
Pheromone Communication in Social Insects

Ants, Wasps, Bees, and Termites

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Pheromone Communication in Social Insects: Sources and Secretions

Johan Billen and E. David Morgan

Introduction

Although the general descriptions of insect anatomy and structure as found in entomological textbooks equally apply to social insects, the development of the exocrine apparatus in the latter clearly distinguishes them from solitary insects (Figure 1.1). An extremely diverse array of exocrine glands is found in all social insects, with 63 different glands described so far (39 if only considering the Formicidae, 21 for the Apidae, 14 for the Vespidae and 11 for the Isoptera) (Billen, 1994). Several of these glands serve 'individual' functions as the source of digestive enzymes or lubricant compounds, although the majority has a clear function related to the social organization of the colony (Hölldobler and Wilson, 1990). Some have a role in producing building material like the wax glands in bees, others secrete antibiotics like the metapleural glands of the ants, or elaborate sticky defensive substances like the frontal glands of some termite species. A major social function of exocrine glands, however, is the production of pheromones, for which many glands have become specialized.

The study of exocrine glands in general, and of pheromone producing glands in particular, has long been faced with a number of practical difficulties. Because of their ectodermal origin, all exocrine glands are associated with cuticle, which has put considerable constraints on the study of gland structure. The development of plastic embedding techniques has allowed much better sectioning conditions, which have resulted in a clearer picture of the structural organization of the exocrine system compared with the information obtained from paraffin

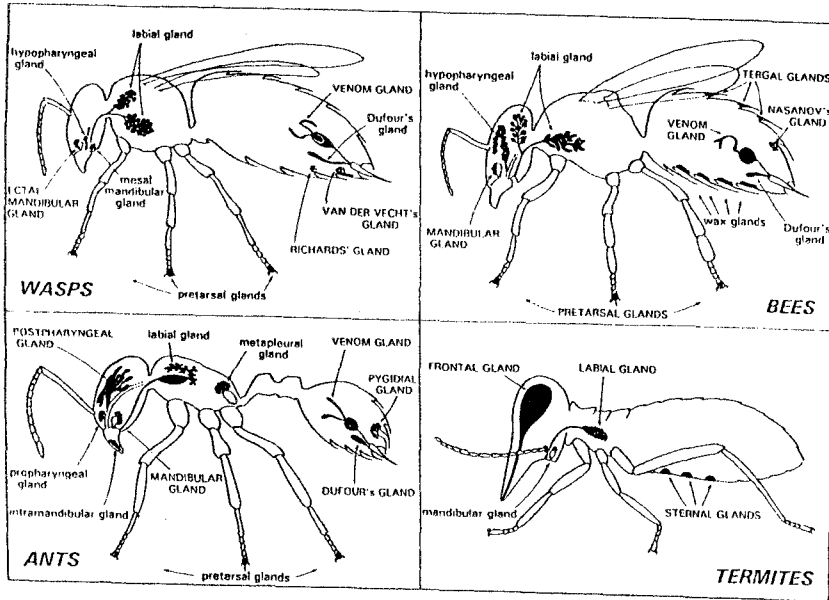


FIGURE 1.1 Schematical profile drawings showing the commonly found exocrine glands in wasps, bees, ants and termites. Glands with a pheromonal function are indicated with capital lettering.

sections. The small size of insects, on the other hand, for long represented a considerable drawback in our chemical understanding of the glandular secretions. The availability of more sophisticated equipment and techniques in the past decades has made analysis at the nanogram level possible, thus resulting in the identification of many glandular products. In this chapter, we focus on the structural and chemical complexity of the pheromone producing exocrine glands of the social insects.

Structural Organization of Pheromone-Producing Glands

Exocrine glands can be classified into two major types according to the structural organization of their secretory cells (Billen, 1991), corresponding with types I and III in the pioneer paper on insect glands by Noirot and Quennedey (1974). Glands with secretory cells of the first type (type I) are directly derived from the tegumental epidermis as is reflected in their epithelial organization. The secretory cells form a monolayered epithelium either as part of the external body tegument through which they directly discharge their secretory products (e.g.

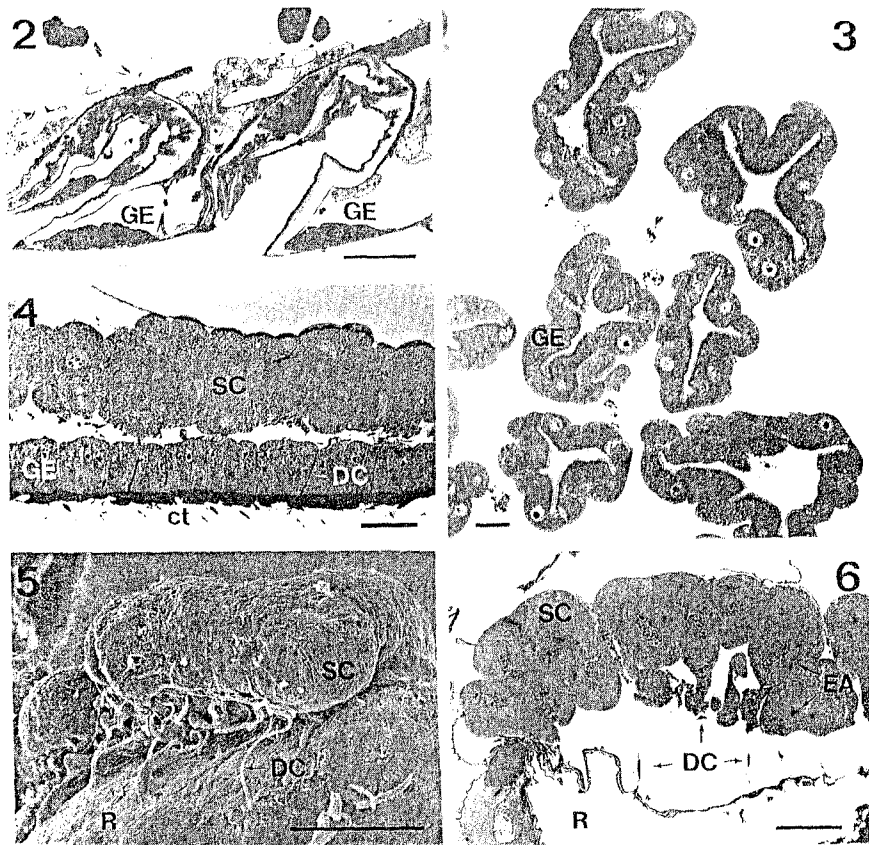
the trail producing sternal glands of termites, Figure 1.2), or as the lining of an internalized reservoir where secretion can be temporarily stored (e.g. the postpharyngeal gland in ants, that is involved in recognition mechanisms, Figure 1.3).

More complicated are the glands with secretory cells of the second type (type III according to Noirot and Quennedey, 1974), where the gland is formed by a variable number of bicellular units, each comprising a secretory cell and a duct cell. Each unit of this gland type originates via tetrad formation by an epithelial stem cell through two mitoses, and subsequent differentiation of one daughter cell into a slender duct cell and one into the secretory cell, while the remaining two daughter cells degenerate (Sreng and Quennedey, 1976). The contact area between the remaining duct cell and secretory cell is known as the 'end apparatus', that represents a specialized region to allow secretory products to find their way to the outside. Glands of this type can equally open, by means of their duct cells, either directly through the tegument (e.g. the Van der Vecht gland in wasps, which is the source of repellent substances, Figure 1.4), or into a reservoir (e.g. the mandibular gland, which is the source of alarm substances in many social insects, Figures 1.5,1.6). The reservoir in these glands is formed by flattened, generally non-secretory epithelial cells.

Secretory cells of type II were described as basally located epithelial cells without contact with the apical cuticle, as is sometimes found in sternal glands of termites (Noirot and Quennedey, 1974). They probably do not represent another secretory cell type, but are to be considered as oenocytes (Noirot and Quennedey, 1991).

Many glands are common to all social insects and occur in the queens, workers and males, as is for example the case for the mandibular and salivary glands. Apart from these standard exocrine glands that are also found in solitary insects, some glands represent neof ormations that are characteristic for the family, subfamily, genus or even for the species. In this way, wax glands are characteristic for the Apidae, and are not found elsewhere. Likewise, Van der Vecht's gland and Richards' glands are specifically found in wasps, while ants are characterized by the presence of postpharyngeal, metapleural and pygidial glands. The exocrine glands of the heterometabolous termites cannot be homologized with the glands of the Hymenoptera, and therefore can be considered as rather specific.

The number of known exocrine glands in social insects becomes more and more impressive, and reflects the evolution of sectioning techniques. Several hitherto unknown glands have recently been described (Figure 1.7), although their function often still remains unknown so far. Because of this steadily increasing variety of exocrine



FIGURES 1.2-1.6 Semi-thin sections through various types of pheromone producing glands. 1.2 - epithelial glands directly opening through the tegument (sternal glands of the termite *Schedorhinotermes lamanius*, scale bar 50 μm); 1.3 - epithelial gland with reservoir (postpharyngeal gland of the ant *Cataglyphis niger*, scale bar 20 μm); 1.4 - bicellular units opening directly through the tegument (Van der Vecht's gland of a worker of the wasp *Polistes annularis*. Also note associated epithelial gland, scale bar 20 μm); 1.5 - scanning micrograph showing the mandibular gland of a worker of the ant *Formica sanguinea* with bicellular units opening into common reservoir (scale bar 50 μm); 1.6 - bicellular units opening into common reservoir (mandibular gland of a worker of the bumblebee *Bombus pratorum*, scale bar 50 μm). ct: cuticle, DC: duct cell, EA: end apparatus, GE:glandular epithelium, R reservoir, SC: secretory cell.

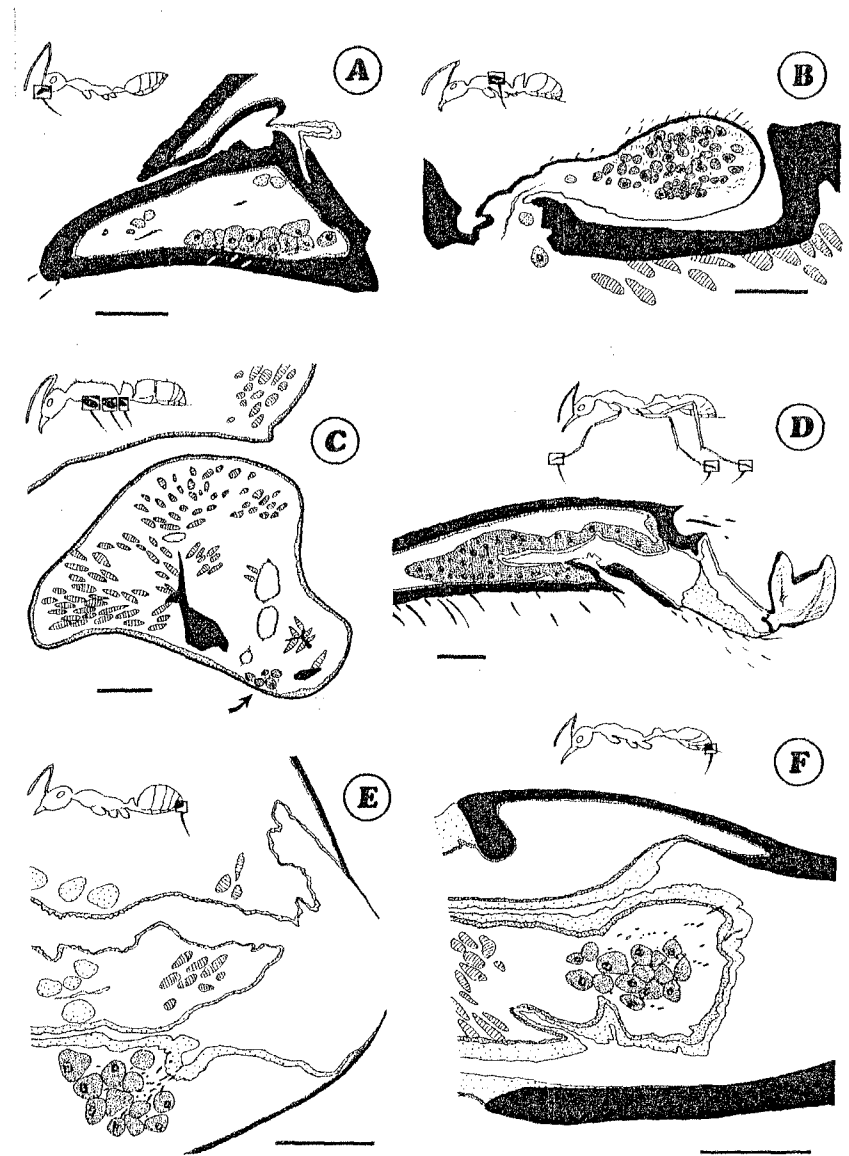


FIGURE 1.7 Examples of recently discovered "new" exocrine glands in the Formicidae (A. intramandibular gland; B. gemma gland *Diacamma*; C. coxal glands *Pachycondyla*; D. pretarsal glands; E. cloacal gland *Cataglyphis*; F. sting bulb gland *Myrmecia* and *Nothomyrmecia*). Scale bar 100 μm .

glands, a clear designation of the various glands is necessary. The terminology 'sternal glands', for instance, includes at least 6 different glands in the Formicidae, and also in the wasps, a plethora of glands associated with the abdominal sternites is found (Downing, 1991). Similarly, recent research has revealed the existence of a much broader variety of 'leg glands' than was originally thought.

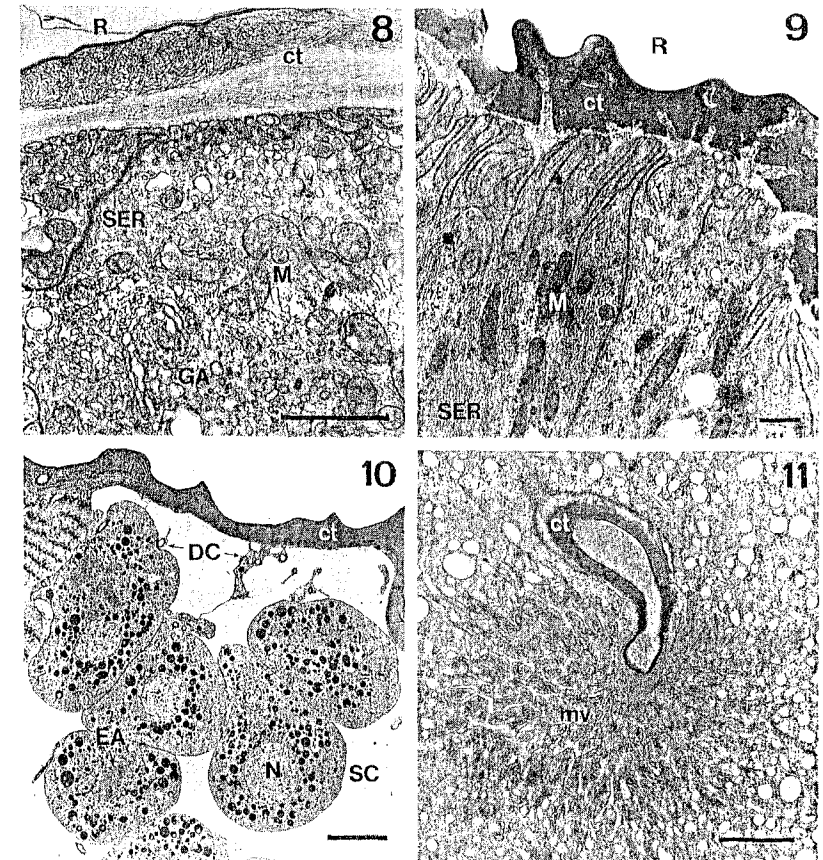
Ultrastructure of Pheromone-Producing Glands

Glands are structures specialized for the storage and the emission, spreading or evaporation of their secretory products (Noirot and Quennedey, 1991). This implies the glandular cells display a specific capacity for the uptake of precursor molecules and the subsequent biosynthesis of the secretory products, and can effectively regulate the release of secretion.

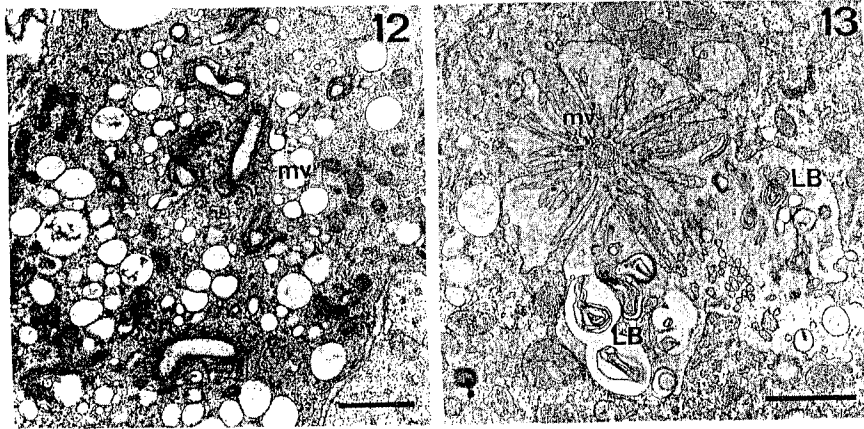
Uptake of precursor substances in general is facilitated by the invaginations of the cell membrane that increase the surface. This is found for the basal cell membrane of the epithelial glands and the peripheral cell membrane of the secretory cells of the bicellular unit glands, which in both cases represent the area that is in contact with the haemolymph, from where precursor molecules are obtained. The cytoplasm of the secretory cells of pheromone producing glands is generally characterized by the presence of a well developed Golgi apparatus and smooth endoplasmic reticulum (Figs. 1.8,1.9). This is in agreement with the production of non-proteinaceous and low molecular weight substances, which are characteristic of many pheromone-producing glands (Noirot and Quennedey, 1974; Billen, 1991). Another common feature in the cytoplasm of pheromone producing glands is the occurrence of various inclusions, of which lamellated bodies are the most conspicuous (Figure 1.13). These probably correspond with secretory material, as may be concluded from autoradiography studies in vertebrates, where lipid secretions were found to occur as lamellar inclusions within the glandular cells (Boudreau et al., 1983; Hidalgo et al., 1985). Mitochondria generally are also abundant among the cellular organelles in pheromone producing glands.

Once the secretory products are formed via the metabolic machinery of the glandular cell, they are ready for transport to the outside for immediate release or for temporary storage in a reservoir. This pathway, however, produces for these molecules a double barrier as they have to cross both the cell membrane of the secretory cell and the cuticle. In epithelial glands, the apical cell membrane is generally differentiated into microvilli that create a considerable increase of surface (Figs. 1.8,1.9). Tubular extensions of smooth endoplasmic

reticulum may continue into the microvilli and thus bring the secretory products straight to the site of release (Billen, 1991; Figure 1.8). The cuticle overlying the epithelium may either be permeable for the secretory molecules to diffuse through (Figure 1.8), or may display conspicuous cuticular pores (Figure 1.9; see Blomquist et al., this book). Glands formed by bicellular secretory units have the end apparatus in



FIGURES 1.8-1.11 Electron micrographs illustrating the ultrastructural organization of pheromone-producing gland cells. 1.8 - apical region in the postpharyngeal gland of a callow worker of *Formica sanguinea* (scale bar 1 μ m); 1.9 - apical region in the tibial gland of *Crematogaster scutellaris* showing cuticular pores (scale bar 1 μ m); 1.10 - secretory cells of the pygidial gland of the amazon ant *Polyergus rufescens* (scale bar 10 μ m); 1.11 - contact area between duct cell and secretory cell in Nasanov's gland of a *Apis mellifera carnica* worker, showing end apparatus with regular microvillar organization (scale bar 1 μ m).



FIGURES 1.12-1.13. 1.12 - Branched end apparatus with blunt microvilli in the venom gland of a *Vespula vulgaris* worker (scale bar 1 μm); 1.13 - end apparatus with distorted microvillar pattern and multilamellar bodies, venom gland *Nothomyrmecia macrops* (scale bar 1 μm) ct: cuticle, DC: duct cell, EA: end apparatus, GA: Golgi apparatus, LB: lamellar bodies, M: mitochondria, mv: microvilli, N: nucleus, R: reservoir, SC: secretory cell, SER: smooth endoplasmic reticulum.

each unit to assure efficient release of the secretory products from the glandular cells, from where associated duct cells carry these products to the reservoir or to the outside (Figure 1.10). This specialized region is formed by an invaginated microvillar differentiation of the cell membrane of the secretory cell, surrounding a central cuticular canal that continues into the duct cell (Figure 1.11). The microvillar sheath again offers a considerable surface increase, while the cuticular lining of the end apparatus always shows pore canals. The lining cuticle considerably thickens where the end apparatus joins the duct cell, thus forming an efficient porous structure in the secretory cell and a discontinuous sheath in the duct cell to prevent leaking (Figure 1.11). The end apparatus often has a sinuous course in the secretory cells, while it may occur with many branches only in exocrine glands of the Vespidae (Delfino et al., 1979; Figure 1.12). The microvillar pattern may vary from an orderly arrangement (Figure 1.11) to very distorted (Figure 1.13). This appearance of extracellular space may create an additional storage capacity for the secretory cells, as was first documented by Bazire-Bénazet and Zylberberg (1979). Lamellar bodies often accumulate in these intermicrovillar spaces of the end apparatus (Figure 1.13), which supports their secretory nature. Also in epithelial glands, lamellar bodies can be found in association with the apical

microvilli and cuticle, from where they reach the reservoir or the outside (Billen, 1991).

The mechanisms that regulate the discharge of secretion are not very well known. Glands without reservoir release their secretion directly to the outside, and do not appear to have a direct control mechanism over their secretory activity. Glands with a reservoir often have muscle fibers surrounding the latter, contraction of which will result in discharge of secretion. In addition to the reservoir musculature, also the duct region may be equipped with a muscular supply that may open or close the duct, and so affecting the secretory activity of the gland, as is the case for the venom gland and Dufour's gland (Billen, 1982). The mandibular gland of bees (Nedel, 1960) and wasps (Hermann et al., 1971) appears to possess a muscular sphincter around its duct, although in ants we could not find any muscular equipment in association with the mandibular gland duct (Billen and Schoeters, 1994). Nervous control of the discharge of secretion by social insect glands is poorly understood. Innervation is fairly commonly found, although the physiological mechanisms remain unknown. A very complex 'multiple innervation' system has been described for the salivary glands of *Kaloterme*s, with mixed nerves containing up to 6 different categories of axons (Alibert, 1983).

Occurrence of Pheromone-Producing Glands

Pheromone producing glands are distributed over all regions of the social insect's body, with a location that is directly linked with their function. Alarm pheromones are primarily produced by the exocrine glands associated with the mandibles and sting, that are the defensive weapons par excellence. The mandibular gland has been reported to be the source for alarm substances in a wide variety of ants and bees (Maschwitz, 1964). In termites, the mandibles themselves also appear to be used as mechanical weapons (Deligne et al., 1981), although no pheromonal function could be attributed to their mandibular glands so far. Many termite species, however, possess an unpaired frontal gland, which opens on the anterior part of the head (Noirot, 1969). The gland is especially developed in the soldiers, and is known as an efficient ant repellent that also offers protection to the termites in their galleries (Kaib, 1985). The evolution of the hymenopteran ovipositor towards a sting equally resulted in a change of the venom and Dufour's gland, being the female accessory reproductive glands, from an initially reproductive function to a defensive role. The venom gland, which always releases its secretion through the sting, especially plays an important role in the alarm-defence system. The complex

compartmentalized organization of the venom gland, that comprises an internalized convoluted gland, may prevent self-toxication (Schoeters and Billen, 1995a,b). The Dufour's gland of wasps and bees opens ventrally of the sting, so that secretion is released into the oviduct (Billen, 1987). This anatomical organization may explain why a pheromonal function for Dufour's gland in these social insects is generally not known. In ants, on the other hand, both the venom and Dufour's gland open through the sting, which represents an excellent anatomical position for the production of trail pheromones.

The use of trail pheromones represents a commonly known communication system for terrestrial social insects like ants and termites. In termites, these are elaborated in the sternal glands. The variety of exocrine glands producing trail substances in the Formicidae is absolutely astonishing. At least 9 different abdominal glands may be involved, where their anatomical position allows efficient deposition of the active substances onto the substrate (Figure 1.14). Several of these glands open through the sting or the anal opening, or occur in association with the sternites. Also tergal glands may produce trail substances, however, although in this case their opening site is very close to the abdominal tip, as is found for the pygidial gland in some

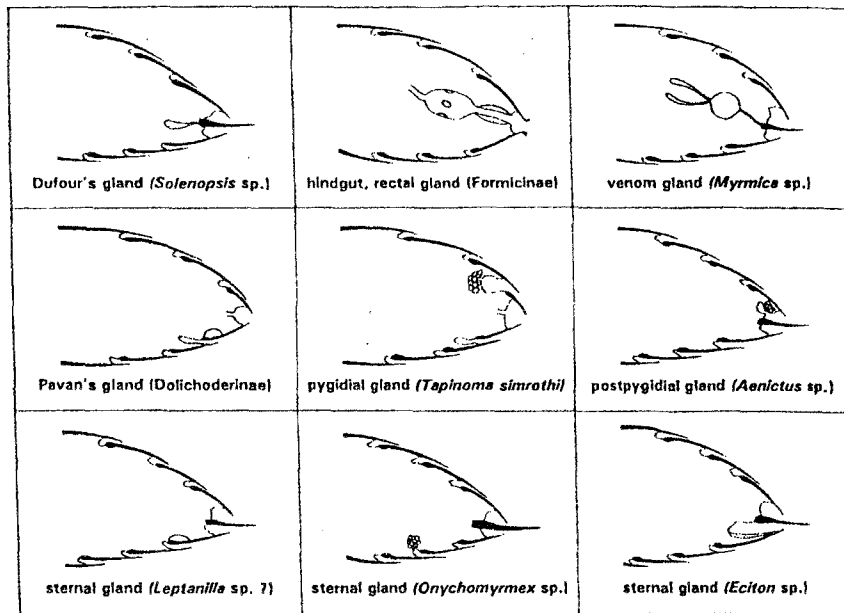


FIGURE 1.14 Schematical illustration of the various abdominal glands in ants that are known to produce trail pheromones.

dolichoderines (Simon and Hefetz, 1991) or the postpygidial gland in *Aenictus* army ants (Oldham et al., 1994a). Besides abdominal glands, the legs represent an obvious position for the eventual production and release of trail substances. Until recently, the tibial gland in *Crematogaster* (Figure 1.9; Leuthold, 1968; Billen, 1984) was reported as the only non-abdominal source for trail pheromones in ants. A combination of behavioural and morphological examination, however, has also revealed a trail marking function in some ponerine ants for the pretarsal glands (Hölldobler and Palmer, 1989) and for the newly discovered basitarsal glands (Hölldobler et al., 1992).

A gland that has very much come into focus recently is the postpharyngeal gland, that represents a unique exocrine structure for the Formicidae only. Although earlier papers generally attributed digestive functions to the postpharyngeal gland (Delage-Darchen, 1976), the finding that the chemical profile of the gland matches to that of the epicuticular hydrocarbons (Bagnères and Morgan, 1991) has raised the question on the role of this gland in nestmate recognition (Soroker et al., 1994; Vienne et al., 1995; Meskali et al., 1995). For this function, the postpharyngeal glands occupy an obviously suited position as their secretion can conveniently be exchanged via oral contact during grooming with nestmates.

Chemical Aspects of Pheromone-Producing Glands

In an attempt to reduce the various kinds of pheromones encountered in insects to some kind of order, they have been classified in various ways. That of Dethier et al. (1960) is heavily based on movement, and was intended for other kinds of semiochemicals in addition to pheromones. It classifies them as locomotory stimulant, arrestant, attractant, repellent, a stimulant (for feeding, mating, oviposition, etc.) and a deterrent (for similar behaviours). Another more widely used system divides pheromones first into primer pheromones and releaser pheromones (Wilson and Bossert, 1963). So few primer pheromones have as yet been identified, that they have not been further divided into categories. Most of the pheromones we know are releaser pheromones, and at present, these are usually divided into nine groups: sex; aggregation; dispersal; alarm; recruitment or trail following; territorial or home range; surface; funeral and invitation pheromones. For a detailed discussion, see Ali and Morgan (1990).

These categories were largely devised for simple types of behaviour, exemplified by the excitement and attraction of males by the female lepidopteran sex pheromones. Compared to the relative simplicity of the sexual attraction of many lepidopterans and dipterans, the

behaviour induced by pheromones of the social insects is intricate and relatively little understood. The potential complexity of social insect signalling is indicated by the great variety of glands described here that are capable of emitting pheromones even after making allowance for many exocrine glands which serve individual functions as indicated earlier.

Pheromone signals may be multicomponent in all insect orders and that is equally true for social insects, but the latter may have a blend of substances from more than one gland (Silverstein and Young, 1976; Hölldobler and Carlin, 1987; Hölldobler, 1995). For example, the trail pheromone of the ant *Messor bouvieri* is derived from both the poison gland (3-ethyl-2,5-dimethylpyrazine) and the Dufour gland (a mixture of C₁₃ to C₁₉ alkenes with one, two or three double bonds) (Jackson et al., 1991). Similarly, queen dominance in *Apis* appears to involve the tergal and pretarsal glands as well as the mandibular glands (Vierling and Renner, 1977; Lensky and Slabezski, 1981). Some pheromone blends may comprise a primer and a releaser component, as we have encountered in the trail pheromone of an *Aenictus* species (Oldham et al., 1994a) where methyl nicotinate acts as a primer substance which must be detected before ants can follow the releaser component methyl anthranilate. Neither substance is effective without the other but they do not have to be released at the same time.

The simple picture of a lepidopteran imago coming to maturity emitting or detecting pheromone, leading to fertilization, bears little resemblance to the situation in social insects where worker castes are normally separated from reproduction and may perform different tasks, dependant upon their size or age. Tengö et al. (1991) have recorded that the Dufour gland secretion of bumblebees varies with time of year. We have observed changes in the mandibular gland and Dufour gland secretions of the ant *Formica sanguinea* (Moens et al., 1990; Ali et al., 1988) as these workers grow older.

We have also found in *Atta sexdens rubropilosa*, a sharp variation in mandibular gland pheromone with size of workers (do Nascimento et al., 1993). There are numerous examples where there are distinct differences between worker and soldier caste, or between sexuals and workers.

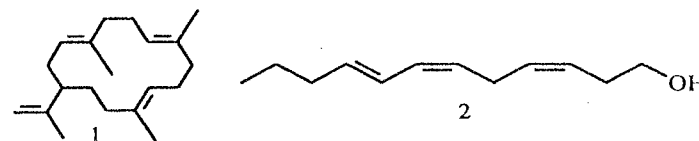
On the other hand, we have examples where different glands contain the same or very similar blends of substances. Bagnères and Morgan (1991) showed that the postpharyngeal gland of several ant species selected randomly contained the same hydrocarbon substances as found on the cuticle, which receives its hydrocarbons from the epidermal glands (Blomquist et al., Chapter 2, this book). Oldham et al. (1994b) have found the mixture of hydrocarbons on the cuticle of

some bumblebee species is the same as that found in the Dufour glands. The pheromonal function is understood in neither case.

Trail or Recruitment Pheromones

Among social insects, the trail following or recruitment pheromones are probably the most clearly defined because they still form a fairly small group. They have been reviewed from a chemical viewpoint by Morgan (1990). Ant trail pheromones have been reviewed by Attygalle and Morgan (1985) and Jackson and Morgan (1993).

The first of these to be studied were termite trail pheromones, in the 1960's when three substances were identified. The first to be isolated was Neocembrene A **1** (now (E)-6-cembrene A) from *Nasutitermes exitiosus* (Moore, 1966). Evidence at present suggests that termite trail pheromones are by no means species specific. This same cembrene is apparently used by *N. walkeri* and *N. graveolus* (Moore, 1966) and by



Trinervitermes bettonianus (McDowell and Oloo, 1984). (3Z,6Z,8E)-3,6,8-dodecatrienol **2** was first identified as the trail pheromone of *Reticulitermes virginiacus* (Matsumura et al., 1968; Tai et al., 1969).

There was then a long pause in the study of termites, but recently the subject has regained attention. Toroko et al. (1989) identified the same dodecatrienol as the pheromone of *Coptotermes formosanus*, and of *Reticulitermes speratus* (Toroko et al., 1991), while Laduguie et al. (1994) identified it in *Reticulitermes santonensis*, and Grace et al. (1995) have identified it in *R. hesperus*. These two compounds remain the only firmly identified trail pheromones of Isoptera.

The number of trail pheromones of ants that has been identified is relatively large. They are gathered together in Table 1.1 and Figure 1.15 (3 to 27). Some of them appear to be species-specific, others are widely distributed through several genera. The claim of identification of the trail pheromone of *Lasius fuliginosus* by Huwyler et al. (1975) has been dropped from the list since Quinet and Pasteels (1995) have shown the mixture of fatty acids is inactive for this species. Species specificity is probably imparted by other glandular secretions, such as the Dufour gland contents laid down at the same time. It is noteworthy how frequently the alkylpyrazines 4,13,14 occur among the Myrmicinae and that the polar (water soluble) compounds come from the venom

gland while the non-polar (lipophilic) compounds tend to come from the Dufour gland. Almost all the substances are volatile, with small molecules, and only geranylgeranyl acetate 22 and possibly hexadecenal 25 (relatively large molecules) and *N,N*-dimethyluracil 23 (relatively involatile) are exceptions to these rules so far. These substances are, in every case recorded, present only in nanogram quantities. Those in the venom glands are little more than impurities in the venom. The small quantities would make them very difficult to identify were it not for the relatively simple bioassays available. See also Vander Meer and Alonso, Chapter 7 this book.

There are numerous reports of spot-marking by male bumblebees close to good food sources, but these are essentially to attract females. Similar marking behaviour has been observed in stingless bees, using the mandibular gland. The mandibular pheromone of *Trigona*

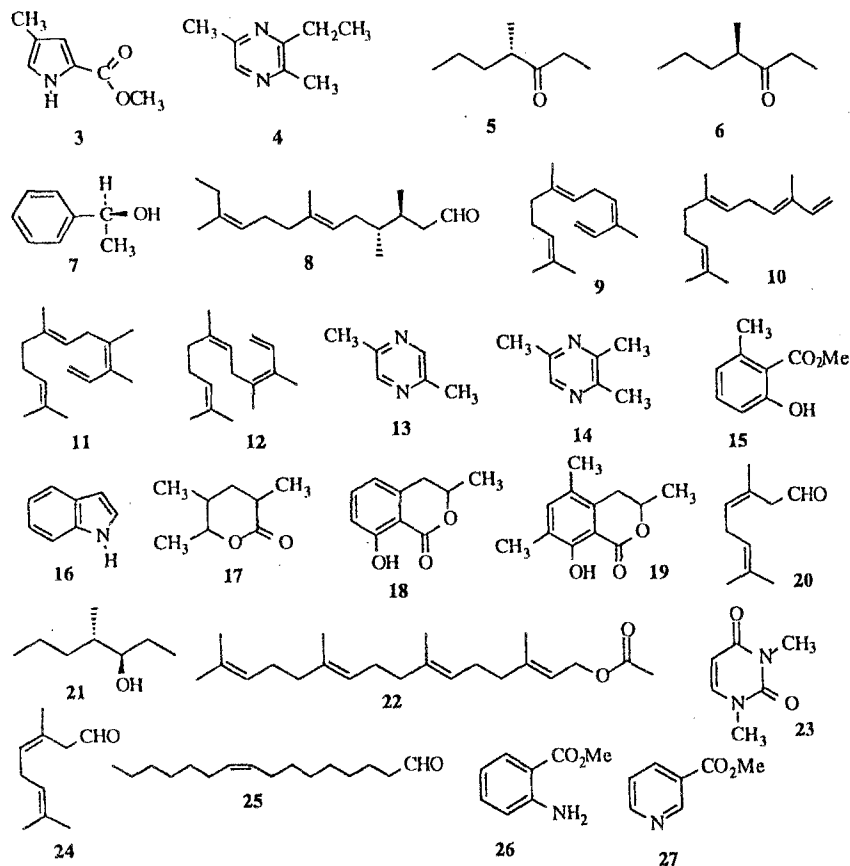


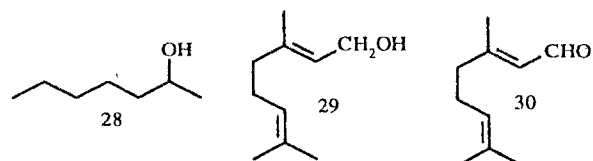
FIGURE 1.15 Trail pheromone substances of ants.

TABLE 1.1 Trail pheromone substances identified in ants.

SPECIES	COMPOUND ¹	SOURCE ²	NOTE/REFERENCE
Myrmicinae			
<i>Acromyrmex octospinosus</i>	M4MPC 3	VG	Robinson <i>et al.</i> 1974,
<i>A. subterraneus subterraneus</i>	M4MPC 3	VG	a/do Nascimento <i>et al.</i> 1994
<i>Aphaenogaster albisetosus</i>	4-Methyl-3-heptanone 5,6	VG	b/Hölldobler <i>et al.</i> 1995A
<i>A. cockerelli</i>	(R)-1-Phenylethanol 7	VG	Hölldobler <i>et al.</i> 1995a
<i>Atta cephalotes</i>	M4MPC 3	VG	Riley <i>et al.</i> 1974
<i>A. sexdens rubropilosa</i>	EDMP 4	VG	Cross <i>et al.</i> 1979
<i>A. sexdens sexdens</i>	EDMP 4	VG	Evershed and Morgan 1983
<i>A. texana</i>	M4MPC 3	VG	Tumlinson <i>et al.</i> 1972
<i>Manica rubida</i>	EDMP 4	VG	Attygalle <i>et al.</i> 1986
<i>Messor bouvieri</i>	EDMP 4	VG	c/Jackson <i>et al.</i> 1989, 1991
<i>Monomorium pharaonis</i>	Faranal 8	DG	Ritter <i>et al.</i> 1977
<i>Myrmica rubra</i>	EDMP 4	VG	d/Cammaerts-Tricot <i>et al.</i> 1977
<i>M. lobicornis</i> , <i>M. lonae</i> , <i>M. ruginodis</i> , <i>M. rugulosa</i> , <i>M. sabuleti</i> , <i>M. scabrinodis</i> , <i>M. schencki</i> , <i>M. sulcinodis</i>	EDMP 4	VG	Evershed <i>et al.</i> 1982
<i>M. gallieni</i> , <i>M. speciosides</i>	EDMP 4	VG	Jackson <i>et al.</i> 1989
<i>M. sp. nova</i> from Idaho	EDMP 4	VG	Jackson <i>et al.</i> 1991
<i>Pheidole pallidula</i>	EDMP 4	VG	e/Ali <i>et al.</i> 1988
<i>Solenopsis invicta</i>	(Z,E)- α -farnesene 9 (E,E)- α -farnesene 10 (Z,E)-homofarnesene 11, (E,E)-homofarnesene 12 homoselenine	DG	Vander Meer <i>et al.</i> 1981, 1988 f/Vander Meer, pers. comm.
<i>Tetramorium caespitum</i>	EDMP 4, DMP 13	VG	Attygalle and Morgan 1984
<i>T. impurum</i>	Methyl-6-methylsalicylate 15	VG	Morgan and Ollett 1987
<i>T. meridionale</i>	Indole 16	VG	g/Jackson <i>et al.</i> 1990
Formicinae			
<i>Camponotus herculeanus</i>	2,4-dimethyl Mellein 18	Hindgut	Bestmann <i>et al.</i> 1992
<i>Formica rufa</i>	(R)-8-hydroxy-3,5,7-trimethyl dihydro-isocoumarin 19	Hindgut	Bestmann <i>et al.</i> 1992
<i>Lasius niger</i>			
Ponerinae			
<i>Leptogenys diminuta</i>	(3R,4S)-4-methyl-3-heptanol 21	Pyg	g/Attygalle <i>et al.</i> 1988, Steghaus-Kovac <i>et al.</i> 1992
<i>Megaponera foetens</i>	<i>N,N</i> -dimethyluracil 23	Pyg	Janssen <i>et al.</i> 1995
<i>Pachycondyla marginata</i>	Citronellal 24	Pyg	h/Hölldobler <i>et al.</i> 1995b
Dolichoderinae			
<i>Linepithema humile</i>	(Z)-9-Hexadecenal 25	PavG	Cavill <i>et al.</i> 1979, 1980
Aenictinae			
<i>Aenictus sp. nova</i>	methyl anthranilate 26, methyl nicotinate 27	Postpyg	i/Oldham <i>et al.</i> 1994a

¹For structures, see Fig. 1.15. ²VG is venom gland, DG is Dufour gland, Pyg is pygidial gland, PavG is Pavan's gland and Postpyg is postpygidial gland. a=1.2 ng/ant; b=8:2 mixture of *S* and *R* enantiomers; c=Dufour gland is more active; d=5 ng/ant; e=other unidentified substances are active; f=structure not yet complete; g=small quantities of DMP 13, TMP 14 and EDMP 4 also present and active; h=*C. ligniperda*, *C. vagus*, *C. pennsylvanicus* and *C. socius* followed same substance; g=cis-isogeraniol 20 acts as a recruitment pheromone, Attygalle *et al.* 1991; h=isopulegol may act as a synergist; i=methyl nicotinate is a primer, required either before or with methyl anthranilate.

subterranea is dominated by geranial and neral (Blum et al., 1970) but the necessary proof that these are the pheromone is lacking. A number of volatile substances are found in the mandibular glands of *Trigona spinipes*, of which only 2-heptanol **28** successfully marked trails that were followed by *T. spinipes* workers.

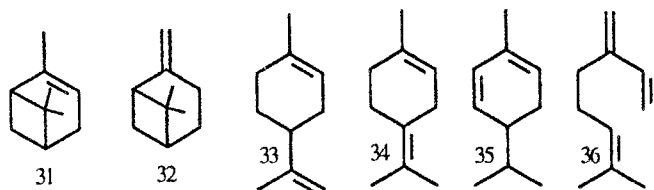


The Nasanov gland in *Apis mellifera* is a well recognized source of a marking pheromone, consisting of geraniol **29** and geranial **30** and other minor related components (Pickett et al., 1980). No other trail of marking pheromones of *Apis* species, nor of any wasps have yet been identified.

Alarm Pheromones

Alarm pheromones are a rather ill-defined class because it is not easy to distinguish or define alarm in a bioassay. The effect might as correctly be called alerting, dispersing or attracting in some of the bioassays recorded and many substances in a concentrated form, placed near a group of insects can agitate or alarm them (but see Vander Meer and Alonso, Chapter 7 this book).

From early work on nasute termites, it was learned that large amounts of monoterpenes (α -pinene **31**, β -pinene **32**, limonene **33**, terpinolene **34**, α -phellandrene **35** and myrcene **36**) are present in the frontal gland secretion of soldiers, in addition to the rapidly oxidizing sesquiterpenes which immobilize predators. It was suggested by Moore (1968) that the monoterpenes may function as alarm pheromones. It would appear that this idea has not been followed up by any recent experimental work.



Hölldobler and Wilson (1990, p. 263,264) have assembled a table of those substances identified as alarm pheromones of ants, together with

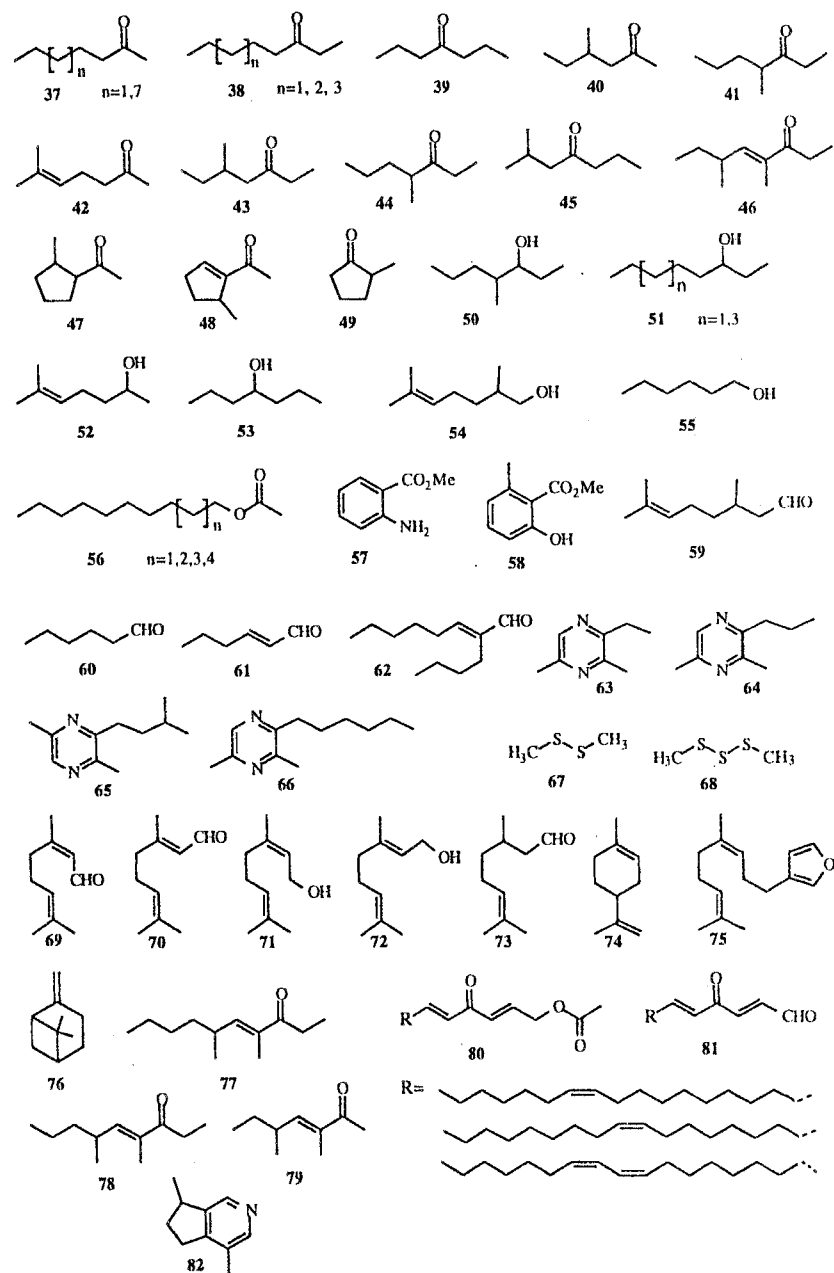
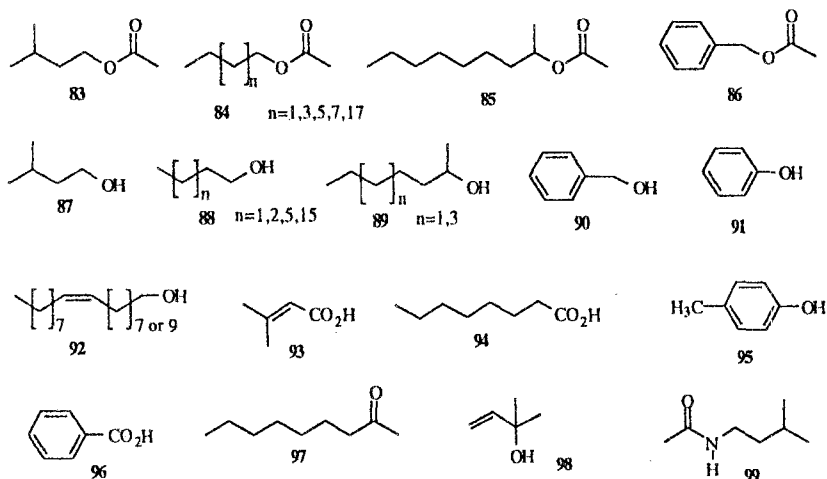


FIGURE 1.16 Alarm pheromone substances in ants.

their glandular sources. The data is not reproduced here since it is comparatively up-to-date and readily available. The list contains 16 ketones 37-49, 7 alcohols 50-55, 6 esters 56-58, 4 aldehydes 59-62 and 4 alkylpyrazines 63-66, together with dimethyl disulphide 67 and trisulphide 68, 8 terpenes 69-76, formic acid, decane and undecane (Figure 1.16). The mandibular gland is the commonest source, followed by the pygidial gland. Although mandibular gland secretions are generally thought to be alarm substances, Cammaerts and Mori (1987) found that the 3-octanol (38, n=1) of *Myrmica* mandibular glands was an attractant or arrestant, rather than causing alarm. There is no correlation between type of compound and glandular source, except that the cyclopentyl compounds 47-49, which are related to iridoids, come from the pygidial gland of a dolichoderine. Three additions are necessary to the list of Hölldobler and Wilson (1990), first, the unusual group of compounds 77-79, represented by manicone 46, in the mandibular glands of *Manica rubida* (Bestmann et al., 1988), and secondly, the unusual contact poison and alarm pheromone 80, 81 from the Dufour glands of *Crematogaster scutellaris* (Pasteels et al., 1989). Thirdly, Janssen et al. (1995) recently found that actinidine 82 from the pygidial gland of *Megaponera foetens* stimulated workers to leave the nest and possibly functions as an alarm pheromone.

Alarm behaviour was long recognized in bees and the pheromone elicits an easily recognized response. The composition of the secretion, from the Koschevnikov's gland in the sting apparatus presents more of a problem. The principal component was soon identified as isopentyl acetate 83 (Boch et al., 1962) but another 22 components have been subsequently identified, including 7 acetate esters 84-86, 11 alcohols 87-



90, 3 acids 93, 94, 96 and phenol 91 and p-cresol 95, identified either by Blum (1982) or by Pickett et al. (1982). 2-Heptanone from *A. mellifera* worker mandibular glands is widely quoted as an alarm pheromone, but Vallet et al. (1991) have shown that it does not induce alarm and claim that it is a forage marking pheromone.

Only one alarm pheromone from a stingless bee has been identified certainly. The mandibular secretion of *Trigona pectoralis* contains 2-heptanol, 2-heptanone, 2-nonanol and 2-nonanone. The 2-nonanone 97 alone released attacking response (quoted in Free, 1987; without source). Veith et al. (1984) have identified the major component of the alarm pheromone of the hornet *Vespa crabro* as 2-methyl-3-buten-2-ol 98. N-3-Methylbutylacetamide 99 has been recognized as an alarm pheromone from the venom sac in two species of wasps, *Vespula squamosa* (Heath and Landolt, 1988) and *Vespula maculifrons* (Landolt et al., 1995).

Sexual Pheromones

The trail pheromone substance 3,6,8-dodecatrien-1-ol (2) already described as a trail pheromone of termites from the sternal gland, has been suggested to be also the sexual attractant pheromone of the termites *Pseudacanthotermes spiniger* (Bordereau et al., 1991) and *Reticulitermes santonensis* (Laduguie et al., 1994). *P. spiniger* has ten times more of the substance in the glands of alate females than of males, and more than in the glands of workers.

Although a number of substances have been identified in the mandibular glands of sexual ants, where these are different from the substances found similarly in workers, we are not aware of any examples where these have definitely been shown to act as sexual attractant pheromones. On the other hand there is plenty of evidence that they exist and for their origins. The difficulties of bioassays have held back their isolation.

By far the best understood of the sexual pheromones of social insects is the queen substance of honeybees. By a fortunate chance the queen substance used for "queen control" inside the nest is also the substance used by virgin queens to attract drones for mating. (E)-9-Oxodec-2-enoic acid (9-ODA) 100 was identified in queen mandibular glands by Callow and Johnston (1960) and Barbier and Lederer (1960). The pure 9-ODA is not as attractive to the drones as whole mandibular glands. Later Callow et al. (1964) identified 13 further components from crushed heads of queens, to which Barbier added methyl p-hydroxybenzoate 101. Bioassay of these showed activity only in 9-ODA and (E)-9-hydroxydec-2-enoic acid 102, 103, a secondary component in the gland.

active alone but the blend was active at as little as 10^{-7} queen equivalents (Slessor et al., 1988). Both isomers of the hydroxydecanoic acid are necessary to give highest activity. On the other hand, Velthuis (1985) has shown that the queen pheromone is a product of the tergal glands and the tarsal or Arnhard glands, in addition to the mandibular glands. Worker ovary development is normally inhibited by the queen. It is noteworthy that in queenless colonies the worker ovaries are inhibited from development by the presence of larvae. Arnold et al. (1994) have shown this inhibition is caused by methyl and ethyl esters of the common fatty acids on the larval cuticle. They describe it as a primer brood pheromone. In ants, several categories of 'brood pheromones' have been defined that are involved in brood discrimination, but the chemical nature of these could not yet be identified (Vander Meer and Morel, 1988).

Home Range Marking

There are territorial social insects that defend a territory marked with their glandular secretions. To demonstrate that secretion is deposited is not to conclude it is the necessary pheromone. There are also home range pheromones that mark a territory without implying defence. Cammaerts has shown that first foragers of *Myrmica rubra*, *M. rugulosa* and *M. schencki* mark territories with their Dufour glands so that subsequent foragers move more quickly over areas marked with their own secretion (Attygalle et al., 1983a). The secretion of *M. rubra* consists of C_{13} to C_{19} hydrocarbons with a small amount of (Z,E)- α -farnesene (9) and two higher homologues. That of *M. rugulosa* consists of almost equal proportions of the terpenes and the same hydrocarbons and the *M. schencki* Dufour gland contains almost only terpenes (Attygalle et al., 1983b). Recent work on the desert ant *Cataglyphis niger* revealed that secretion from the cloacal gland, which is a common exocrine structure in the genus *Cataglyphis*, acts as a home marking pheromone (Wenseleers et al., 1996).

This brief review has, of necessity, confined itself to identified pheromone compounds and excluded venoms, defensive compounds and secretions which may serve to maintain the exoskeleton in a healthy and waterproof condition. The variety of compounds used as pheromones and their various biosynthetic origins are striking. But it is also noteworthy that the same odorous compounds turn up frequently in insects, and sometimes in plants, serving different functions.

Concluding Remarks

The exocrine system in social insects is extremely well developed. Thanks to the modern sectioning techniques, new glands will probably continue to be discovered, and thanks to micro-chemical techniques, the composition of more secretions will be identified. Unraveling their respective functions will remain a major challenge for the entomologist. Pheromonal communication, as illustrated in this book, will need further and more careful study, both of the contribution of the active substances of individual glands, as well as of the synergistic effects of multicomponent pheromones, that may occur as sister components within a particular gland or that find their origin in different glands. Attention must also be directed to investigate the effect on pheromonal messages of acoustic and tactile communication cues. In a rapidly advancing subject we can expect many more exciting discoveries ahead.

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