Physiological Entomology (2009) **34,** 1–17 DOI: 10.1111/j.1365-3032.2008.00658.x

REVIEW ARTICLE

Trail pheromones of ants

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Abstract. The study of trail laying, recruitment of workers and trail-following by worker ants comprises a co-operative study of entomologists and chemists that has resulted in the identification of the chemical nature of such pheromones in many species of five subfamilies of ants. These pheromones may comprise a single compound or, in one exceptional case, a blend of as many as 14 compounds, they may come from a single gland, or in some cases, a combination of two glands. They may be peculiar to a single species or may be shared by a number of species. They exist in the glandular secretion in nanogram to picogram quantities and are detected by workers in minute amounts on a trail. The present state of knowledge of these pheromones and their chemical structures is reviewed. Suitable bioassays and odour perception are discussed and the stereobiology of a few examples is considered.

Key words. Bioassay, chemical identification, exocrine glands, odour perception, recruitment, stereobiology, trail pheromone.

Introduction

Any observant person cannot but marvel at the sight of a column of ants moving back and forth along a trail between a source of food and their nest. The ability to exploit a food source in this way is one of the marks of social organization. Wilson (1971) said that the odour trail system is the most elaborate of all known forms of chemical communication. Darwin (1871), 100 years before Wilson, on considering the abilities of ants, had observed that '… yet their cerebral ganglia are not so large as a quarter of a small pin's head … yet the brain of an ant is one of the most marvellous atoms of matter in the world'. Taking these statements together, it is not surprising that the use of foraging trails by ants has attracted so much curiosity and investigation.

Much of our knowledge of behaviour of ants in foraging and its variation between species has been summarized by Hölldobler (1978), by Brian (1983) in his chapter on 'Foraging in groups' and also by Hölldobler & Wilson (1990) in their monumental book. The more theoretical studies of ant behaviour on trails by Deneubourg and others (Beckers *et al.*, 1989, 1992; Fourcassie & Deneubourg, 1994; Jeanson *et al.*, 2003; Sumpter & Beckman, 2003; Dussutour *et al.*, 2006; Jackson & Chaline, 2007; Montgomery *et al.*, 2008) are beyond the scope of this review.

Trail laying has been defined as a field activity in which an insect marks a route with scent or odour traces such that other insects of the same community are able to follow it (Sudd, 1959). The process of creating a trail begins with a scout ant finding food. After it has fed, it deposits a trail of chemicals as it returns to the nest. Inside or just outside the nest, it attracts other workers in a variety of ways that are not always understood. It may include antennal contact, regurgitation, jerking movements or the emission of another odour that recruits workers to leave the nest. It has become clear that this act of recruitment can be an integral part of the overall chemical process of trail-following. Wilson (1971: 548) defines recruitment as communication that brings nestmates to some point in space where work is required. The recruited workers then follow the trail to the food source. Workers returning laden with food re-enforce the trail. When the food is exhausted and the workers return empty, they no longer re-apply the trail substance, and the odour evaporates. The trail therefore exists only as long as it is useful. The process of mass foraging trails, as we now understand them, is illustrated in Figure 1.

It had long been recognized that the trails the ants follow must have an odour content subsequent to the simple scientific experiment of drawing a finger across a trail causing the foragers to temporarily lose the trail and search around for it (Bonnet, 1779). Forel (1886, 1908) proposed that the ants use their antennae to follow the odour. Eidmann (1927) used a laboratory colony of *Myrmica* to show that, after a single worker had found some sugar solution and returned to the nest, a number of ants took the same route that the finder had used in returning, even in its detailed turnings. If a clean floor was substituted after the first returned, the following ants wandered about and reached the food only by chance. Santschi (1911, 1930), using *Tapinoma nigerrimum*, found

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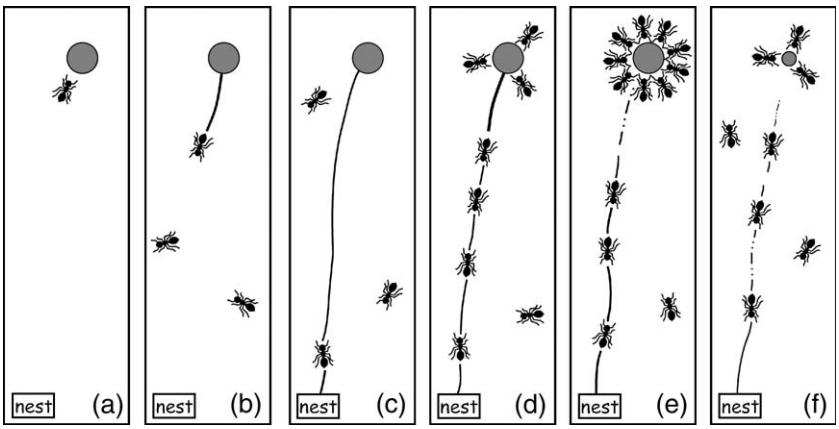

Fig. 1. Outline of the life of a foraging trail. (a) A worker randomly searching finds food. (b) As it returns to the nest, it lays a chemical trail. (c) Inside the nest, other workers are stimulated in various ways to emerge and follow the trail. (d) Replete workers continue to reinforce the trail with their secretion. (e) When the food is fully exploited, unsuccessful workers no longer re-enforce the trail. (f) The food is consumed, hungry workers do not lay secretion. The thickness of the line indicates the strength of the odour. Broken lines represent weakening odour as the trail evaporates. The trail is efficient because the number of workers using it is proportional to the amount of food available (Billen, 2006; reproduced with permission of the Netherlands Entomological Society).

that the finder ant rubbed her gaster against the floor. When he examined the area near the nest with a hand lens, he saw tiny droplets in the line she had taken, suggesting that something from the anal gland had been deposited when the gaster was rubbed against the floor. Goetsch (1934) showed that a trail could be laid with the gaster of a newly-killed ant. Carthy (1951) noted that a following ant closely followed the unseen trail with its antenna, and conducted experiments from which he concluded there was no directional information in the trail. Furthermore, he was able to make the trails of *Acanthomyops (Lasius) fuliginosus* visible by dusting them with *Lycopodium* spores (Carthy, 1951).

The foraging strategy of 98 species of ant and their colony size are related in a table by Beckers *et al.* (1989). In species that have a smaller number of workers, the system of tandem running is often used (Möglich *et al.*, 1974; Hölldobler & Wilson, 1990: 273; Richardson *et al.*, 2007). In this strategy, the first scout to find the food lays a trail back to the nest and recruits another worker. The scout follows its own odour trail, whereas the recruited worker keeps in contact with the first by antennal contact until the food is reached. Both can feed and then lay trails back to the nest, and both can recruit one more nestmate on the return journey (Fig. 2). The antenna contact encourages the lead ant to continue running, and the chemical odour entices the follower to keep contact. It is tempting to speculate that the odour trail developed from an ability to detect one or more metabolic by-products in a worker that had just ingested and was digesting food. As yet,

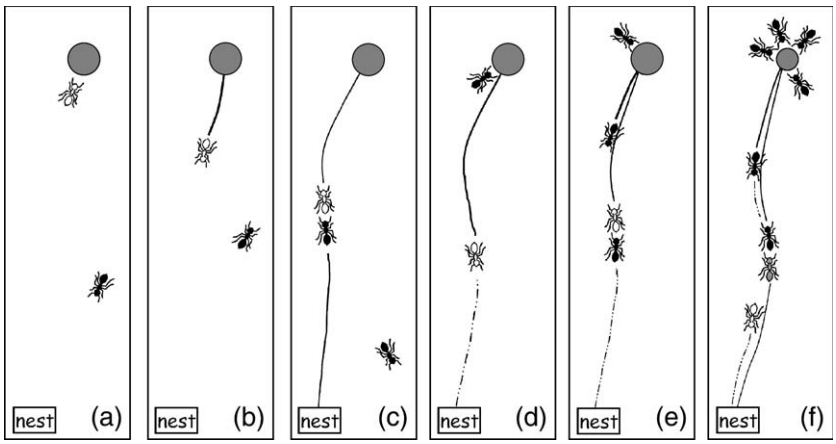

Fig. 2. Scheme of tandem running in ants. (a, b) The randomly searching scout lays a trail to the nest after finding food. (c) It recruits another worker, which follows it by antennal contact and odour of the food. (d) Both ants lay odour trails as they return to the nest. (e, f) Each returning worker can recruit one more. The original finder is shown in white, recruited ants are black. Broken lines indicate older, evaporating trails (Billen, 2006; reproduced with permission of the Netherlands Entomological Society).

because of the difficulty in testing them, no substances used in tandem running have been identified.

The subject of ant trail pheromones has been reviewed a number of times, (Parry & Morgan, 1979; Attygalle & Morgan, 1985; Ali & Morgan, 1990; Morgan, 1990; do Nascimento & Morgan, 1996) but it has not received attention recently. It is appropriate to review the subject now, to include and honour the great work done in discovery of formicine trail pheromones, by the late Professor Dr Hans-Juergen Bestmann of the University of Erlangen-Nürnberg, in collaboration with Professor Bert Hölldobler of Würzburg.

Glandular sources

The glandular sources of trail pheromones are variable. The subject has been reviewed by Attygalle & Morgan (1985) and by Billen & Morgan (1998). Those pheromones known at present come either from various abdominal glands, or in the special case of the genus *Crematogaster*, paired tibial glands. In myrmicine ants, the venom gland is usually the source, with the exceptions of *Monomorium pharaonis* and *Solenopsis invicta*, where it is found in the Dufour gland. In those formicine ants so far studied, it has been the hind gut, also described as the anal sac.

Foraging behaviour varies widely among poneromorph ants. Some forage with visual cues, others use tandem running, some behave like army ants, and some do use trails, either to exploit large prey or for colony migration. In the few species studied so far, the venom, Dufour and pygidial glands have all been found as sources. The few examples from the Dolichoderinae produce it in the Pavan gland (Cavill *et al.*, 1979, 1980; Attygalle *et al.*, 1998a) and the sole identified pheromone from the army ants, an *Aenictus* species from the Aneuritinae (Oldham *et al.*, 1994a), is stored in a postpygidial gland. In all but *Crematogaster*, the pheromone is applied to the substrate by dragging the sting lance or the abdomen along the ground as the ant returns to the nest. The shape of the gaster of *Crematogaster* workers does not permit the touching of the sting lance to the ground.

Billen *et al*., (2005) have identified a footprint gland on the hind pretarsi of the queenless ant *Amblyopone reclinate*, and have shown by bioassay that it contains a trail pheromone, but no isolation studies have been made yet.

Bioassays

The study of trail pheromones appeals particularly to chemists with an interest in insects because the bioassays are relatively easy to carry out, can be performed in the laboratory and can quickly provide a result. The difficulties to be overcome are the minute amounts of substance involved, the necessity to take great care against contamination because worker ants are extremely sensitive to the presence of part per billion of the chemicals, and the possibility that sometimes a pheromone may consists of several components. In the case of *S. invicta*, difficulty was encountered because it consisted of a following element and a recruitment element. Other complications are encountered below, but most notably, as in *Tetramorium meridionale* or *Aphaenogaster rudis*, when any one component may show little or no activity and only the complete mixture has activity comparable to a glandular extract.

The first bioassay methods with glandular extracts were described by Wilson (1959) using *Solenopsis saevissima*. Today, three types of bioassay are generally used: the straight line, the Y-shaped choice assay and the circular trail. The first two have variations between laboratories. The straight line test has the advantage of simplicity. A length of paper or plastic marked with a gland extract or fraction, or synthetic compound can be inserted into an existing natural trail and the behaviour of the ants is observed as they reach the inserted section (Fletcher & Brand, 1968; Barlin *et al.*, 1976). In the Y-choice, solutions of the material (usually a solvent extraction of some part of an insect) and a control (either the solvent used for the extract or an alternative candidate compound) are streaked along from the origin in the lower part of the Y-tube (approximately 3 cm). The arms of the Y-tube diverge at 30–40° with the solvent or alternative compound continuing into one arm of the Y-tube, whereas the extract continues into the other arm. The course taken by an insect placed at the origin at the bottom of the Y is then observed. It either moves into one of the arms of the tube (i.e. a positive result) or it does not move (i.e. a negative result). Accumulation of data from many such observations indicates whether the insects can follow an active trail or indifferently choose both branches (Vander Meer *et al.*, 1988; Kohl *et al.*, 2003). This method has the advantage of showing a clear preference between two competing extracts or compounds, and is useful for studying ageing of trails by comparing older and newer trails. The circular trail, first described by Pasteels & Verhaeghe (1974), requires the drawing of a circle, with a radius of 5 cm, on paper with pencil and marking off 1-cm divisions on the circumference (Evershed *et al.*, 1983; Billen *et al.*, 1992; see also Sonnet & Moser, 1973). A solution of the extract to be studied is then evenly spread on the circumference with a special pen or a pipette. Hand application has been found sufficiently accurate but a mechanical device for even application has been described (Gerardy & Verhaeghe, 1988). Ants are allowed one at a time onto the paper, placed in their foraging area and when they contact the circumference of the circle, the number of 1-cm arcs they follow before they leave the circumference (and presumably can no longer detect the odour) is counted and analysed statistically. In this test, usually an amount equal to one-tenth of the amount of that compound found in the gland gives the maximum trail-following. More or less than that amount gives poorer following of the trail. It is therefore important to test compounds at the correct physiological concentration. This method has the advantage of providing a quantitative answer for the activity of each extract or compound. Somewhere between these latter two methods is the use of a circle or ellipse, with the test substance applied to the circumference on one side and the control on the circumference of the other. This can be extended to have two ellipses touching, with the

sides to which the test substance is applied reversed at the point of contact (Bestmann *et al.*, 1999; Blatrix *et al.*, 2002).

For a long time, the analytical methods available to chemists were insufficiently sensitive to detect the minute amounts of chemicals deposited on trails, until the introduction of the technique of gas chromatography (GC) (for separation of components) linked to mass spectrometry (MS) (for identification of those components) approximately 40 years ago. This coincided with the growth of the subject of insect pheromones. In 1959, the identification of the first insect pheromone was announced with the isolation of bombykol, the sexual attractant of the female silk moth *Bombyx mori* (Butenandt & Karlson, 1954; Butenandt *et al.*, 1959). This was soon followed by other examples and the word 'pheromone' was devise to describe chemicals that carry information between individuals of the same species (Karlson & Butenandt, 1959), by analogy with the word hormone, which is a chemical messenger within an individual. Linked GC-MS was the best tool for pheromone study, and it was soon applied to examining chemical trails. Even with that technique, the first identification of chemicals from trails required large quantities of ants for extraction. A method of solvent-free injection of the whole gland was evolved for the analysis of small samples that permits the quantification of nanogram amounts of pheromone in the gland of a single worker (Morgan & Wadhams, 1972). Although GC-MS is the essential technique, other chromatographic methods are useful (Morgan, 1990) and the final clinching achievement is the synthesis of a proposed structure and proving its activity in a bioassay.

Venom gland

The first ant trail pheromone chemically identified was that of *Atta texana* (Tumlinson *et al.*, 1971, 1972). Its identification required 3.7 kg of dried ants. The compound was identified as methyl 4-methylpyrrole-2-carboxylate (**1**; Fig. 3) found in the venom gland, although there were other compounds contributing to the trail that were not identified. Compound **1** had a detection threshold of 80 fg cm^{-1} of trail (8×10^{-14} g cm^{-1}), which illustrates the extreme sensitivity of detection of this kind of pheromone. The same compound was identified in *Atta cephalotes* (Riley *et al.*, 1974), and *Acromyrmex octospinosus* (Cross *et al.*, 1982). These were quickly followed by identifications in some other leaf-cutting ants, where the compound 3-ethyl-2,5,-dimethylpyrazine **2** (Fig. 3) was identified in *Atta sexdens rubropilosa* (Cross *et al.*, 1979) and *Atta sexdens sexdens* (Evershed & Morgan, 1983). There was some confusion caused by cross-species trail testing in early work. For example it was found that *Atta sexdens, A. cephalotes* and *A. octospinosus* could all follow trails made from *A. texana* venom glands, which led to the mistaken conclusion that all leaf-cutting species used the same compound (Riley *et al.*, 1974). We later showed that there were very small amounts of compound **1**, accompanying **2** in the venom reservoirs of *A. sexdens sexdens*, *A. s. rubropilosa* and *A. cephalotes* (Evershed & Morgan, 1983), which explained the cross activity between some species. We also found that, for *A. s. sexdens*, the pheromone consisted of

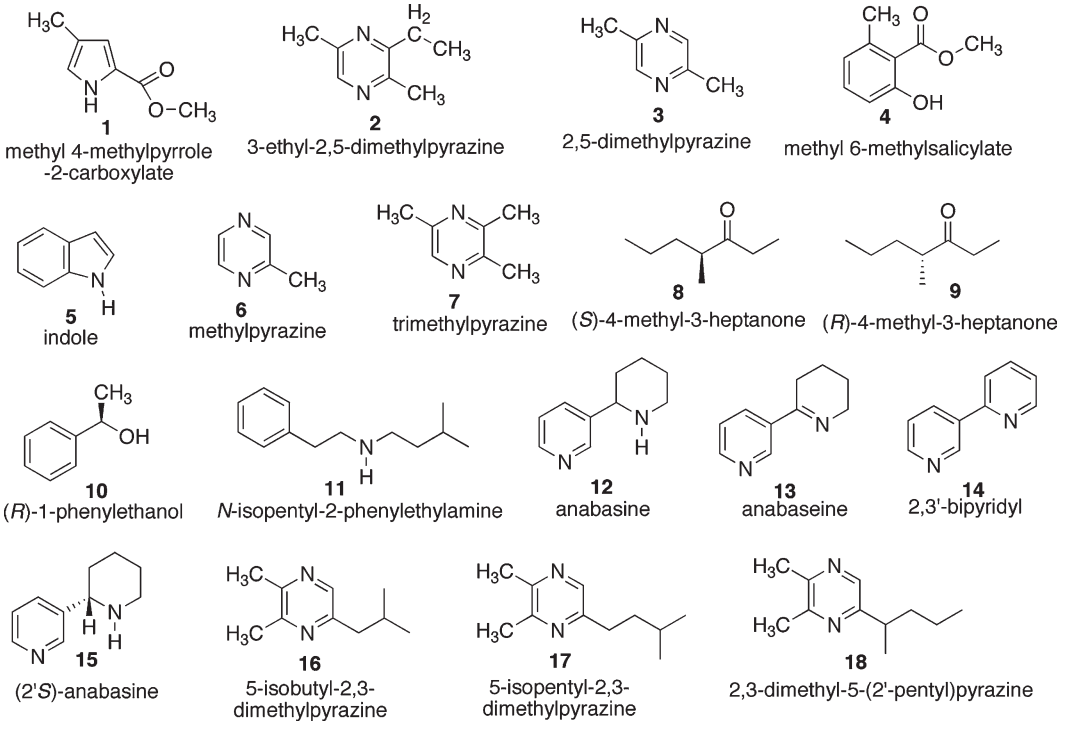

Fig. 3. Compounds from venom reservoirs of ants found to be components of trail pheromones.

both compounds, **2** and **1**, in an optimal ratio of 14 : 1 (Billen *et al.*, 1992).

In 13 species of *Myrmica* examined, just the one substance **2** was found to provide the trail pheromone (Evershed *et al.*, 1983; Morgan, 1990). From measurement of the amount in individual workers of *Myrmica rubra*, an average of 5.8 ± 1.7 ng of **2** was found in the venom reservoirs of workers (Evershed *et al.*, 1981).

The first clear example of a mixture of two substances comprising the pheromone was *Tetramorium caespitum* from the U.K., which has a 7 : 3 mixture of **2** and 2,5-dimethylpyrazine **3** in its venom reservoir, and this 7 : 3 mixture of the synthetic compounds also gave the optimum trail-following (Attygalle & Morgan, 1983). Unlike the simple case of *Myrmica* species, which share one compound and one pheromone between several species, of three species of *Tetramorium* examined, all had quite different active compounds. Methyl 6-methylsalicylate **4** (approximately 1 ng per gland) a compound already and later found in other glands of ants with other functions, was the pheromone of *Tetramorium c.f. impurum*, (Morgan & Ollett, 1987), a species morphologically very close to *Tetramorium caepitum*. It now appears that what was then though to be a single species may now be a complex of several, so it is difficult to give correct species names. Using the most sensitive MS techniques then available, no trace of **2** or **3** could be found in *T. impurum.* In *T. meridionale* yet another mixture was found. The major substance was indole **5** (approximately 1 ng per gland), but it alone had little activity. Further examination showed the presence of smaller amounts (approximately 100 pg gland^{-1}) of methylpyrazine **6**, 2,5-dimethylpyrazine **3**, trimethylpyrazine **7** and 3-ethyl-2,5-dimethylpyrazine **2**. A mixture of 1 ng **5** and 0.1 ng of each of the four pyrazines gave activity comparable to a single venom reservoir (Jackson *et al.*, 1990). This was the first example where the mixture, rather than any one compound comprised the pheromone. By contrast, both **2** and **3** are present in the glands of *Manica rubida* but only **2** (mean of 7 ng per gland) is active as the trail pheromone (Attygalle *et al.*, 1985). The seed-collecting ant *Messor bouvieri* also uses compound **2** in its trails, but it appears that the full pheromone consists of **2** from the venom gland and some unidentified compound from the Dufour gland (Jackson *et al.*, 1989).

The subject of cross-activity between related species was of much interest in early work on trails, reviewed by Attygalle & Morgan (1985). Blum & Portocarrero (1966) failed to find trail-following in *Daceton armigerum* (Myrmicinae) but found that *S. invicta* would follow a trail of its Dufour gland extract; and its venom glands were followed by *Trachymyrmex septentrionalis*, *A. texana*, *A. cephalotes*, *A. sexdens* and *A. octospinosus* (Blum & Portocarrero, 1966). Later Hölldobler *et al.* (1990) demonstrated that workers of *D. armigerum* were recruited to foraging trails when termite food was provided, and they observed workers extending their sting at irregular intervals. They showed that the trail-following response came from the venom gland and to a lesser extent from a sternal gland. In a chemical examination of the Dufour gland, venom gland and pygidial gland, the now familiar mixture of 2,5-dimethylpyrazine **3**, trimethylpyrazine **7** and 3-ethyl-2,5-dimethylpyrazine **2** were found in small (approximately 1 ng) and variable quantities in the venom gland (Morgan *et al.*, 1992). This explains the cross-activity with several species. The pygidial gland contained no detectable volatile compounds and the Dufour gland contained large amounts of hydrocarbons, and small quantities of compounds called tetramorines, related to farnesal, but nothing that had been related to trail pheromones of *Solenopsis*; therefore, the cross-activity with *Solenopsis* remains unexplained (Morgan *et al.*, 1992).

Although methyl 6-methylsalicylate **4** is found with various functions in mandibular glands and pygidial glands (Morgan, 2008), only the second species to use the compound as trail pheromone is *Mayreilla overbecki* from Australia. Colonies of this tiny ant were found to migrate by well-defined trails, or bud off new colonies when their nest chamber becomes too small. The venom gland was identified as the source (Kohl *et al.*, 2000). A trace of methyl 6-methylsalicylate **4** was identified as the only active compound. Large amounts of farnesenes in the gland had no activity (Kohl *et al.*, 2000).

Aphenogaster albisetosus uses a mixture of (*S*)- and (*R*)-4-methyl-3-heptanone **8**, **9** from their venom glands, as pheromone in the ratio of 8 : 2 (Hölldobler *et al.*, 1995). The mixture attracts workers of *A. albisetosus* and orients them along the trail. The venom glands of *Aphenogaster cockerelli* contain a mixture of (*R*)-1-phenylethanol **10** and (*S*)-4-methyl-3-heptanone **8** in the proportions of 88 : 11. Synthetic (*R*)-1-phenylethanol induces trail-following in *A. cockerelli* workers even at 0.01 equivalents of a venom gland but the 4-methyl-3-heptanone does not have any following effect but it attracts the workers and induces them to leave the nest (Hölldobler *et al.*, 1995). The mixture in *A. cockerelli* explains why *A. albisetosus* can follow the trails of *A. cockerelli,* but *A. cockerelli* cannot follow those of *A. albisetosus.* A quite different mixture has been found in *Aphenogaster rudis*. Its venom glands contain four volatile compounds, *N*-isopentylphenylethylamine **11**, anabasine (also known as neonicotine) **12**, anabaseine **13** (also known as nornicotine), and 2,3′-bipyridyl **14.** The last three are well-known alkaloids in tobacco and other plants. None of the four compounds singly show much activity in trail-following. Mixtures of **11** and **12**, or **11** and **13** show activity of about 80% of that of a venom gland, whereas a mixture of **11** and **14** has little activity. A mixture of all four compounds is almost equal to that of a venom gland, but the 2,3′-bipyridyl **14** does not appear to contribute much to the effect (Attygalle *et al.*, 1998b). Anabasine **12**, anabaseine **13** and 2,3′-bipyridyl **14** are also found in the venom gland of *M. bouvieri* (see above) but there have no recognized pheromone function (Jackson *et al.*, 1989). Anabasine, alone among this group of compounds is chiral. It has been shown to be (2′*S*)-anabasine **15** in *Messor sanctus*, and this form in excess over (2′*R*)-anabasine in *Aphaenogaster subterraneana* and *Aphaenogaster miamiana* (Leclercq *et al.*, 2001) but its chirality has not been demonstrated for any species in which it acts as pheromone or part of the pheromone.

Eutetramorium mocquerysi (Myrmicinae) from Madagascar lays trails for recruitment and nest migration. Three more complex alkylpyrazines were identified in the venom gland, 5-isobutyl-2,3-dimethylpyrazine **16**, 5-isopentyl-2,3-dimethylpyrazine **17** and 2,3-dimethyl-5-(2′-pentyl)pyrazine **18** (Tentschert *et al.*, 2000). Only **16** was found to be active, and the investigators were unable to explore whether the other compounds had any synergistic function. After the initial identification of methyl 4-methylpyrrole-2-carboxylate **1** in *Atta* and *Acromyrmex* species, there were no further reports of this compound as pheromone until recently. It has now been identified in two termite-hunting species of *Metapone* (Myrmicinae), *Metapone madagascarica* and *Metapone* new species (Hölldobler *et al.*, 2002). It is the only active compound in the venom glands of both species.

The poneromorph ant *Leptogenys diminuta* uses (3*R*,4*S*)-4-methyl-3-heptanol **19** (Fig. 4) as the trail pheromone from the venom gland (Attygalle *et al.*, 1988), although it requires the stimulation of the pygidial gland secretion to search for the trail (Attygalle *et al.*, 1991). Synthetic methylheptanol **19** completely reproduced the effect of the gland extract. It is the only volatile compound detected in worker glands (Maile *et al.*, 2000). Queens have more of the same compound and some 4-methyl-3-heptanone in their venom glands. It is noted that old virgin ergatoid queens perform similar tasks to workers in this species, and foraging by virgin ergatoid queens is occasionally observed in the field (Maile *et al.*, 2000). Both workers and mated and virgin queens also contain, in the same gland, long-chain esters of 4-methyl-3-heptanol, which will have the effect of reducing the volatility of 4-methyl-3-heptanol (Maile *et al.*, 2000). The same compound **19** has been identified in the venom gland of another species, at present identified as *Leptogenys* species 5 (Janssen *et al.*, 1997b). However, in another species, *Leptogenys peuqueti*, a quite different situation was encountered. This species has the most complex trail pheromone mixture yet encountered.

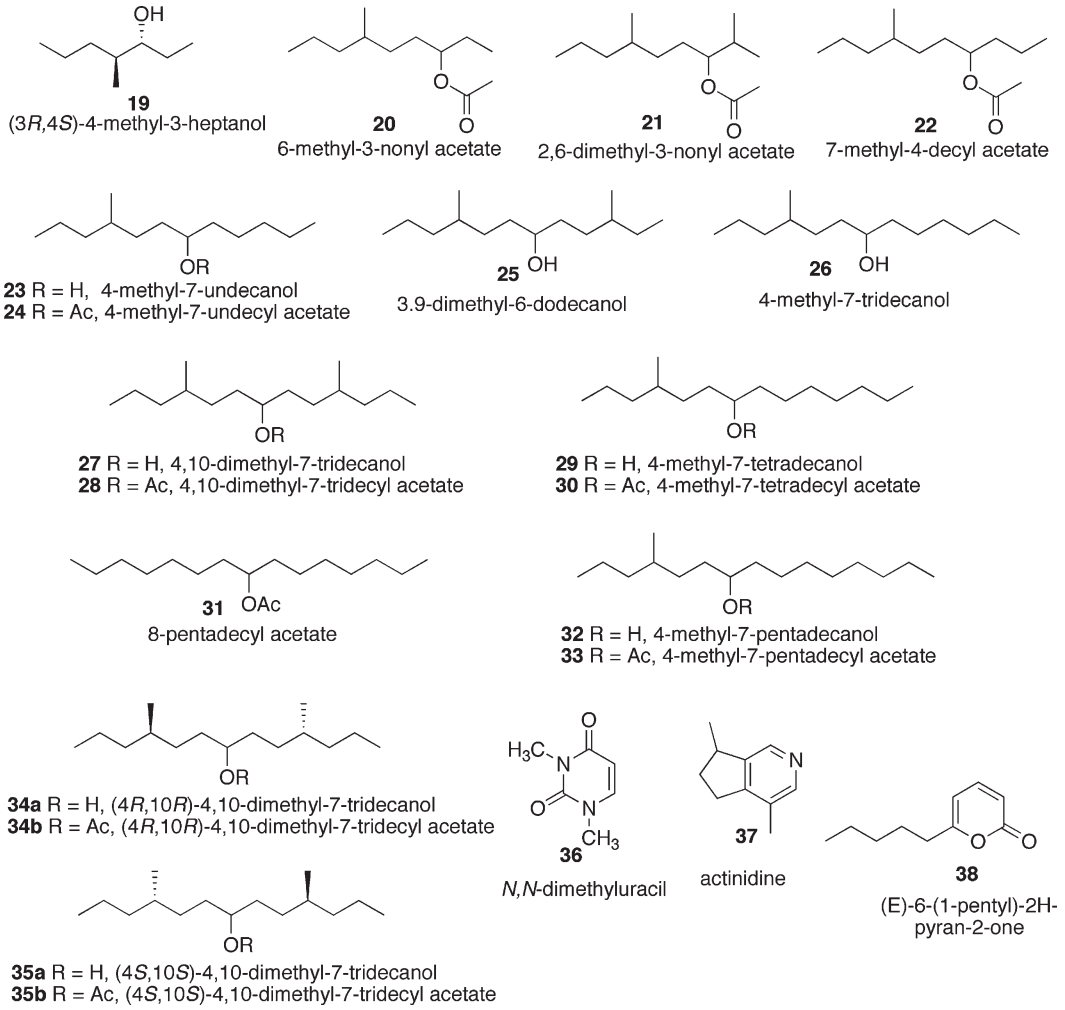

Fig. 4. Structures of further trail pheromones from venom reservoirs of ants.

Fourteen low-molecular-mass alcohols and acetates **20** to **33** were identified in the venom gland (Janssen *et al.*, 1997a). Each compound was synthesized and tested in a trail-following test at 0.1 equivalents of the average amount of that compound found in the gland. All compounds showed significantly higher activity than controls and a mixture of all the compounds was equal to 90% of the activity of a venom gland extract, suggesting that all compounds are necessary to stimulate trail-following (Janssen *et al.*, 1997a). Each of the compounds in this species has two or three chiral centres, but only the (4*R*, 10*R*) and the (4*S*,10*S*) chiral forms of 4,10-dimethyl-7-tridecanol and their acetates, **34** and **35**, could be tested. The workers preferred to follow trails of (4*R*, 10*R*)-isomers **34** over those of the (4*S*, 10*S*)-isomers **35** or the racemic mixture of all possible chiral forms (Janssen *et al.*, 1997a). It should be noted that both **34** and **35** are symmetrical about the OH and OAc groups, but that other enantiomers exist that were not tested. All these compounds belong to the same biosynthetic group containing simple branched alkyl chains.

Pachycondyla analis (= *Megaponera foetens*), an African termite-raiding species is an example where secretions from two glands are used together. It was first demonstrated that, of the possible abdominal glands, the venom gland had a weaker but longer-lasting trail effect, whereas the pygidial gland has a stronger but less persistent effect (Hölldobler *et al.*, 1994). Through the work of Bestmann's group, the true trail pheromone was identified as *N,N*-dimethyluracil **36** (a less volatile compound) present in the venom gland, whereas the more volatile actinidine **37** from the pygidial gland stimulates the ants to forage (Janssen *et al.*, 1995).

Guest ants are social parasites, with their nests located on the periphery of their host colonies. *Formicoxenus provancheri* is a guest in the colonies of *Myrmica incompleta* (both myrmicines). The pheromone of *M. incompleta* has been confirmed to be 2-ethyl-3,5-dimethylpyrazine **2** (Barbazanges, 1989), conforming to the pattern already noted for *Myrmica*. The parasite *F. provancheri* followed the trails of *M. incompleta* as well as the hosts did themselves, down to 0.1 venom gland equivalents, and lower. The *F. provancheri* were not found to reinforce the trails, and as far as is known do not lay trails themselves (Lenoir *et al.*, 1992).

It had been claimed earlier that the pheromone of *Pristomyrmex pungens* was a mixture of C_{14} to C_{20} saturated and unsaturated acids (Hayashi & Komae, 1977) but, because of the ubiquity of these acids, this was doubtful. The Bestmann group showed that the venom gland contains 6-pentyl-2-pyrone **38**, and a number of monoterpenes (α-pinene, β-pinene, camphene, myrcene, α-phellandrine, α-terpinene and limonene). In trail-following tests, and electroantennography (EAG) tests, **38** is the pheromone. The monoterpenes were inactive and, when added to **38**, had little additional effect (Janssen *et al.*, 1997b).

Workers of *Solenopsis* species forage along well-established trunk routes but they are recruited to foraging or directed from one trunk route to another by emission of the familiar three pyrazines **2**, **3** and **7** from the venom glands, with compound **2** being the main recruitment pheromone (Hölldobler *et al.*, 2001). The routes are marked with colony-specific hydrocarbons from the Dufour glands (Hölldobler *et al.*, 2004). There is a further refinement in *P. barbatus* where patrolling workers direct foraging to particular routes by laying Dufour gland secretion on approximately the first 20 cm of those routes to be used that day (Greene & Gordon, 2007).

Dufour gland

Monomorium pharaonis or pharaoh's ant is a tramp species that has become an important pest in permanently warm buildings, such as hospitals. It was an early target for identification of its trail pheromone to be possibly used in control measures. After initial mis-identification of two compounds in its venom gland, called monomorines I and II, the true trail pheromone, faranal **39** (Fig. 5) was identified in its Dufour gland (Ritter *et al.*, 1977). Faranal is classed as a sesquiterpene with two extra carbon atoms, typical of many terpenoid compounds made by insects using homomevalonic acid instead of mevalonic acid (Morgan, 2004). The juvenile hormone compounds are other examples of this type.

Wilson (1959) showed clearly that the pheromone of *S. saevissima* was present in the 'sting accessory gland' (Dufour gland). Also in *S. invicta*, the imported fire ant of the U.S.A., the pheromone was in the Dufour gland. It was identified first as (*Z,Z,Z*)-allofarnesene **40** (Fig. 5) by Williams *et al.* (1981), with optimal activity at 100–500 fg cm^{-1} of trail, but this was synthesized and found not to have activity by Vander Meer *et al.* (1981). The latter group found that the pheromone had two components, both in the Dufour gland. The recruitment part of the pheromone consisted of (*Z,E*)-α-farnesene **41** (approximately 6 ng $gland^{-1}$), acting as a trail-following stimulant, and a still incompletely identified homosesquiterpene containing three rings and one double bond (75 pg $gland^{-1}$) (Vander Meer *et al.*, 1988). The final structure of this compound remains unsolved. However, this mixture alone would not induce trail-following. It was necessary to put Dufour gland extract on the first few centimetres of trail to induce workers to begin following. It is possible that it requires approximately 250-fold more pheromone to begin the arrractant and inducer part of the process than is required once trail-following has begun (Vander Meer *et al.*, 1990). The trail pheromones of *Solenopsis richteri*, *Solenopsis xyloni* and *Solenopsis geminata* appear to be closely-related terpene compounds (Barlin *et al.*, 1976) but their structures have not yet been solved.

Ectatomma ruidum was the first poneromorph ant found to have its trail pheromone in the Dufour gland. The major volatile compound in the gland is all-*trans*-geranylgeranyl acetate **42** (approximately 140 ng per gland) accompanied by a trace of geranylgeraniol **43** (approximately 1–10 pg per gland) (Bestmann *et al.*, 1995a). Both compounds showed activity in trail-following bioassay and EAG experiments, and the acetate was more active in both. It is noted that, for *E. ruidum*, drawing a trail from the nest with Dufour secretion does not induce massive recruitment to the trail but, if a successful scout ant having fed on a sugar solution enters the

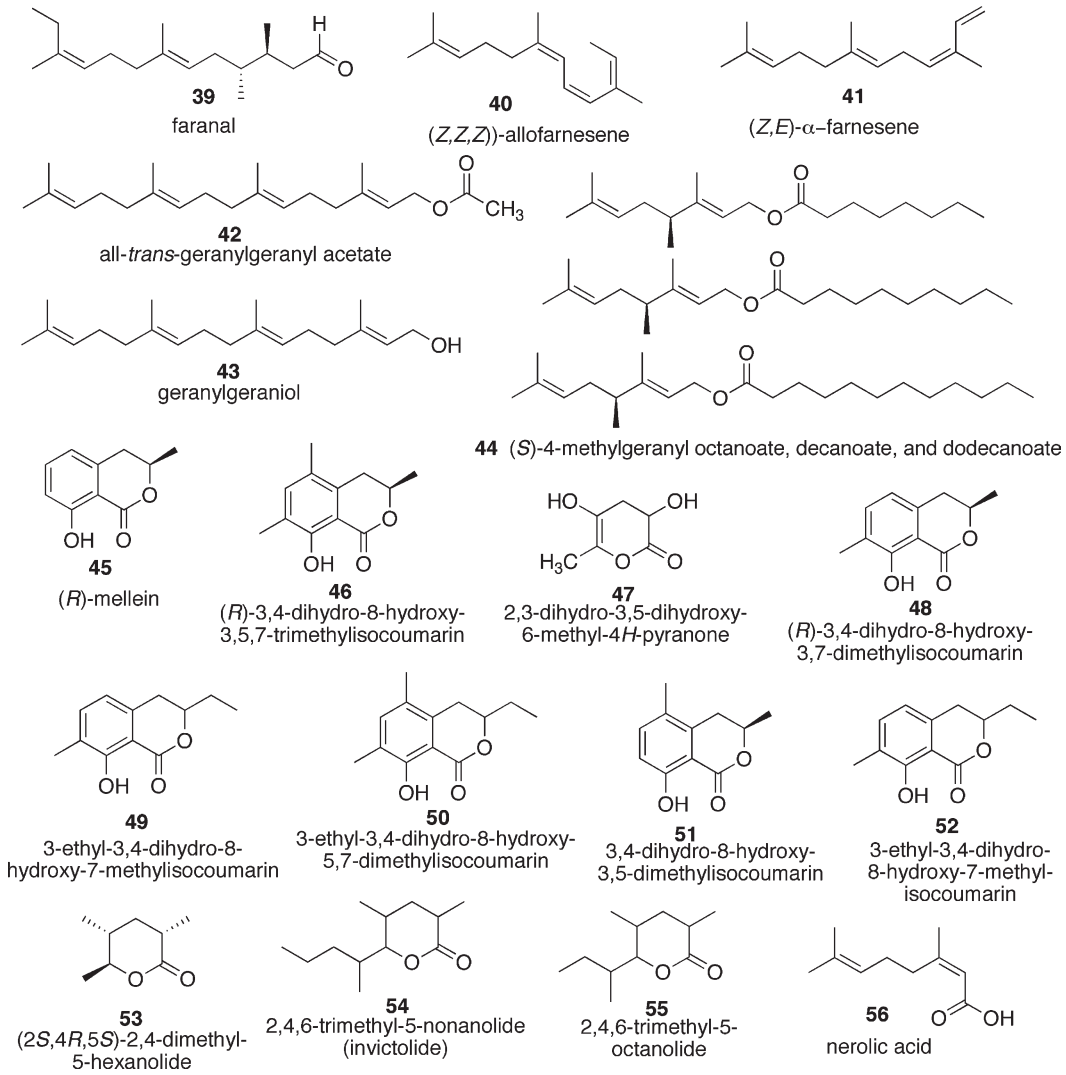

Fig. 5. Compounds from Dufour glands and the hind gut, which serve as trail pheromones. The (*R*)-enantiomer of mellein, as shown, has been found to be the correct form of the pheromone in all cases. The (*R*)-enantiomer of **46** and **51** have been shown to be the active isomer in some, but not all, species where the compound is the pheromone.

nest and a trail is then laid, there is a much greater response with many workers leaving the nest and following the trail (Pratt, 1989).

The poneromorph ant *Gnamptogenys striatula* employs a mixture of 4-methylgeranyl esters of fatty acids **44**, found in the Dufour gland (Blatrix *et al.*, 2002). Bishomogeranyl esters are also present in the gland but display little or no activity. A mixture of octanoate, decanoate and dodecanoate esters of 4-methylgeraniol gave activity comparable to a gland. These three compounds comprise approximately 85% of the total secretion (Blatrix *et al.*, 2002). Compounds **42**, **43** and **44** all have larger molecules and are less volatile than compounds encountered up to now. It is also interesting that all the compounds so far identified as pheromones in the Dufour gland belong to the class of terpenoids.

Hind gut

At present, all the trail pheromomes found in formicine species originate in the hindgut (sometimes called the rectal sac). At first, they proved very difficult to identify until the very careful chemical work of Bestmann and his group. With the experience of many years working on insect substances, using the solid injection method, skill in organic synthesis, and the collaboration and field experience of Bert Hölldobler, they made great strides in the advancement of the subject, and all our knowledge of trail pheromones of formicine species is due to them. Their first successes were in identifying the pheromones of *Formica rufa* and *Lasius niger* (Bestmann *et al.*, 1992). The compound in *F. rufa* was (*R*)-3,4-dihydro-8-hydroxy-3-methylisocoumarin, commonly called mellein,

45 (Fig. 5), a compound found widely in nature, not only in ants but other insects and micro-organisms. The pheromone of *L. niger* was identified as the related compound 3,4-dihydro-8-hydroxy-3,5,7-trimethylisocoumarin **46**. This compound was active at 500 pg per trail. Both in trail assays and in EAG tests, there was a stronger response to (*R*)-3,4-dihydro-8-hydroxy-3,5,7-trimethylisocoumarin **46** than to the (*S*)-enantiomer (Kern & Bestmann, 1994). Other compounds accompanying it in *L. niger* in comparable quantities were dodecyl acetate, palmitic and oleic acids but these were not active. There was too little of the material available from the rectal sac to make a complete identification so they had to synthesize a number of compounds and compare their chromatographic retentions and mass spectra with the isolated substances. They were then able to confirm their identifications with bioassays and EAG experiments with the synthetic compounds (Bestmann *et al.*, 1992).

The possession of synthetic samples of several related compounds enabled them then to go on to identify these and other isocoumarins in other formicine species. It had earlier been claimed that the trail pheromone of *Lasius fuliginosus* was a mixture of C_6-C_{10} and C_{12} fatty acids (Huwyler *et al.*, 1975) but this always seemed doubtful. It was now shown to be (*R*)-mellein **45**, present at only 50–100 pg per gland (Kern *et al.*, 1997). 2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyranone **47** is also present in the hindgut (0.6–1.1 ng) and shows some slight activity. Apparently other components, not yet identified, are present in the hindgut of *L. fuliginosus* because **45** and **47**, individually or in combination, do not equal the activity of a hindgut extract (Kern *et al.*, 1997). These ants feed on honeydew produced by aphids and the pyranone **47** is present in the honeydew. It is possible that the pyranone **47** is sequestered by the ants from their food, as happens in other cases with insects feeding on certain plants (Morgan, 2004).

In two *Formica* species, no identification was achieved. Mellein **45** and 3,4-dihydro-8-hydroxy-3,5,7-trimethylisocoumarin **46** were identified in *Formica fusca*, but neither compound showed any trail-following activity for this species, although they gave positive responses in the EAG using *F. fusca* antennae; also the (*R*)-3,4-dihydro-8-hydroxy-3,5,7-trimethylisocoumarin **46** gave a greater response than the (*S*)-enantiomer (Bestmann *et al.*, 1992; Kern & Bestmann, 1994). In *Formica sanguinea*, they found two isocoumarins they had synthesized, 3,4-dihydro-7-hydroxy-3,7-dimethylisocoumarin **48** and 3-ethyl-3,4-dihydro-8-hydroxy-7-methylisocoumarin **49**, but again, though EAG-active, neither compound was active in trail-following (Bestmann *et al.*, 1992).

They were able to show that the trimethylisocoumarin **46** was the main component of the pheromone for *Camponotus silvicola* from Peru. Compounds **45**, **50**, **51** and **52** were all present and EAG-active but only **46** gave trail-following (Übler *et al.*, 1995). Compound **46** was also the principal pheromone component of *Camponotus inaequalis* (Bestmann *et al.*, 1997). The dimethylisocoumarin **48** was the main pheromone component for *Camponotus rufipes* from Brazil (Übler *et al.*, 1995). Compounds **45**, **46** and **49** were all present in *C. rufipes* and EAG-active but only **48** was active in bioassay.

A different group of compounds, δ-lactones, was first discovered in *Camponotus herculeanus.* Two trail pheromone components were identified; 2,4-dimethyl-5-hexanolide **53** was the principal one, but there was a less strong contribution from 3,5-dimethyl-6-(2 -pentyl)-2*H*-pyran-2-one **54** (Bestmann *et al.*, 1995b). Dimethylhexanolide **53** can exist in eight possible isomeric forms (four enantiomeric pairs). Without synthesizing all possible forms but, by a clever combination of synthesis, EAG tests and nuclear magnetic resonance spectroscopy, Bestmann *et al.* (1999) eliminated down to two possible isomers, one of which in a trail bioassay with *C. herculeanus* workers showed no activity, the other, (2*S*, 4*R*, 5*S*)-2,4-dimethyl-5-hexanolide **53** showed strong activity, confirming that it was the pheromone. It is interesting to chemists that nature uses the thermodynamically most stable isomer for the pheromone. The second compound **54** is known as invictolide because it was discovered earlier as a queen recognition substance in the venom glands of queens of *S. invicta* (Rocca *et al.*, 1983). 2,4-Dimethyl-5-hexanolide **53** was also found to be the principal trail pheromone component of several more *Camponotus* species; *Camponotus pennsylvanicus*, *Camponotus socius* and *Camponotus vagus* (Haak, 1995; Bestmann *et al.*, 1997; Kohl *et al.*, 2001). Following their success with *C. herculeanus,* Bestmann *et al.* (1999) were then able to show that the same isomer of dimethylhexanolide **53** was also the pheromone of *Camponotus ligniperda*, *C. pennsylvanicus*, *C. socius* and *C. vagus.*

Camponotus atriceps (= *Camponotus abdominalis)* uses 3,5-dimethyl-6-(2′-butyl)tetrahydro-2H-pyran-2-one (also called 2,4,6-trimethyl-5-octanolide) **55**, another of this group (Haak *et al.*, 1996). *Camponotus floridanus* is very close to *C. atriceps*, indeed, it was once considered a synonym for *C. atriceps* but it uses a quite different substance. It uses nerolic acid **56**, the only example of a free carboxylic acid that is a trail pheromone (Haak *et al.*, 1996). This species and pheromone are the subject of a recent study of olfactory reception in the ant brain (Zube *et al.*, 2008). The pheromone of *C. ligniperdus* consists of at least three compounds: **53**, **54** and **55** (Bestmann *et al.*, 1997).

Three further species of *Camponotus* proved difficult problems because of the tiny amount of pheromones present. The active compounds were found by trapping fractions from the gas chromatograph and testing each fraction in a bioassay. In that way, the part of the chromatogram to be examined carefully was located and examined by MS. In *Camponotus castaneus*, the identified pheromone compound was a tiny amount of 3,5-dimethyl-6-(2′-butyl)-tetrahydro-2*H*-pyran-2-one **55** (Kohl *et al.*, 2003). The major compound in the rectal sac, eicosanol, was inactive. From trapping and testing of fractions from chromatography of hindgut extracts of *Camponotus balzani*, it was revealed that only one short section of the chromatogram was active. This was identified by comparison with the synthetic compound as 3,4-dihydro-8-hydroxy-3,5,7-trimethylisocoumarin **46** (Kohl *et al.*, 2003). The main compound in the secretion, octyl hexanoate, was inactive. There was a tiny amount of mellein present but it too was inactive. The same trimethylisocoumarin **46** was the only active pheromone component identified in *Camponotus*

sericeiventris (Kohl *et al.*, 2003). The authors point out that the major recruitment signal in all of these last three species appears to be the formic acid of the venom gland (Kohl *et al.*, 2001, 2003). The first, and as yet, only study on the biosynthesis of trail pheromones was conducted by deuterium labelling of acetic acid on a number of species of *Formica, Lasius* and *Camponotus* (Bestmann *et al.*, 1997). It showed that all the compounds **45** to **55** are built up from acetate and propionate units linked together in a chain, with modification and cyclization to the final products, probably by a polyketide pathway (Morgan, 2004).

Pygidial gland

We have already seen that trail-following in the termite-raiding *P. analis* (= *M. foetens*) requires a following component *N,N*-dimethyluracil **36** from the venom gland, and actinidine from the pygidial gland (Janssen *et al.*, 1995). A similar situation is found with *L. diminuta*. The trail-following component (3*R*, 4*S*)-4-methyl-3-heptanol from the venom gland requires *cis*-isogeraniol **57** (Fig. 6) from the pygidial gland, released inside the nest, for recruitment to the trail (Attygalle *et al.*, 1991). Another termite-raiding poneromorph, *Pachycondyla* (= *Termitopone*) *marginata* has a trail pheromone in the pygidial gland. The pheromone is used both in raiding and in nest migration. The active compound is citronellal **58**, a very common monoterpene aldehyde found in insects and in plants (Hölldobler *et al.*, 1996). The related compound isopulegol **59** elicits an increase in locomotory activity and may function synergistically in recruitment. The recruiting ant also gives a shaking display to encourage recruitment (Hölldobler *et al.*, 1996). The myrmicinae *Ocymyrmex laticeps* uses 2-(3′-indolyl)-ethanol **60** from its pygidial gland to lay trails (Morgan *et al.*, 2006).

The pygidial glands of dolichoderine ants usually contain mixtures of monoterpenes called iridoids. These act as alarm and defense substances, and are effective against other insects. The pygidial gland of *Tapinoma simrothii*, which contains iridodials **61** and iridomyrmecin **62**, has a double function. At high emission rates from a point source, these compounds cause alarm. Workers rush to the source with open mandibles and raised abdomens. When a lower concentration was drawn along a line, workers followed the secretion calmly. These trails are long-lasting (Simon & Hefetz, 1990).

Pavan gland

The first substance reported as a trail pheromone of a dolichoderine ant was (*Z*)-9-hexadecenal **63** (Fig. 6) (Cavill *et al.*, 1979) from the Pavan gland of *Linepithema*

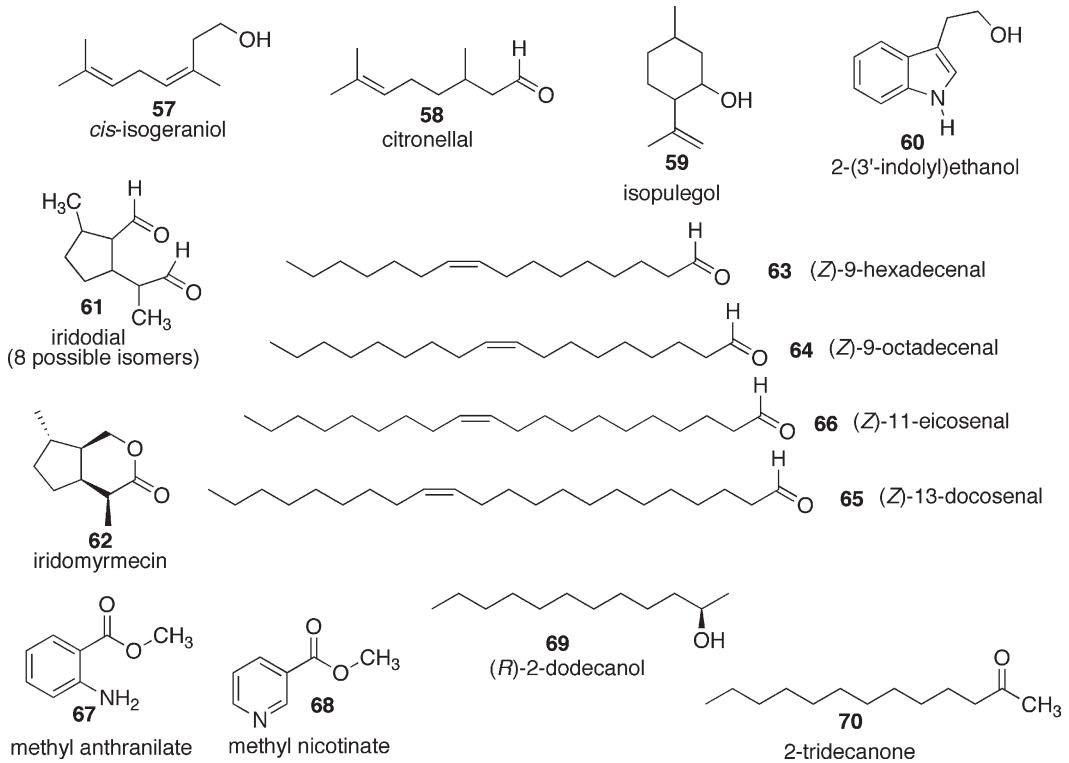

Fig. 6. Compounds from the trail pheromones of ants found in the pygidial gland, Pavan gland, postpygidial gland and tibial gland.

(*Iridomyrmex*) *humilis*, although the compound was reported to be inferior in test to a glandular extract (Van Vorhis Key & Baker, 1982). For the arboreal east Asian ant *Dolichoderus thoracicus*, only the Pavan gland was found active. Analysis showed the gland contained many compounds, including a number of aldehydes (Attygalle *et al.*, 1998a). By concentrating on the aldehydes, (*Z*)-9-octadecenal **64** (approximately 10–20 ng per gland) and (*Z*)-13-docosenal **65** (approximately 2–4 ng) were identified as the major compounds. In all, 18 aldehydes from C_{16} to C_{24} were identified. In trail tests at 1 ng cm^{-1}, (*Z*)-9-hexadecenal **63**, (*Z*)-9-octadecenal **64**, (*Z*)-11-eicosenal **66** and (*Z*)-13-docosenal **65** all showed some activity (**63** and **64** were most active, **65** and **66** were less so). However, when pairs of compounds were tested together (**63** and **64**, **64** and **65**, **64** and **66**), they showed greater activity and a mixture of all four had activity almost equal to that of a single gland (Attygalle *et al.*, 1998a). In a preliminary examination, the authors found that several other *Dolichoderus* species also contained aldehydes and suggested that their trail pheromones may not be highly species-specific.

Postpygidial gland

Army ants are difficult to maintain in the laboratory in the ways required for pheromone studies, so little is known of that aspect of them. In the one example known from the subfamily Aneuretinae, an unidentified species of *Aenictus*, the trail pheromone has been found in a postpygidial gland (Oldham *et al.*, 1994a). The ants were taken from a raiding column in Hong Kong, and the work carried out swiftly when it was still possible to keep them in good condition. On an artifical trail made from a 100-fold dilution of an extract of one gland of a worker, most of the workers ($> 90\%$) followed a trail on reaching it and continued following it for several hours, making quantification impossible. The simple compound methyl anthranilate **67** was identified at approximately 100 ng per gland. However, when trails made of synthetic **67** were tried, they induced little activity, unless the ants were transferred from an trail made from glandular extract, whereupon they followed very well. There could even be a lapse of several hours between contact with the natural and synthetic trails. This led to the discovery of a second component in the gland at much smaller concentration (1% of the total secretion). The second compound was identified as methyl nicotinate **68.** Artificial trails made with methyl nicotinate induce no trail-following but, when combined with methyl anthranilate in the ratio 1 : 100 and even when only 10 pg **68** and 1 ng **67** were used per trail, the workers ran persistently on the trail. Methyl nicotinate can be considered a primer pheromone because, if given to the ants even 6 h before the methyl anthranilate, it primes them to follow the anthranilate trail (Oldham *et al.*, 1994a). Methyl anthranilate **67** was also found in the postpygidial gland of *Aenictus rotundatus*, but the fragment of the colony could not be maintained in good condition long enough to perform trail bioassays (Oldham *et al.*, 1994b).

Tibial glands

In the genus *Crematogaster*, workers raise their gasters forward over the thorax when alarmed and use their spatulate stings to spread defensive secretions on attackers. As a consequence, they are unable to bring the tip of the gaster into contact with the ground. Yet they lay foraging trails. Leuthold (1968) showed that, in *Crematogaster ashmeadi*, they laid a secretion with their feet from glands in their hind tibia, connected to their tarsi by a tubular duct. Fletcher & Brand (1968) independently, by extracting different body parts of *Crematogaster peringueyi*, showed that the pheromone was located in the hind legs. They also found that, when laying a trail, the worker walked with a curious gait with their hind legs held closer together. The morphology of the gland and the duct to the tarsi were described by Billen (1984). A number of 2-alkanols and methyl ketones from C_{11} to C_{15} have been identified in the tibial glands of several species (Ollett, 1989) but, at present, the pheromone of only one species, *Crematogaster castanea*, has been fully identified as (*R*)-2-dodecanol **69** (Morgan *et al.*, 2004). Both (*R*)- and (*S*)-2-dodecanol showed strong activity, too great to measure accurately down to 0.1 ng per trail of 32 cm but, below that, the (*R*)-enantiomer was approximately 100-fold more active than the (*S*)-enantiomer, and the activity of the latter can be accounted for by the small amount of the (*R*)-enantiomer it contained. It has proven difficult to obtain reproducible results with other species. The major pheromone component of *Crematogaster scutellaris* and *Crematogaster liengmei* is 2-tridecanone **70** (J. M. Brand & E. D. Morgan, unpublished results). There appears to be some cross-activity of trails within groups of species of *Crematogaster* (Espadeler & Martí, 1994).

Perception and stereobiology

We do not understand how the olfactory system perceives odours, although the subject is advancing rapidly and there are some clues available that help. Using individual mouse olfactory receptors and a group of simple aliphatic compounds of related structures, Malnic *et al.* (1999) were able to show that any one receptor can recognize a number of chemically related odorant compounds, and any odorant can be recognized by multiple receptors. Therefore, different odorants are recognized by different combinations of receptors. Moreover, a receptor can respond with different affinities to different compounds. For example, a receptor might bind most strongly to pentanol but it might also bind to butanol, hexanol and heptanol with lower affinities. A slight change in the structure of a compound or a change in its concentration can change the pattern of which receptors respond, and also change the intensity. According to Malnic *et al.* (1999), there are approximately 1000 odorant receptor genes in mammals. If each odorant were detected by only three receptors, theoretically, the system would be able to discriminate almost 10^{12} odours. Insects have far fewer odorant receptor genes; approximately 60 in *Drosophila* (Nozawa & Nei, 2007), more in the honeybee,

approximately 170 (Robertson & Wanner, 2006), and still more in ants. The number of genes corresponds closely to the number of olfactory glomeruli, and *C. floridanus* has approximately 460 glomeruli (Zube *et al.*, 2008) and therefore approximately also the same number of olfactory genes. The number of receptors corresponding to each gene is probably small but variable with the sensitivity for a given compound. Each axon extends from the receptor through the antennal nerve to the antennal lobe of the brain. Antennal lobes are particularly large in ants because: (i) ants rely strongly on olfaction (other insects use visual clues more) and (ii) pheromone communication is more advanced and elaborate in ants compared with other social insects (Gronenberg, 2008). It is particularly interesting that the response of *C. floridanus* glomeruli to the trail pheromone nerolic acid **56** was stable over a wide range of concentrations, seven to eight powers of ten, and the intensity of response was measured in the duration of the response (Zube *et al.*, 2008).

Without knowledge of the structure of any odorant receptors, we presume that they have three-dimensional surfaces onto which odour molecules of specific shape can be attached, rather as a substrate is attached to the active site of an enzyme. This means that the three-dimensional shape of a molecule, and hence the absolute configuration of a chiral molecule, affects which, if any, receptors accept that molecule. This helps to explain the importance of chirality, or handedness, of perceived molecules. Insects may respond in a variety of ways to pure enantiomers or mixtures of them. From the discussion above, it can be seen that frequently one enantiomer of a chiral structure is used as a trail pheromone by an ant species, whereas the other enantiomer is not present. Frequently, the presence of the unnatural isomer in a synthetic mixture does not affect the detection of the natural isomer. Mori (2007), in a review on chirality in pheromones, discusses examples where the presence of the unnatural isomer can inhibit the detection of the natural isomer, or the pheromone may consist of a specific blend of the isomers, and response is greatest to that blend, whereas others are less effective. Examples exist where the pheromone consists of both isomers, and the insect does not respond to either alone. In all, Mori (2007) lists ten ways in which insects may respond to enantiomeric pheromones.

Stereobiology refers to the shape of the receptors on the antenna that detect the volatile compounds. They can be very precise in the shape of the molecules that they accept, or they can be more accommodating, in binding a range of molecules of similar shape and polarity. There have been a few studies to see how specific the chemical structure must be for a compound to show activity as a trail pheromone. When the first trail pheromone (methyl 4-methylpyrrole-2-carboxylate **1**, Fig. 7) was isolated, considerable effort was given to examining similar structures that might show activity. The molecule of **1** is planar and rather rigid, and lends itself well to study. Over 30 compounds were synthesized and tested, but only two compounds, **71** and **72**, where the methyl group on the ring was replaced by a chlorine or bromine atom, showed comparable activity (Sonnet & Moser, 1972, 1973). For the pheromone of *T. caespitum*, consisting of the two pyrazine compounds **2** and **3**, the position and size of the alkyl groups is critical. When either 2,3-dimethylpyrazine **73**, or 2,6-dimethylpyrazine **74** replaced 2,5-dimethylpyrazine **3**, the pheromone mixture was inactive but, if trimethylpyrazine **7** replaced 3-ethyl-2,5-dimethyl pyrazine in the mixture, there was some activity (Attygalle & Morgan, 1984). The structure of methyl 6-methylsalicylate **4** is also largely rigid and is useful for examining the effects of small alterations in the structure or position of atoms. Thirty eight compounds of similar structure to **4** were examined in trail bioassays with *Tetramorium impurum* (Morgan *et al.*, 1990). Removal of the methyl ester group to give 6-methylsalicylic acid **75** totally removed activity, but *m*-cresol **76** (i.e. removal of the whole ester group) showed some activity. Addition of an extra methyl group to the benzene ring at position 5 (compound **77**) destroyed activity, but addition of an extra methyl group at positions 4 or 3 (compounds **78** and **79**) had no effect, and gave activity similar to the natural compound **4**. In the case of faranal **39** (Fig. 5), the 3-epimer (3*R*, 4*R*)-faranal **80** shows weak activity and does not interfere with the activity of the natural (3*S*, 4R)-enantiomer **39** if a trail is made of the mixture, and the C-10 double bond can be *cis* or *trans* without affecting activity (Kobayashi *et al.*, 1980; Koyama *et al.*, 1983). Efforts to predict the effects of a change of structure on pheromone activity remain ineffective at present.

Other insects

The ability to exploit a food source co-operatively or to organize the move of a whole colony to a new home are characteristics of social organization. In many respects, ants have attained the highest level of social organization and, consequently, have developed this use of scent trails to a high degree, although the habit of trail-following is not confined to ants. A number of trail pheromones, different in structure from those of ants, and tending to be less volatile, have been identified in termites (Morgan, 1990; Sillam-Dusses *et al.*, 2007). The aerial marking of foraging routes by some bees by intermittant deposits of odour marks on plants along their routes or at the flowers is also well known (Jarau *et al*, 2004). Bumblebees release pheromones in the nest to recruit to foraging (Granero *et al.*, 2005). Sub-social tent caterpillars of *Malacosoma* (Lepidoptera: Lasiocampidae) lay trails of 5β-cholestan-3-one **81**, a relatively involatile substance (Fitzgerald & Webster, 1993; Colasurdo & Despland, 2005). Caterpillars of *Eriogaster lanestris* (Lepidoptera: Lasiocampidae) also follow trails of compound **81** (Ruf *et al.*, 2001). Recently, it was reported that a female shield bug *Parastrachia japonensis* (Hemiptera: Parastrachiidae) orients towards its burrow by odour when bringing back food for its young (Hironaka *et al.*, 2007).

Conclusions

The investigation of trail pheromones has lagged after the active period in the 1990s when many formicine pheromones

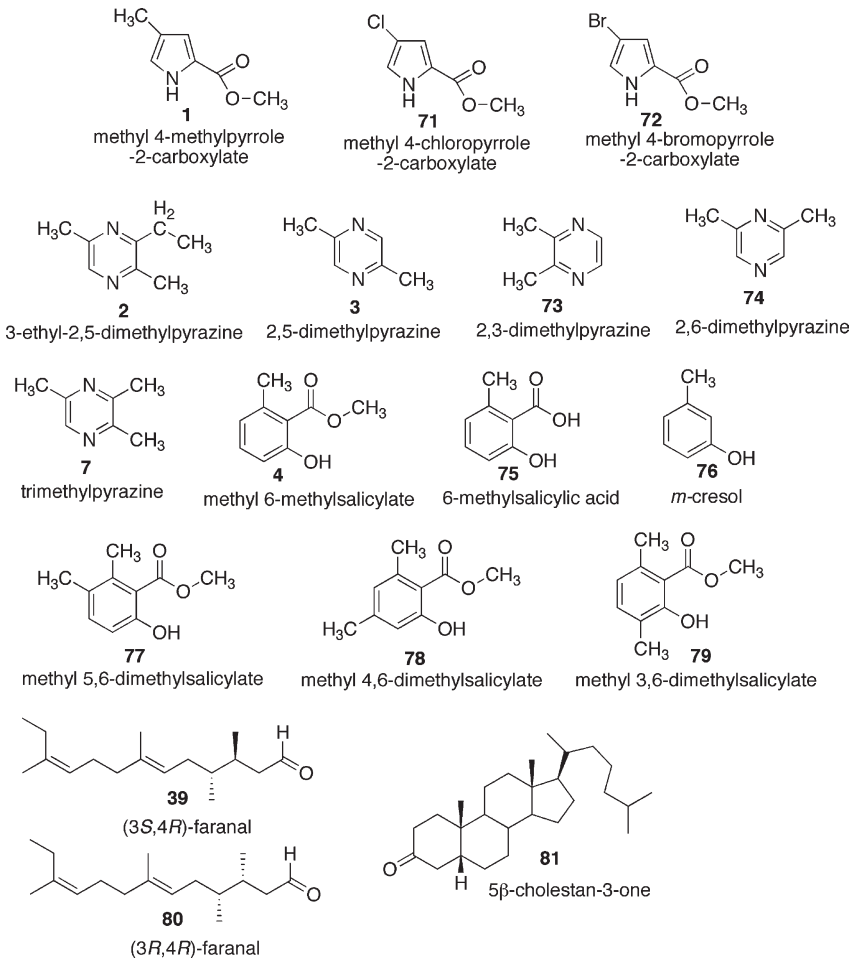

Fig. 7. Some examples of compounds studied in attempts to understand the specificity of olfactory receptors.

were identified. Subsequrent to the first trail pheromone discoveries, MS has advanced steadily, and its sensitivity has increased at least 100-fold. That should provide some encouragement. Further progress will depend upon the co-operation and dedication of chemists but will also require even more the acute observation and design of bioassays by entomologists. It is probable that yet more complex forms of recruitment and trail use will emerge for their work.

The importance of efficiency of foraging through recruitment to trails is illustrated by the story of the crazy ant *Paratrechina longicornis* (Formicinae), which infested the Biosphere 2 Center in Oracle, Arizona, shortly after it was built. It excluded virtually every animal that was not a mutualist or which was not protected in some way (Wetterer *et al.*, 1999). Its dominance inside the Biosphere appears to be related to its excellent exploitative ability rather than to any physical aggressiveness or defensive strategies (Witte *et al.*, 2007). It was simply faster to recruitment than competing species. The Dufour gland has an attracting and exciting effect (Witte *et al.*, 2007). It contains chiefly undecane and 2-tridecanone, and small amounts of ethyl phenylacetate and other compounds (Morgan *et al.*, 2005), but the trail pheromone, located in the hindgut as expected (Blum & Wilson, 1964), has not yet been identified (Witte *et al.*, 2007).

Acknowledgement

I thank Dr W. Gronenberg for guidance on understanding the perception of odours by ants.

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Accepted 25 September 2008
First published online 2 December 2008

