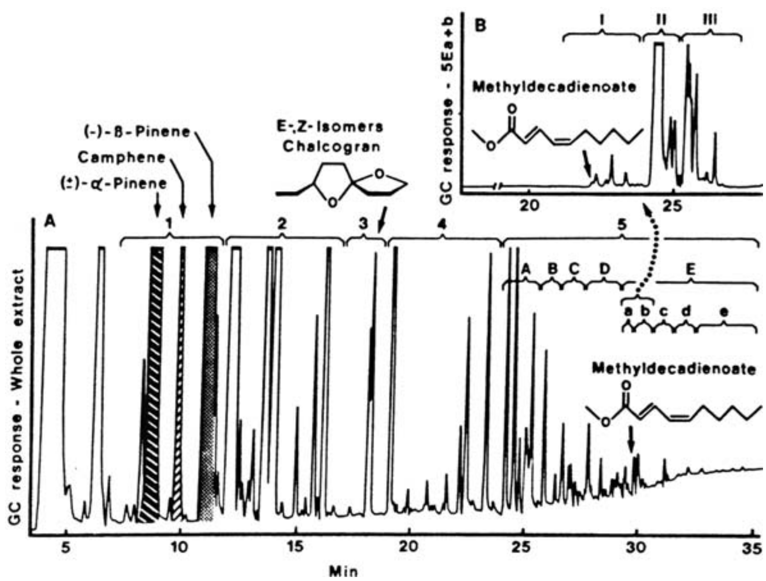


Fig. 23 High resolution capillary gas chromatograms of an extract (body washing) of a single *Pityogenes chalcographus* male. The two active pheromone components, acting in strong synergy, were identified as "chalcogran" and an isomer of methyl decadienoate, see molecular formulas and arrows. Most of the volatile materials, all the large peaks, are mono- and sesquiterpenes from the tree (*Picea abies*). Identifications are done by repeated fractionations and recombinations, followed by behavioural testing.

activity, including potential practical application such as monitoring sawfly outbreaks.

The female produces primarily an alcohol precursor of the active pheromone, which occurs in 1 ng quantities per individual female. It is an analytical challenge because of the small amounts of active compounds and interfering large amounts of non-active volatile compounds.[92–104] The pheromones represent structural variations on a theme. The active compounds are acetate or propanoate esters of the straight chain methyl branched secondary alcohols (the precursors), Fig. 24. We have found that specific stereoisomers—and in two species, *Microdiprion pallipes* and *Macrodiprion nemoralis*, there are four chiral centres and consequently 16 possible chiral isomers!—give strong electrophysiological response from the male antenna, and full behavioural reaction. Our organic chemistry colleagues have synthesized all the possible stereoisomers, in high stereochemical purity, for tests in the laboratory and in the field. In *Neodiprion sertifer* we found a structural analogue, Fig. 24 second from above, present in the insect, which acts as an inhibitor (antagonist) decreasing attractivity.

The pine sawflies have occasional outbreaks, especially in central and southern Europe, which can be monitored by traps baited with the pheromone (as in the case of the bark beetle).

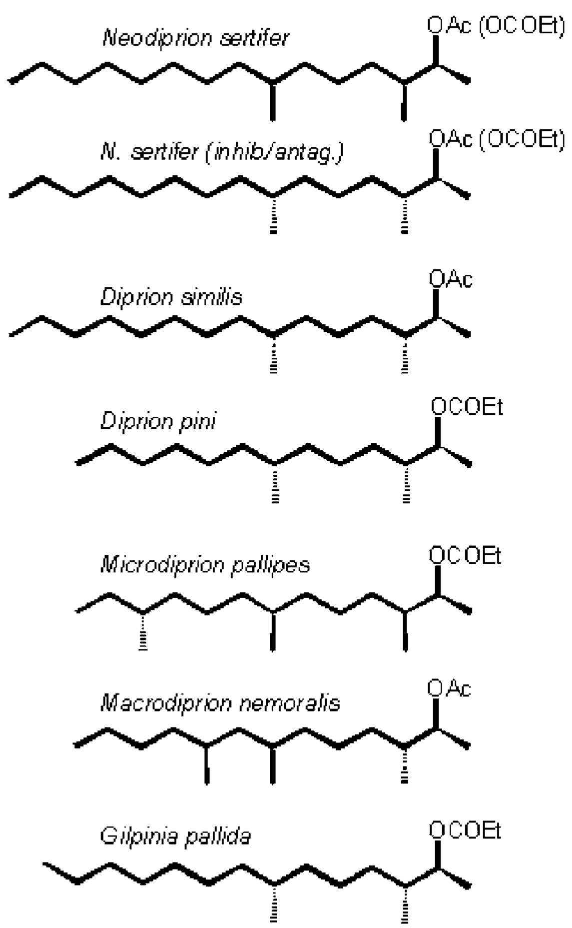


Fig. 24 Sex pheromones of Diprionid sawflies: esters of methyl branched secondary alcohols.

So the pheromones are interesting both from the applied and theoretical points of view.

There is a good review of the pheromone biology of sawflies (Diprionidae) by Anderbrant[105] and one focusing on semiochemistry by Keeling *et al.*[106] Hilker and coworkers have studied the kairomonal effects (gives advantage for the receiver) of sawfly sex pheromones on egg parasitoids.[107] They found that *Chrysonotomyia ruforum* (the egg parasitoid) was arrested when perceiving the major sex pheromones of *Diprion pini* or *Neodiprion sertifer*. This tritrophic effect turned out to be stereospecific. They have also found that plants are able to "notice" insect egg deposition and respond by activating direct and indirect defences.[108] This seems to be quite a general phenomenon, true for many different plants and herbivore insects.

The pine sawfly sex pheromones really represent an interesting extreme case of achieving species specificity by stereochemical isomerism. In four of the species we have studied (see Fig. 24), there are three stereochemical centra producing eight possible isomers, and in the two species with four chiral centra there are 16 possibilities. For two other Gilpinia species we have preliminary results indicating simpler structures with two active centra producing four isomers.

We carried out a study of the emission of volatiles from *Diprion pini* females which showed that precursor alcohols are released together with short chain acids. Evidently, esterification to the active esters takes place at the moment of release.

7. Larval defence: pine sawflies, larch sawflies and monarch caterpillars

Many insects spend a large part of their lives in larval form, with much of the chemistry and biology differing from the adult. In so-called pest insects it is often the larva that is the damaging agent, as it is foraging on leaves, needles, wood, *etc*. As many female insects deposit their eggs on specific plants, the larvae develop there. The larvae use many protective devices including chemicals to avoid detection and disruption. We have studied defensive secretions of pine sawfly larvae, larch sawfly larvae and monarch caterpillars.

Pine sawfly larvae

Larvae of the pine sawfly *Neodiprion sertifer* sequester, and store selectively, from the host trees *Pinus silvestris* and *Pinus contorta* (*R*,*S*)-5-germacradien-4-ol. Its function is not fully understood but it might be an important part of the protective discharge of the larvae and pupa,[109] Fig. 25a–c, 26A–D.

The larval regurgitate, Fig. 25c, may serve as an additional visual deterrent. The chromatograms in Fig. 26A–D show that the germacradienol present in the complex blend from *P. contorta* needles is selectively enriched in the larval regurgitate (A and B as compared to C and D).

Larch sawfly larvae

In the larch sawflies larvae one species, *Pristiphora erichsonii*, is colonial and another, *P. wesmaeli*, is solitary, Fig. 27. Both

† Covered by patents. Commercial product: Chalcoprax (BASF, Germany).

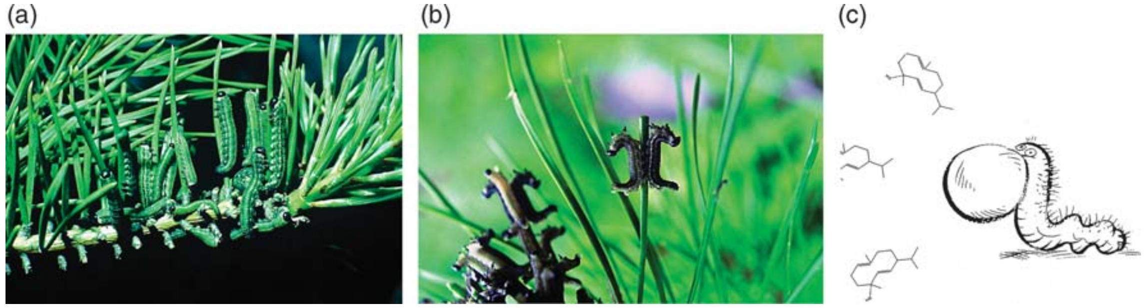


Fig. 25 (a)–(c). Pine sawfly larvae (*N. sertifer*) feeding on needles of *P. contorta*. In (a) they are calmly eating; in (b) they react with a behaviour called "snap-bending" in response to a disturbance. Repellant chemicals are emitted concurrently. A major component is the germacradienol. (c) A cartoon showing secretion of the larval regurgitate.

exhibit a "snap bending" when disturbed, and this behaviour seems linked to the emission of species-specific odours.

Chemical analyses showed that the two species give off partly related (five of them are monofunctional monoterpenes) but clearly different volatiles, three major compounds in each species, Fig. 28.[110]

Monarch caterpillars

The Monarch butterfly (*Danaus plexippus*) caterpillar gives off highly volatile compounds when threatened. Major compounds are 3-hydroxy-2-butanone, which is not given off by adults, and *Z*-3-hexenol which is present in the food plants *Asclepias curassavica* and *A. syriaca*. The discharge of these compounds accompanies violent paroxysms elicited by predators.[111] Low-flying aircraft (such as Hawker Harrier jets, which we experienced in the greenhouses of the late Dame Dr Miriam Rothschild at Ashton Wold, Peterborough, UK) can also elicit this behaviour. It was quite alarming even to us human bystanders!

The chemical relationship between plants, used for egg-laying and subsequent foraging, and insects is an intricate one, often referred to as an arms race. The plant often produces defensive compounds and the insect develops detoxification mechanisms to deal with them.

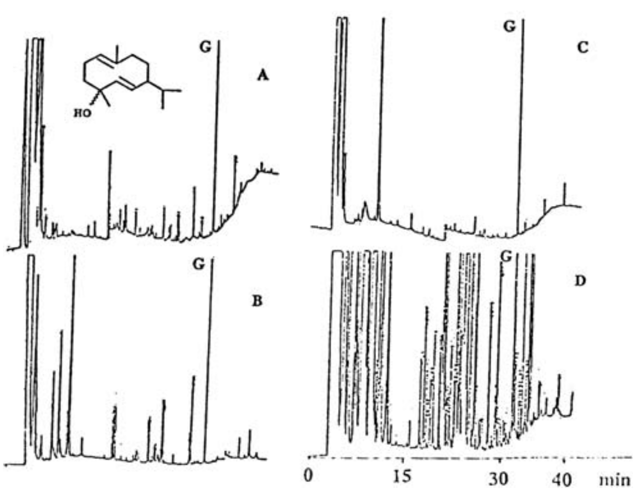


Fig. 26 (A)–(D). Gas chromatograms of extracts containing 1,6-germacradien-5-ol. (A) LC fraction 6 of extracted *N. sertifer* pupae; (B) *N. sertifer* larval regurgitate; (C) LC fraction 6 of *P. contorta* needle extract; (D) recombined LC fractions 1–10 of the *P. contorta* needle extract.

8. Chemical communication and flowering plants: pollination attractants/stimulants

Flowering plants produce and give off a multitude of volatile compounds. It is generally thought that this emission primarily acted as defence and secondarily, during the evolutionary

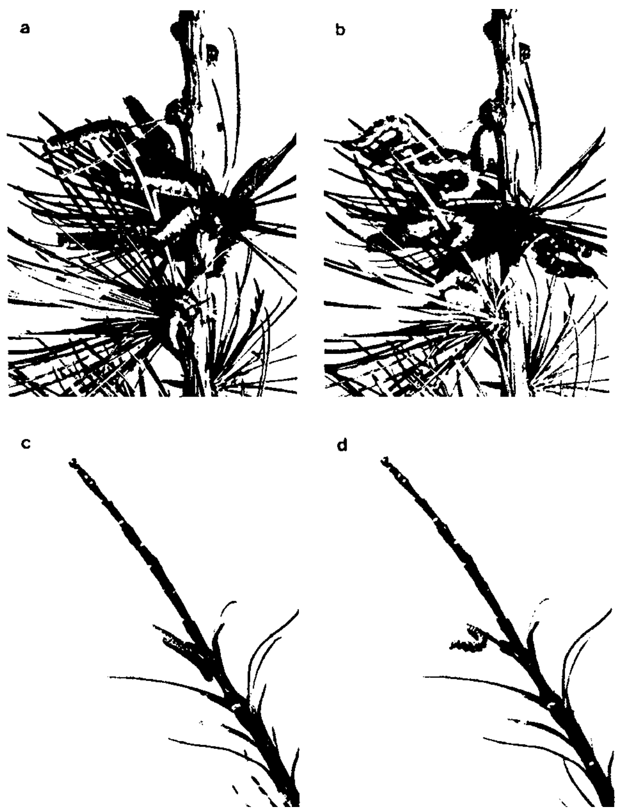


Fig. 27 Behaviour pictures of *Pristiphora* larvae. Upper left: *P. erichsonii*, calmly eating; upper right: alarm position – snap bending; lower left: *P. wesmaeli*, single individual calmly eating; lower right: alarm position. Reproduced from: S. Jonsson, G. Bergstrom, B. S. Lanne and U. Stensdotter, *J. Chem. Ecol.*, 1988, **14**, 714, with kind permission of Springer Science and Business Media.

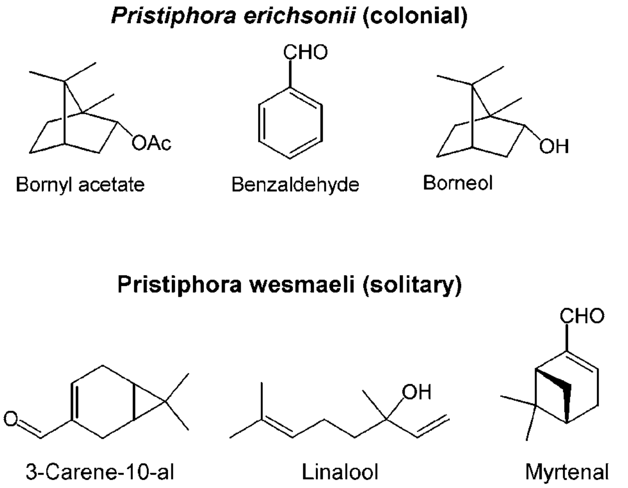


Fig. 28 Volatile compounds given off by larvae of the two species.

process, for attracting pollinators, especially insects. The flowering plants attract and guide pollinators by visual and chemical stimuli, and often—but not always—offer them a reward in the form of nectar and/or pollen. The flower organs are often intricately adapted for the pollinator in their morphological organisation. This is especially true for flowers specialized on one major type of pollinator. In this case the flower can be optimalized to accommodate the insect, see for instance Fig. 31, 32 and 38. This is a mutual interdependence. One can say that the insect has made the flower and the flower has made the insect. The flower scent is usually a complex blend of volatiles, which are produced through the three major biosynthetic pathways: the acetogenic (fatty acid derivatives), mevalogenic (isoprenoids) and also the aromatic (benzenoid) routes. Here are a few examples of results from studies of plant volatiles.

Actaea and *Rosa*

We have found that besides the flower parts sepals and petals, pollen, in many species, produces and emits specific volatile compounds, which are different from those emitted by other flower parts. We noted this first in *Actaea*.[112,113] Distinct chemical profiles were also shown by the pollen volatiles from *Rosa rugosa*, Fig. 29,[114,115] with fatty acid derivatives (particularly aldehydes, ketones and esters), simple monoterpenes

Fig. 29 *Rosa rugosa* exposing the anthers, which carry pollen.

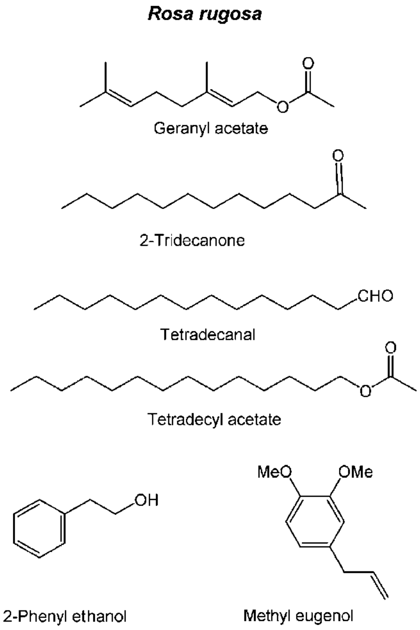


Fig. 30 Compounds identified exclusively, or dominantly, from pollen.

(geranyl acetate) and a few benzenoids (2-phenylethanol and methyl eugenol), Fig. 30, giving characteristic fragrances. These compounds may act primarily as pollen allepathic (defensive) compounds, and maybe as close-up-guidance for pollinating insects.

Cypripedium

In three species of *Cypripedium* (Orchidaceae) we found extreme chemical disparity between the three variants (sometimes discerned as species) *C. calceolus*, *C. parviflora* and *C. pubescens*, Fig. 31. Each one is dominated by compounds of one of the three major classes of volatiles: octyl and decyl acetate; *cis*-β- and *trans*-β-ocimene; 1,3,5- trimethoxybenzene, Fig. 32. They represent fatty acid derivatives, isoprenoids, and benzenoids, respectively.[116] The great chemical difference between the three variants may reflect functional evolution in relation to different bee faunas, which act as pollinators by deception. The flowers do not offer food for the insect. Instead this flower is specialized to lure young, inexperienced females of certain bee genera to act as pollinators. They are attracted by the scent and by the intense yellow colour. Through the morphology of the flower, the so-called lady's slipper, the bee is trapped inside the labellum, and must exit by passing the pollen, which attaches to the body.

Ranunculus and some other species

In *Ranunculus acris*,[117] Fig. 33 and 34, α-farnesene and the small lactone 5-methylene-2(5*H*)-furanone (protoanemonin) are characteristic pollen volatiles. The pollen odour is markedly different from that of other flower parts with relatively few components. It may serve as a signal to pollen feeding insects. Protoanemonin is a skin irritant and responsible for the fact that many animals, such as cattle, avoid eating this plant. It is a protective device.

Fig. 31 Left: *Cypripedium calceolus* (lady's slipper). Flower with *Andrena haemorrhoa* female stuck inside the labellum. Centre: Bee creeps out with pollen on its front thorax. Right: Whole flower. Observe the "windows" on the sides of the labellum, which are not damage but guides for the pollinator. They serve to direct the insect so as to pass the pollinia.

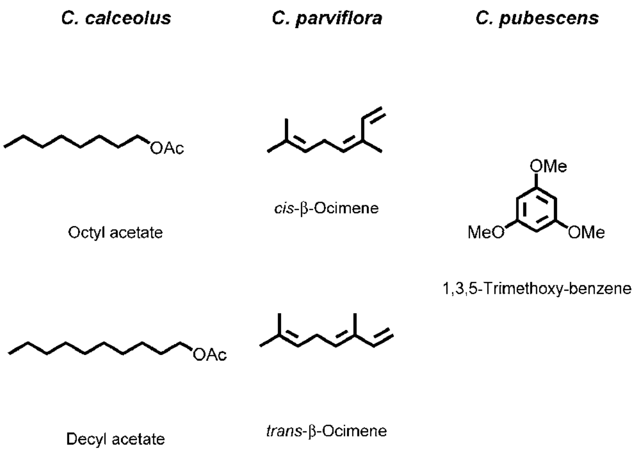


Fig. 32 The three species (also called forms) of *Cypripedium*: *calceolus*, *parviflora*, and *pubescens*, with drastically different emitted volatiles, acting as pollinator attractants/stimulants.

Another comparative study was carried out for pollen and the remainder of the flower in *Papaver rhoeas*, *Filipendula vulgaris*, and *Lupinus polyphyllus*.[118] In the first one, the difference between pollen and whole-flower volatiles was very subtle. In *F. vulgaris*, a large amount of 2-heptadecanone is characteristic for the pollen, and in *L. polyphyllus* pronounced amounts of hexanol and limonene distinguish the pollen odour.

From these and other studies, it can now be firmly established that pollen produces and emits characteristic scent profiles. Although this can be comprehended from the biochemical and cytological point of view, the full functional and evolutionary meaning remains to be studied.

Other studies were aimed at investigating the role of volatile signals in evolutionary old plant–pollinator systems, where floral structures and scents serve as mating sites and food, as brood substrate, or as potent herbivore deterrents. In the first category, scents, including short esters, of primitive Winteraceae plants (trees occurring in New Caledonia) in relationships with likewise primitive *Sabatinca* moths, were studied.[119] The *Sabatinca–Zygogynum* (one genus of Winteraceae) relationship represents an ancient and primitive pollination strategy. The trees keep the insects in the flowers for some time by short chain chemicals, such as ethyl and methylpropyl acetates, Fig. 35. Thereby the insects behave as if intoxicated and pollination is stimulated.

Fig. 33 *Ranunculus acris*.

In another study chiral esters were found to attract pollinating beetles (genus *Elleschodes*, Coleoptera Curculionidae) of *Eupomatia* (a magnoliid genus with just two species, studied in New Caledonia).[120] *Eupomatia* are pollinated by these weevils, which may have their entire life cycle linked to the host plant. The short chain, chiral esters serve as species-specific attractants, Fig. 36. Plant–pollinator systems where floral structures

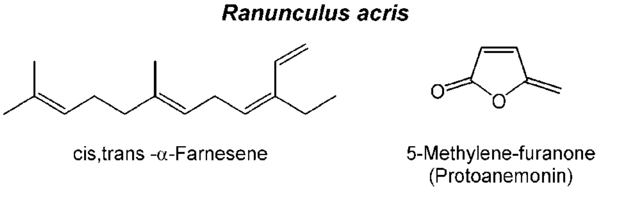


Fig. 34 Two major components of the volatile emission from the flowers.

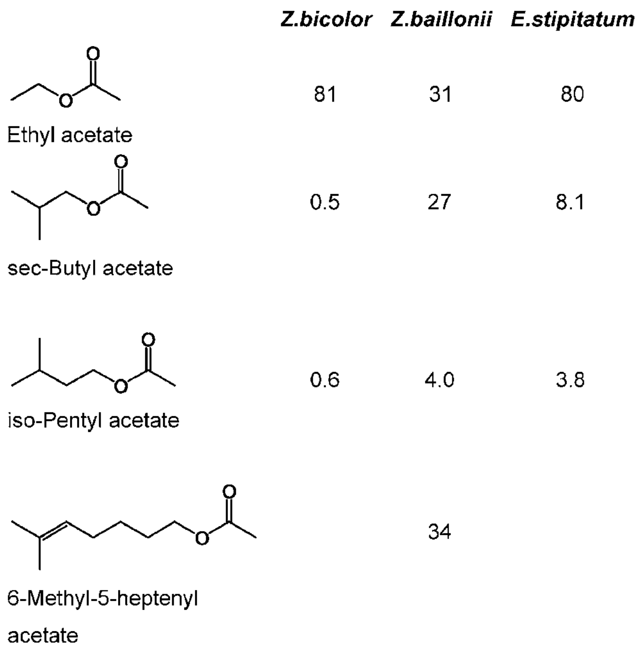


Fig. 35 Volatiles identified from flowers of *Zygogynum* and *Exospermum* species.

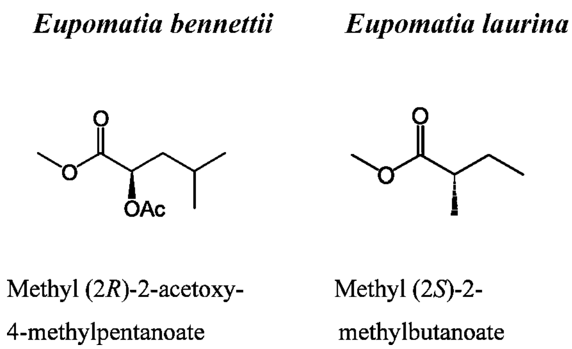


Fig. 36 Chiral esters, acting as attractant in *Eupomatia*.

serve as pollinator brood substrate through ovule consumption are usually highly specific mutualisms.

The hypothesis that early chemical attractants for pollinators evolved from herbivore deterrents was augmented by analyses of scents from cones and flowers of four insect-pollinated cycad (Cycadales) species.[121] In the flowers, all three major classes of volatile compounds made up the odour bouquets. The compound classes found in the cycads are also potent herbivore deterrents. The results suggest convergent evolution in the gymnospermous cycads, and in the magnoliid angiosperms, of the olfactory cues that attract pollinating insects.

For general reviews of plant volatiles, see refs. 122 and 123.

The examples show a case of pollinator-attraction by deceit, *viz*. in *Cypripedium*. The same phenomenon occurs in *Ophrys*, another genus (see below) of the species-rich Orchidaceae family. These specializations *vis-á-vis* the pollinators call for a highly developed system for attracting and exciting the insects, including the chemical signalling.

The pollination of *Ophrys* orchids

Orchids of the genus *Ophrys*, distributed mainly in the Mediterranean region and with some species northwards in Europe, have one of the most specialized ways of pollination known. They are visited only by males of certain species of bees and sphecid wasps, which do not obtain food on the flower but are attracted by volatile compounds which mimic sex attractants of the pollinators in combination with visual and tactile stimuli, Fig. 37a, b, and c. Each *Ophrys* species—the taxonomy is complicated—is pollinated only by a few bee or wasp species, a highly specialized assortative pollination strategy which calls for a highly adapted communication system.[124–130]

Chemical analyses of flower volatiles have been made in several *Ophrys* species and for some species of pollinators. Potential attractants are fatty acid derivatives and isoprenoids. Sesquiterpenes, with varying ring structures, Fig. 38, have a

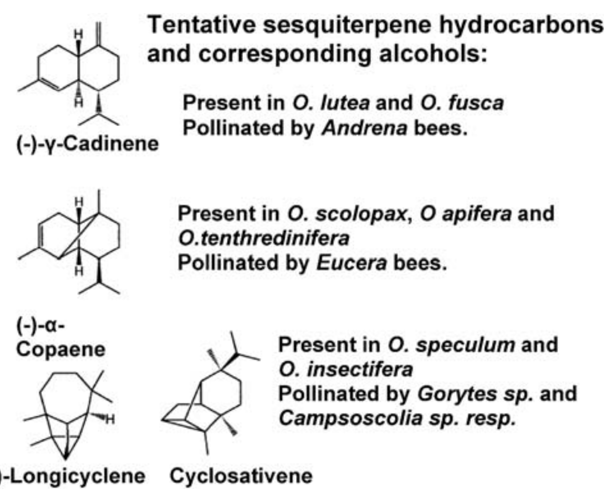


Fig. 38 Tentative structures of sesquiterpene hydrocarbons which, together with their corresponding alcohols, are characteristic for different groups of visiting/pollinating insects.

Fig. 37 (a) *Ophrys speculum* (left), (b) *Ophrys insectifera* with *Argogorytes* male (centre), and (c) *Ophrys tenthredinifera* with visitors.

distribution in different species which matches the pollinator visiting pattern. This led us to believe that it was these compounds which were mainly responsible for attraction and excitation. These studies were carried out by Bertil Kullenberg, Uppsala for many years. We did not find the sesquiterpenes in the females corresponding to the pollinating males though; however, the GC–EAD technique was not available in earlier days. Some behavioural tests and some electrophysiological studies were carried out. They were later continued by colleagues, who have found highly potent fatty acid derivatives, including hydrocarbons. Still, some short, straight-chain hydrocarbons and particularly some cyclic sesquiterpenes may be active mimetic compounds.

Studies by colleagues have focused first on the *Ophrys sphecodes* group[131–135] and then on the *O. exaltata*/*Colletes cunicularius* relationship[136] and the *O. fusca* group.[137]

Conclusion and outlook

For many years there has been an increasing proliferation and diversification of science into new disciplines and subdisciplines. Several of them represent combinations of subjects and they are truly interdisciplinary, for example the interfacing between chemistry and biology, with designations such as Molecular Biology, Biological Chemistry and Biochemistry. Chemical Ecology is one such area that focuses on the chemical communication among organisms. In the evolution of scientific developments it represents something new and constructive, an "anastrophe",[138] which joins together chemical interactions studied in a collaborative way on the molecular and the organismic levels. It not only adds to chemical and biological knowledge and approaches, it is truly synergistic in approaching complex phenomena *via* interconnected methods, techniques and thinking.

Herein we summarize the present understanding of behaviour-guiding olfactory signals studied in chemical ecology with some general statements:

1. The systems consist of sender, signal, receiver, and the environment, which can be air, water or direct contact.

2. It is a prerequisite that sender and receiver have "agreed upon" the signal, through the evolutionary process—by inheritance, or learning, or a combination thereof.

3. All living organisms (possibly without exception) employ exocrine chemical signals to guide behaviours linked to various vital needs.

4. Chemical signals very likely were also important in the origin of life.

5. Chemical communication is one means through which ecological relationships are maintained.

6. Chemical signals can represent evolutionary quanta—*i.e.* minimal changes in chemical structures—by which micro-evolutionary steps can be studied. Thus, they have also an importance for systematics on lower taxonomic levels, especially on the species and genus levels.

7. Practical application in control of organisms in a non-toxic and precise, selective way through monitoring, mating disruption, or population reduction.

8. The volatile signal usually consists of few components produced by one or more of the three major biosynthetic pathways.

9. Living organisms often have special structures which assist in the release of signals. Likewise, the reception of a signal is made possible by the arrangements of receptor cells, like the sencilla of insects.

10. A sequence of biochemical events involving reception, transduction (to a coded electrical signal), conduction, discrimination (in the olfactory bulb), and perception so as to produce sensitive and meaningful information about the environment, as well as guiding behaviour.

11. Possible combinations with signals from other sensory modalities: visual, acoustic and tactile stimuli, and CNS coordination with memory.

An attempt to define and relate these facts and conditions by a series of statements can be used to form an outline towards a theory of chemical signals.[139]

The examples given in the eight previous sections of this article should give some idea of what chemical communication and chemical ecology are about, the chemicals and the behaviours involved, which should specifically depict the following phenomena.

1. Chemical mimetism in bees. In bees, bumblebees and ants (all are hymenopteran insects) the volatile chemical signals are predominantly produced and emitted from special glands of which there are several, in different parts of the body. These insects produce complex blends of chemicals which are often members of a homologous series. Bees and bumblebees are important pollinators and there has been, over time, a strong co-evolution between them and flowering plants. Mimicry is quite a common phenomenon in Nature, often visual, when it can be referred to as protective disguise. Chemical mimetism, described for the first time in insects,[28–31] may also be quite common. It has been known in the relationships between plants (orchids) and insects for some time, see section 8.

2. Dual functions in musk bees. The dual function expressed by polymerization and cyclization, respectively, of ω-hydroxy acids is a good example of the economy of Nature. It points to a stepwise functional adaptation. In a group of related organisms the chemical signals evolved often represent a variation on a theme, quite clear when one compares species-specific signals, *e.g.* inside a genus.

3. Species specificity in male bumblebees. In the multi-component species specific volatile compounds deposited along a repetitive flight route by marking males were found to have evolved their specificity through a combination of molecular parameters like chain length, double bond position, functional group together with a characteristic proportion between components. In this way specificity is achieved for a number of species (we found all the 38 Scandinavian species to be specific) based on combinations of relatively simple compounds. The discovery of sympatric speciation founded on separate volatile substances in two of the species makes this assortment even more distinct.

4. Sex pheromones in antlions. The two-component male sex pheromones of these archaic species (they belong to Neuroptera, which was more common in the late Palaeozoicum and early Mezozoicum) represent another clear case of

chemical variation. Small chemical differences give species specificity, based on the high discriminatory power of the olfactory receptors.

5. Bark beetle pheromones. The insect order Coleoptera is the most numerous and widespread order. Defence compounds have been studied previously and Coleoptera includes many species like bark beetles, which are important tree destructors, and therefore of considerable economic importance. We found the bark beetle behavioural ecology most interesting, especially the distinct differences between the two species we studied in depth. Their species-specific sex pheromones are the products of the acetogenic and the mevalogenic pathways. Again, their signals are made up by two components each.

6. Sex pheromones of pine sawflies. Sawflies also belong in the order Hymenoptera, but are clearly different from bees and ants. They have a pronounced sexual dimorphism in the female sex pheromone and the highly sensitive receptors located on the large, featherlike antenna of the male. Also here the species-specific sex pheromones represent variations on biochemical themes, and here their stereochemistry plays a fundamental role, giving up to 16 stereoisomers in two of the species studied.

7. Defence compounds of larvae. The three groups of larvae studied were found to have semiochemicals combined with specific defensive behaviours. This serves to deter potential predators. The volatile compounds are aposematic (serving to warn) signals for toxic compounds in the larvae.

8. Volatiles of flowering plants. Most flowering plants have a special scent, and their scents differ characteristically, probably as a result of co-evolution among plants and visiting insects. One can observe products of all the three major biosynthetic routes, often in combination, and often with a rich bouquet of volatiles. The visual, tactile and chemical signals from the flowers combine to give either a more generalistic or a more specific relationship to visiting insects.

The three major ways of using pheromones for control purposes are monitoring (surveillance by pheromone-baited traps), trap catches (for population decrease) and mating disruption (permeating the atmosphere with synthetic pheromone). All three are being used in agriculture and in forestry as a positive alternative to non-specific, destructive methods which may be toxic. For example, the use of mating disruption in forestry, for the gypsy moth, has been used over areas of altogether 230 000 ha; control of the grapevine and grapeberry moths in Europe together over 105 000 ha; for the pink bollworm, which is a pest on cotton, over 50 000 ha; for the codling moth, the oriental fruit moth and leafroller moths (fruit pests), together over 238 000 ha worldwide.[140] There is definitely room for a wider use of all three methods based on selective pheromones to control pests.

What then for the future? New analytical techniques are likely to appear, both for the chemical and biological sides, and combined instrumentation with higher sensitivity and the capacity to provide more information. Through miniaturization, some of these analytical means will be of much lighter weight and functionable directly in the field, which is already developing to some extent. Further improved techniques for studying behaviour both in the laboratory and in the field are needed. Computer search algorithms of reference material (like stored mass spectra) can be further facilitated.

Various genetic techniques and methods will certainly sweep into this field and this is likely to have a major impact to add to our understanding of chemical communication, for instance of the evolutionary processes. There is clearly room for more and in depth studies of the ecological importance and implications of chemical communication. Further studies in synthetic organic chemistry will likely give us access to more precise chemicals, such as stereochemically pure, chiral compounds, their role in pheromone science recently reviewed by Mori.[141] More studies of the biosynthesis of behaviour-releasing compounds may be facilitated by the drastic improvments in genetic methodology. Regarding the study of receptor functions, it is expected to give major advances in our understanding of the biochemistry of the olfactory process, as well as the central nervous level, including the links to memory and behaviour.

Further studies on various aspects of chemical communication in the types of insects and plants which are described in this article have been performed in later years by several groups. Work on behaviour-guiding olfactory signals in bees, bumblebees and flowering plants is among them. Examples are the enzyme genetic analyses by Pamilo *et al.* on the *Bombus lucorum* complex[143] and the study by Bertsch[142] on the scent specificity in *B. cryptarum* and *B. lucorum*. Bumblebee inquilinism (an inquiline is an animal that lives as a visitor in the nest, burrow, or dwelling place of an animal of another species) in *B. sylvestris* and the possible role of mimicking volatiles were investigated by Dronnet *et al.*[144] Savolainen and Vepsalainen[146] discussed the possibility of sympatric speciation through intraspecific social parasitism by a mitochondrial DNA phylogenetic analysis in three inquiline *Myrmica* ant species. Luxova *et al.*[145] determined the absolute configuration of some chiral terpenes, which act as marking pheromones in bumblebees. An important study of intra- and interspecific variability in the labial gland secretion of male *B. ruderarius* and *B. sylvarum* has been reported by Terzo *et al.*[147] Very good sources of further information on these phenomena are on the one hand original articles, especially in the two dedicated publications: *The Journal of Chemical Ecology* and *Chemoecology*, on the other hand *The Proceedings of Annual Meetings of ISCE.*[148–150] Examples from ref. 149 are Ayasse *et al.* who identified, synthesized and bioassayed 16 compounds from cuticle extracts of *B. terrestris* queens. The compounds were found to have an effect as primer pheromone, inhibiting the ovarian development of the workers. They also function as a recognition signal of the queen. The Prague group reported (posters) age-dependent changes in exocrine glands of *B. terrestris* queens, and changes over time in compounds from the labial gland secretion of *B. lucorum*.

Much interest is today focused on the chemical arms race between food plants and insects. Jasmonic and salicylic acids are important for defensive responses of plants to attack by herbivores and pathogens, both in chewing and non-chewing insects. The compounds are often found in high concentrations in eggs, which can be attacked by parasites. Induced defence is being studied, often as a part of tritrophic interactions. These types of studies represent an actual trend, which is likely increasing.

For use in the field one can hope for a wider acceptance and use of control methods based on additional knowledge about chemical signals. In the field of medicine these methods have not yet had a major breakthrough. Biological colleagues blame this to some degree on the basic fact that flies and mosquitos, which belong to the insect order of Diptera (two-wings), notoriously appear in dense groups of both males and females, where it is difficult to penetrate with synthetic chemical signals, although they definitely exist and are known to some extent. Some of the major medical pests call for new methods of attack. Genetic manipulation of vector populations has had some success, and attempts at control with juvenile hormones have also been made.

Some groups of living organisms have not yet been studied to a large extent. There is much room for more studies of mammalian behaviour—including that of Man—in relationship to exocrine chemical signals. Aquatic/marine organisms represent a world quite unknown to us as to the use of behavioural chemical signals. The same is certainly true for microorganisms. One can hope that substantial biological and chemical research will be directed to these large and important groups of organisms. It will give us new fundamental knowledge necessary to expand our understanding of the world we inhabit.

Acknowledgements

The collaboration of all colleagues who have contributed to the basic findings presented in this article is gratefully and collectively acknowledged, in addition to the financial support from Foundations and Research Councils.

I specifically would like to thank the following colleagues for use of photographs (numbers of figures in parentheses): Anders Nilsson (3, 31), Bertil Kullenberg (9, 37), Peter Bergman (10), Inga Thomasson (14), Jan Lofqvist/Rolf Elofsson (1, resp. 16), Andreas Yasseri (15), Goran Birgersson (18, 20, 21), Sten Jonsson (25), Heidi Dobson (29) and Sven-Olof Strandhede (33).

I thank Daniel Pettersen and Goran Hilmersson for skilled help with the computer.

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