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Pickett, J. A., Birkett, M. A., Woodcock, C. M. and Zhou, J-J. 2009. *Scents and sex: insect pheromones.* Portland Press Ltd. doi:10.1042/BIO03102028

The publisher's version can be accessed at:

- https://dx.doi.org/10.1042/BIO03102028

The output can be accessed at: https://repository.rothamsted.ac.uk/item/8q73v/scents-and-sex-insect-pheromones.

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Insect pheromones

Scents and sex

John A. Pickett, Michael A. Birkett, Christine M. Woodcock and Jing-Jiang Zhou (Rothamsted Research, Harpenden, UK)

Pheromones are chemical signals (semiochemicals) that act between members of the same species, sex pheromones being the signals that facilitate sexual reproduction. Many organisms use such semiochemicals, but it is insects to which the main research attention has been directed. This article will therefore concentrate on the insect sex pheromones.

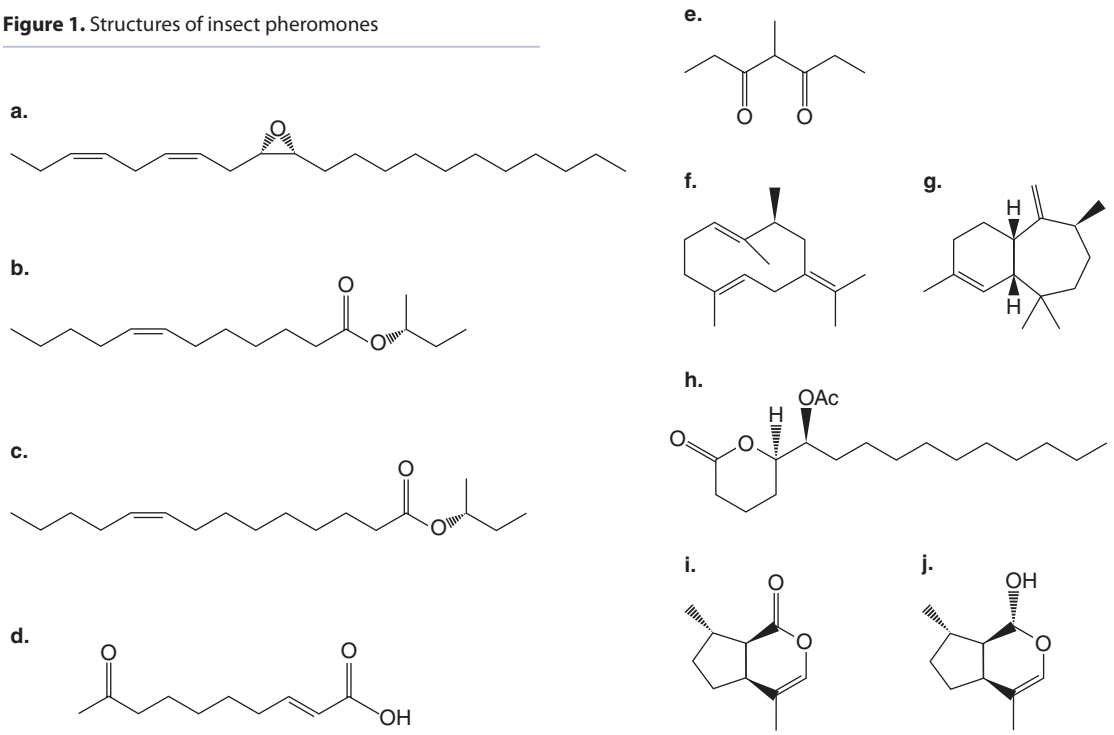
Where pheromones have been discovered in new situations, the term 'pheromone' has not always been applied, for example in quorum sensing by bacteria and with plant activators involving intraspecies interactions. Because pheromones by definition act externally, they need to be relatively robust and mostly involve lower-molecular-mass lipophilic molecules derived from fatty acid, polyketide, isoprenoid, amino acid and phenylpropanol biosynthetic pathways or precursors. They are usually deployed at extremely low (sub-nanogram) levels. Many are volatile, further restricting their physicochemical properties, but even those acting in the rhizosphere or aquatic systems can be lipophilic molecules which achieve sufficient solubility for long-range signalling. Molecular diffusion plays only a minor role in pheromone dispersal and, even in apparently still air or water, this usually involves movement together with the bulk medium.

This year is the 50th anniversary of the first identification, by Butenandt, of a pheromone defined as such, specifically the sex pheromone of the silk moth, *Bombyx mori*, (*E*,*Z*)-10,12-hexadecadienol.

Production

Production of insect sex pheromones is usually by the female of the species and involves either specialized glands secreting to the surface cuticle or direct release to the air, e.g. by pneumatic eversion of the gland. Most sex pheromones for moths and butterflies (Lepidoptera) are derived from fatty acids, as is the case for *B. mori*. For some, e.g. the family of tiger and footman moths

Figure 1. Structures of insect pheromones



Key words: *aphrodisiac, gustation, isoprenoid, olfaction, semiochemical*

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(Arctiidae), the unsaturated double bonds are already established in unsaturated fatty acids derived from primary metabolism. Thus, for the cinnabar moth, *Tyria jacobaeae*, and the scarlet tiger moth, *Callimorpha dominula*, the pheromones are synthesized by chain elongation of linolenic acid in two steps to the corresponding C_{22} compound, which, after decarboxylation and epoxidation, gives the pheromonal compound as the monoepoxide of the hydrocarbon with three (*Z*) double bonds (Figure 1a). These two species are able to use the same pheromonal component because they are normally separated during their mating seasons, although they will mate when confined together (Figure 2). However, for many lepidopterous species, particular desaturases and then reductases are employed to convert fatty acid precursors into novel unsaturated aldehydes and alcohols with the option of esterification. We have found recently that the burnet and forester moths (Zygaenidae) can have the reverse of this structural motif by employing esters of long-chain unsaturated fatty acids, e.g. for the plum moth, *Illiberis rotundata*, which uses a two-component mixture[1] (Figures 1b and 1c).

The family Hymenoptera, which includes the wasps and bees, provides an example of the second insect pheromone to be identified. Colloquially known as the honey bee queen substance and having various roles in regulating the sociality of honey bees (*Apis mellifera*), the main compound, identified by Butler, Callow and Johnston in 1961 as a fatty acid product (Figure 1d), acts as a typical sex pheromone in attracting male honey bees (drones) to the queen.

The beetles (Coleoptera) represent a vast order of insects, but, where pheromones are employed, these mostly act as aggregation pheromones, although often with a sexual bias. A wide range of biosynthetic pathways is involved, with many species employing isoprenoids. Bark beetles were originally thought to sequester isoprenoid precursors from their hosts, particularly those feeding on conifers which are rich in isoprenoidal resins. However, it became evident that *de novo* biosynthesis by these insects is a major route to these pheromonal components, and genes for their biosynthesis are now being identified, e.g. by Blomquist. Another predominant pathway is the polyketide route and, as an example, the male-produced aggregation pheromone of the pea and bean weevil (*Sitona lineatus*) comprises a diketone (Figure 1e).

Dipterous species such as flies often have acute visual capabilities and do not always use pheromones, and mostly not for long range interactions. In this order, there are also interesting variations in the type of sexual behaviour that is mediated. Male New World sandflies, *Lutzomyia longipalpis*, which transmit parasites causing leishmaniasis, have geotypes which release different sex pheromones in different regions, e.g. homogermacrene (Figure 1f) from the Lapinha region of Brazil[2] and a homohimachalene (Figure 1g) from the Jacobina region[3], to affect behaviour of the females. Although difficult and expensive to synthesize, the homogermacrene can be produced cheaply from germacrone, obtained as a major component of the essential oil from a plant, the rock cranesbill (*Geranium macrorrhizum*)[4].

The only pheromone known for mosquitoes is produced by those in the *Culex* genus, which transmit West Nile virus and the filarial parasites that cause elephantiasis. The pheromone is released from eggs maturing on the water surface and attracts further gravid females to lay eggs (Figure 3). In this case, the biochemistry returns to the fatty acid pathway with specificity derived from the stereochemistry of the lactone[5] (Figure 1h).

The bugs (Hemiptera), particularly aphids (Aphididae, Homoptera), produce isoprenoid sex pheromones, which, for many pest aphids[6,7], comprise the nepetalactone (Figure 1i) and the nepetalactol (Figure 1j). These are produced and released from organs in the hind legs of the female aphid and, in spite of a molecular mass of only 166 and 168 atomic mass units, have four asymmetric carbons, with the aphid producing only one of the 16 possible stereoisomers. Although initially thought to act as a short range aphrodisiac, the synthetic compounds can be used to trap aphids from a distance. In addition, the pheromonal components act as foraging stimulants and attractants for parasitic wasps that can severely reduce aphid populations (Figure 4).

Figure 2. Interspecific mating between *Callimorpha dominula* (left) and *Tyria jacobaeae* (right)

Figure 3. Oviposition by *Culex quinquefasciatus*

Figure 4. The aphid parasitoid *Diaeretiella rapae* attacking the peach-potato aphid, *Myzus persicae*, on *Arabidopsis thaliana*

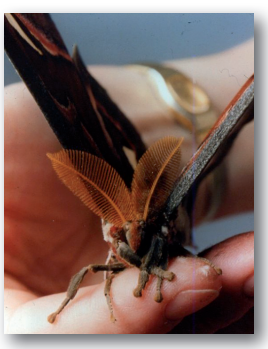

Figure 5. Antennae of the male Atlas moth, *Attacus atlas*

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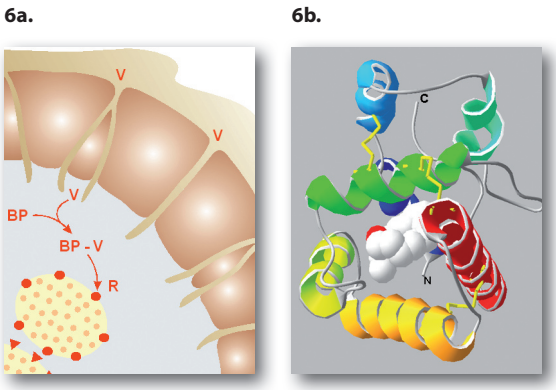

Figure 6. (**a**) Cross-section of antennal sensillum. V, volatile molecule; BP, binding protein; R, receptor. (**b**) Three-dimensional structure of *Bombyx mori* PBP bound with the pheromone (*E*, *Z*)-10,12-hexadecadienol (spheres). N- and C-termini are indicated

Figure 7. Single-cell recording on antenna of the vetch aphid *Megoura viciae*, using a tungsten microelectrode

Detection

A great deal of work has been directed towards the mechanisms by which external signal molecules are detected by animals and the processes often referred to as 'olfaction', for volatile molecules, and 'gustation', for aqueously dissolved signals. For the most sophisticated signals, olfaction is generally employed, and this also appears to be true for organisms in the rhizosphere and in aquatic systems. For plants, we have virtually no knowledge of how external signals, particularly putative phytopheromones, are detected. However, for insects, there is a large scientific literature, not only because of the interest in these organisms due to their impact as pests and disease vectors, but also because they present tractable model animal systems. There are, of course, significant differences between the olfactory apparatus of insects and other arthropods and vertebrates. The sensory organs in insects are mostly situated on the antennae and mouthparts, but can be elsewhere, including on the tarsi (legs and feet). Olfactory organs responsible for detecting sex pheromones are predominantly sensillae situated on the antennae (e.g. Figure 5). Superficially, these sensillae comprise horn-like or plaquoid structures into which the pheromone enters through pores in the cuticle (Figure 6a). On reaching the aqueous lumen beneath, the pheromone then binds to a pheromone-binding protein (PBP) of around 14 kDa mass present at an extremely high concentration, e.g. up to 10 mM. Three-dimensional structures of some PBPs have been determined (Figure 6b). There is an expectation that at least some molecular recognition takes place at this stage, and the main function of the PBP appears to be transport across the aqueous lumen for co-recognition with the bound pheromonal ligand at the pheromone receptor protein[8]. This is embedded in a dendritic extension of the pheromone receptor neuron, the cell body of which is embedded deeper within the antenna. Initially, it was expected that the pheromonal receptor protein would be a transmembrane G-protein-coupled system. However, recent publications demonstrate a receptor protein coupled to an ion channel protein[9,10]>. We have devised an algorithm for searching insect expressed sequence tag (EST) libraries and full genomic sequences by which to identify the odorant-binding protein genes and are studying the mechanisms of molecular recognition at this stage of olfaction[11].

Although much needs to be learned about the mechanism by which pheromonal and other olfactory ligands are recognized by insects, we can already exploit the olfactory system for pheromone identification. This can be accomplished by measuring the standing potential across a whole antenna (the electroantennogram, or EAG) and observing the change in this potential when the olfactory neurons respond to a pheromone or other semiochemical applied to air flowing over the antennal preparation. Alternatively, recordings can be made using extremely fine tungsten or glass electrodes to measure the activity of individual olfactory neurons (single-cell recording, or SCR) (Figure 7). These electrophysiological preparations can be placed in parallel with a gas chromatographic (GC) detector for assessment of activity of components collected from the air above an insect releasing the pheromone (Figure 8). Indeed, most of the pheromones described, after the pioneering studies on the silk moth and honey bees, have been identified by coupled GC–EAG or GC–SCR. A coupled GC–EAG trace is shown for the identification of sex pheromone components of the rosy apple aphid, *Disaphis plantaginea*[7] (Figure 9).

Many pheromones are now known to contain a number of components and there are often olfactory neurons specific for each component. Thus, for the two components of many aphid sex pheromones, recordings can be made from two neurons responding specifically to the individual components, with typically a low amplitude cell for the lactone and a higher amplitude cell for the lactol. Only when both olfactory neurons are stimulated does the aphid central nervous system respond by causing upwind flight, leading eventually to the location by the male of the pheromone-releasing female. Sometimes, the males can be somewhat overenthusiastic (Figure 10).

Use

A major driving force in the investigation of insect sex pheromones has been the control of horticultural and agricultural pests, and also the control of insects acting as vectors for pathogens affecting farm animals and human beings. Clearly, the high specificity of insect sex pheromones would lend itself towards more targeted, and thereby safer, approaches in terms of human health, but also to conserving the environment and species diversity. However, at the same time, this renders the use of sex pheromones more expensive than the deployment of broad spectrum toxicants in pest control. One further advantage of sex pheromones is that they can be deployed to exploit beneficial insects, not only in the management, for example, of honey bees, but also in the attraction of predators and parasites of pest insects. Indeed, the aphid sex pheromones are already

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being developed for their role as foraging stimulants, or 'attractants', with the parasitic wasps attacking aphids[12]. The cost and sustainability of sex pheromone production can be reduced by botanical production and the development of new industrial crops for generating the components directly, or as cheap precursors which can be converted into the pheromone using 'green chemistry'. In addition to the example of the sandfly pheromone mentioned above[4], the mosquito oviposition pheromone[5] and the aphid sex pheromones[12] can also be produced via industrial crops far more cheaply than by *de novo* synthesis using non-renewable feedstock. In 1985, Pickett[13] suggested that pheromones for pest insects could be produced by genetically modified crop plants, and, although not yet proven for sex pheromones, we have demonstrated aphid repellency and increased foraging by an aphid parasitoid using the model plant thale cress, *Arabidopsis thaliana*, genetically modified to release the aphid alarm pheromone[14]. We are now attempting to do the same in wheat and other crops.

Whether the sex pheromone is synthesized conventionally, produced via plant products or released directly by crop or companion plants, there are various ways in which these agents can be deployed[15]. A widely used approach is 'lure and kill', in which a pheromone attracts the insect to a site where a specific contact insecticide, or a mechanical or biological approach to killing the pest, can be employed. In the case of a biological control agent, a pathogen specific to the pest can be brought into contact with the insect when it enters a trap incorporating a pheromone lure. The pathogen can then either kill the pest directly or be transferred thereby to the wider population. This latter system, comprising autodispersion of a pathogen by means of the pheromone, can overcome the problem that many pheromones have in attracting the less relevant sex of the species.

Examples have been given where the behaviour of the female, and even the gravid female, is controlled by a sex pheromone. Release of pheromones from point sources can disrupt mate location by the male and this effect could be delivered by a genetically modified crop. Mating disruption is already widely used, but is achieved most effectively over large areas by incorporating geographic isolation so that mated females do not confound the process by entering from other regions. Some attempts have been made to cause destructive interspecies mating by releasing a pheromonal component that converts the natural release of a sex pheromone from a pest species into that of another, and prevalent, species. However, Baker has shown that males can differentiate between single sources of pheromonal component mixtures and point sources of individual components[16].

Insect sex pheromones can be valuable monitoring tools for more effective deployment of pesticides or biological control agents, but there will be problems if only the less important sex of the pest population is measured.

Future

The continuing identification of insect sex pheromones is providing new control and monitoring tools across an ever-widening range of insect species, incorporating newly emerging pests as a consequence of climate change. Advances in the sensitivity of analytical chemical and particularly spectroscopic equipment, and also electrophysiological techniques, will make a great contribution. Equipment developments, particularly in mass spectrometry, will allow exploitation of insect pheromones in monitoring by directly recording the presence of pests, via their pheromones, without the attendant problem of catching insects at a life cycle stage far removed from the stage at which damage is done. A wider understanding of the mechanisms by which components of insect olfaction recognize pheromonal molecules will allow the construction of new pheromone detection systems. Because of the robust nature of the proteins involved, we believe that the PBPs, and olfactory ligand binding proteins generally, show considerable promise in this respect and can already be produced, pure, on a large scale by expressing the genes in fermentation organisms[17].

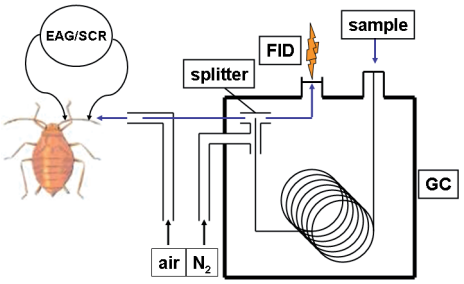

Figure 8. Coupled gas chromatography–electrophysiology. FID, flame ionization detector

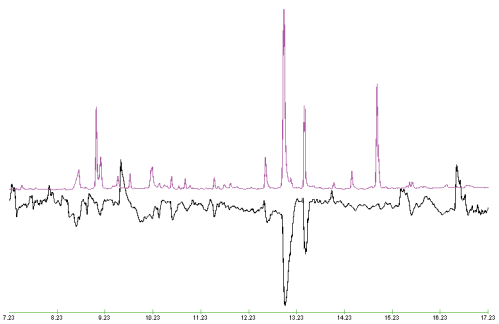

Figure 9. Coupled GC–EAG trace of responses of male *Disaphis plantaginea* to volatiles released by conspecific females. Upper trace: FID response; lower trace: antennal response

Figure 10. Overenthusiastic male vetch aphids, *Megoura viciae*, surround a sexual female

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The study of insect sex pheromones, and the now established interaction between insects and ourselves[18], will facilitate a greater understanding even of human pheromones that may be used in menstrual cycle regulation and in the detection of medical problems and perhaps lifestyle or psychological issues. ■

Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. This work was in part supported by the United Kingdom Department for Environment, Food and Rural Affairs.

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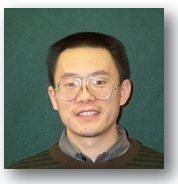

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