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CHEMICAL INVESTIGATIONS OF INSECT EXOCRINE GLANDS

by

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A thesis submitted to the University of Keele for the Degree of Doctor of Philosophy

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The trail-following component of the trail pheromone of *Pheidole pallidula* has been identified as a 3-ethyl-2,5-dimethylpyrazine. It is present in minor but not major workers. Another component which acts as primer appears to be required but it has not been identified.

A time study of the filling of the Dufour gland of *Formica sanguinea* from emergence of adults for several months has shown the gland fills slowly and the composition changes with time. A re-examination of the mandibular gland secretion of *Tetramorium caespitum* has shown it contains chiefly 4-methyl-3-hexanol not 3-octanone.

The Dufour glands of workers and queens of the myrmicine ants *leptothorax acervorum* and *Messor minor* and the formicine ant *Camponotus aethiops* have shown little differences between the castes. Dufour glands of workers of the myrmicine species *leptothorax nylander*!, *Messor capitatus*, *T. caespitum*, *Myrmica londae*, *Myrmica sabulei*, *Myrmica aloba*, and *Myrmica scabrinodis*, and the formicine species *Formica fusca*, *Formica lemani*, *Lasius fuliginosus*, *Camponotus aegyptiacus*, *Camponotus vagus* and *Cataglyphis savignyi* have been carried out. Examination of *T. caespitum* from a wide range of habitats has shown no significant differences in composition. For *F. fusca* and *F. lemani*, the substances of the Dufour gland enable the species to be distinguished chemically. Attempts to use Dufour gland substances to distinguish between a group of uncertain *Myrmica* species was inconclusive. The Dufour glands of major and minor workers of *C. aegyptiacus* have quite different composition, the former contain acetates in quantity.

Attempts to study the sexual pheromone of the beetle *Attagenus*
scalaris were frustrated by the inability to maintain live insects. Hexadecanoic acid, octadecanoic acid and octadecenoic acid were identified in the body in significant quantities and will have to be tested to see if they provide the pheromone.
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Behaviour and Communication

Behaviour can be simply defined as what animals do, or the ways in which an organism adjusts to, and interacts with, its environment (Matthews and Matthews, 1978). Any sensory signal which produces a behavioural change in an animal is an example of communication. The message may emanate from another animal as well as come from a plant. For the signal to evoke a behavioural response, the receiver has also to be in the requisite state of readiness.

Animals communicate between each other by means of 1) visual; 2) acoustic; 3) tactile and 4) chemical signals.

Visual communication as Carthy (1966) pointed out, shares with acoustic the advantage that when used in courtship it permits accurate location of the sender. The disadvantage is that receiver and sender must be close enough to see or hear each other, but the habits of many insects are such that their environment does not permit long visual ranges. Moreover the distance over which communication can occur is governed by the capacity of the insect eye to distinguish patterns and movements. The ranges will therefore be much shorter than those over which birds, for example, can communicate visually. Vision is clearly of limited value to nocturnal species (Lloyd, 1983). In daylight-feeding Acridoidea, for example, sight dominates sound (Otte, 1977).

Acoustic communication is important for those animals where small size means that they are frequently hidden by vegetation and hence
cannot signal visually. Acoustic communication has distinct advantages, for the caller does not have to leave cover to expose himself to possible dangers (Carthy, 1966). Auditory signals are less hindered by obstacles and can provide extended and detailed communication over distances far greater than the compound eye can function; but are less directional than sight (Lewis, 1984). In the Ensifera, sound is generally of greater importance than smell (Otte, 1977). Communication of alarm by acoustic means may be very common in ants. Stridulatory apparatus on the post-petiole and gaster was found in 83% of Myrmicinae and 48% of Ponerinae examined by Markl (1973). The leaf-cutting ants Atta cephalotes and Acromyrmex octospinosus stridulate when fighting or when trapped by part of the body (Markl, 1967). The ponerine ants Megaponera foetens respond to stridulation from sister workers when they encounter its termite prey (Trinervitermes) (Markl, 1973).

Tactile signals have distinctive qualities for very short range communication, for example, the violent kicking among crowded aphids used to achieve immediate spatial orientation (Dixon, 1963) and the zig-zag accelerated running of termites used to raise the general level of colony excitation (Stuart, 1961).

Chemical communication between individuals is widespread among insects, and highly developed among social insects. The chemical compounds may be diffused into the air or water, moved by currents deposited on food or flowers, or passed by trophallaxis.

Insects in general employ all these senses, and the evolution of greater acuity in one mode seems to change the relative importance of the others.

Chemical Communication

Any chemical which conveys information between organisms is
termed a semiochemical (Law and Regnier, 1971). Semiochemicals are divided into two subcategories (Nordlund and Lewis, 1976): i) Allelochemicals (Whittaker and Feeny, 1971) and ii) Pheromones.

Another way to categorize chemicals that modify animal behaviour is in terms of the type of behaviour they induce, according to the scheme of Dethier et al. (1960). This scheme has six categories:

1. **A locomotory stimulant**, a chemical which causes by a kinetic mechanism, insects to disperse from a region more rapidly than if the area did not contain the chemical. The effect may be to increase the speed of locomotion, to cause the insects to carry out avoiding reactions, or to decrease the rate of turning.

2. **An arrestant**, a chemical which causes insects to aggregate in contact with it, the mechanism of aggregation being kinetic. An arrestant may slow the linear progression of the insects by reducing actual speed of locomotion or by increasing turning rate.

3. **An attractant**, a chemical which causes insects to make oriented movements towards its source.

4. **A repellent**, a chemical which causes insects to make oriented movements away from its source.

5. **A feeding, mating, or ovipositional stimulant**, a chemical which elicits feeding or oviposition in insects.

6. **A deterrent**, a chemical which inhibits feeding or oviposition when present in a place where insects would, in its absence, feed or oviposit.

**Allelochemicals**

Allelochemicals are chemicals which convey information between organisms of different species (interspecific). Allelochemicals are categorised according to the advantage of the behavioural response
caused by the releasing compounds; if the receiving individual has the advantage, i.e. a predator locating its prey using prey odour, the allelochemics are called kairomones (Brown et al., 1970), e.g. volatile compounds originating from larvae of Drosophila are attractive for its parasite Leptopilina heterotoma and L. fimbriata (Vet and Hoeven, 1984); but if the advantage is turned to the odour-releasing individual, i.e. secretions deterring competing species from a limited resource or defensive secretions which directly repel predators, the allelochemics are called allomones (Brown et al., 1970), e.g. the formic acid spread by formicine ants against their predators. Another type of allelochemic is called a synomone (Nordlund and Lewis, 1976) which benefits both the releaser and recipient, for example, floral scents that attract pollinating insects (as honey bees do); a separate designator, apneumones (Nordlund and Lewis, 1976) is applied to signals from non-living emitters.

Pheromones

The term pheromone is derived from the Greek pherein, to transfer (carry); homan to excite. Pheromones are defined as substances which are secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction (Karlson and Butenandt, 1959; Karlson and Luscher, 1959).

An early example of the study of chemical communication within a species is given by Fabre (1914), who kept a single female of the emperor or hawk moth (Saturnia pavonia) in a screenwire cage placed in an open window between 8 and 10 p.m. A total of 150 marked males released from kilometers away were attracted to the female within eight subsequent days. Fabre repeated the experiment with a female oak spinner (Lasiocampa quercus) which was exposed in a window between 3 p.m. and sunset. Up to 60 males were attracted daily while the
female within the screen cage usually was completely motionless. Mell (1922) released a few marked male of the Chinese silkworm moth (Actias selene) from a train at different distances of 4.1 and 11 km from caged females in a gauze cage. Respectively 40 and 26% of the males found their way back to the female. It was claimed that males of the gypsy moth (Lymantria dispar) were able to find a female moth from a distance of 3.0 km (Collins and Potts, 1932).

Although it seems obvious now that the sense of smell is involved, Fabre and the others could not deduce this. The scientific interest in olfactory communication did not blossom until the late 1950s when the chemical identification of bombykol, the first insect pheromone was isolated from the silkworm moth (Bombyx mori) in Germany (Butenandt, 1959).

Types of Pheromones

In 1963, Wilson and Bossert proposed dividing pheromones into two categories according to their mode of influence: 1) Primer pheromones which induce relatively long-lasting physiological changes in receiving individuals; 2) releaser pheromones which stimulate the receiving individual to perform immediate behavioural responses.

Primer Pheromones

Primer pheromones are, for example, secreted by queens (ovary and queen cell construction inhibitors) as well as workers (caste inhibitors) in social insects (bees, ants, wasps and termites) and in subsocial insects secreted as growth accelerating agents (as in migratory locusts). One primer pheromone is trans-9-oxo-2-decenoic acid (9ODA) which has been identified as part of the mandibular gland secretions of honeybee queens of Apis mellifera (Butler et al., 1961). It is the most effective dominance and inhibition signal of the queen.
The workers that become egg-layers after removal of the queen also produce 900A in their mandibular glands, therefore inhibiting ovarian development in sister workers (Ruttner et al., 1976; Crewe and Velthuis, 1980). Primer pheromones from queen larvae, queen pupae as well as from adult queens are obtained by workers which distribute it to other bees in transferred food and by antennal contact (Free and Ferguson, 1982; Free et al., 1984).

While pheromones must certainly be involved in queen control in ants (Wilson, 1971), none have yet been identified and there is little direct evidence for their existence, because of the difficulties of assaying for them. With the development of a reliable bioassay, Fletcher and Blum (1983) and Vargo and Fletcher (1986) found that queen pheromones had been involved in control over the production of male and female sexuals in Solenopsis invicta. Queens or freshly killed corpses of egg-laying queens inhibit the numbers of sexuals (male and female) (Vargo and Fletcher, 1986). Also fertile queens of Monomorium pharaonis are known to inhibit the development of both male and female sexuals from queen-laid eggs (Petersen-Braun, 1977). Berndt (1977) indicated that daily rinsing of fertile queens of M. pharaonis in organic solvent removed their ability to inhibit the production of new sexuals. Virgin queens are unable to inhibit the rearing of new sexuals (Berndt and Nitschmann, 1979). In Oecophylla longinoda and O. smaragdina, the queens inhibit workers from laying reproductive eggs (male-producing) (Holldobler and Wilson, 1983). Queens of Plagiolepis pygmaea inhibit the sexualization of female larvae (Passera, 1980).

Primer pheromones also play an important role in caste determination. Adult majors (soldiers) of Pheidole inhibit to a limited extent the induction of development of major workers from larvae by secreting a contact soldier pheromone (Wheeler and Nijhout, 1984). They found that induction of soldier development of Pheidole
bicarinata is inhibited in methoprene-treated larvae and the degree of inhibition increase with increasing contact between larvae and adult soldiers. Greg (1942) found that soldiers of Pheidole morrisi inhibit the production of more soldiers.

Primer pheromones not only act as inhibitor agents but also have the opposite effect, as adult males of the migratory locust Schistocerca gregaria secrete a volatile substance (surface pheromone) that accelerates the growth of both male and female young for synchronizing the maturation within the species (Morris, 1954, 1964).

Releaser Pheromones

Releaser pheromones mediate a wide variety of behavior and we try to categorize them collectively in a simple and broad classification according to the function or the behavior that they elicit in the receiving insect into 1) sex pheromones, 2) aggregation pheromones, 3) dispersal or spacing pheromones, 4) alarm pheromones, and 5) recruitment or trail pheromones (Birch and Haynes, 1982); 6) territorial or home range pheromones (Cammaerts et al., 1977; Holldobler and Wilson, 1977); 7) surface pheromones (Wilson, 1971). 8) funeral pheromones (Wilson et al., 1958; Wilson, 1963a; Matthews and Matthews, 1978) and 9) invitation pheromones (Ahmadi and McClelland, 1985).

Sex Pheromones

Sex pheromones are usually released by the female insect to advertise her presence, i.e. to lure the male for successful mating; if the chemical releases long range mate location it is called a sex attractant, while the chemical which facilitates courtship or prepares the opposite sex for copulation is called an aphrodisiac (Birch, 1974; Birch and Haynes, 1982) and is usually released by males.
Sex Pheromones of Non-Social Insects

Non-social insects (solitary) depend on sex or aggregation pheromones as the major means of bringing individuals together for reproduction and survival.

Production and release of sex pheromones and response to it, depends mainly on time of day and age of insects and differs from one insect species to another. Sex pheromones are produced only at certain times of day and released as they are found, and there is a synchronization between time of release from females and response from male insects of the same species.

Extensive studies have been made on sex pheromones of many species of the orders Lepidoptera and Coleoptera since research started with the identification of bombykol (trans-10, cis-12-hexadecadieno1) (I) (Butenandt, 1959).

Order: Coleoptera, family: Dermestidae

Virgin females exhibit calling behaviour which is associated with pheromone release. There is a characteristic sequence of actions from the responding males, which include: extension of the antennae, elevation of the body by leg extension, rapid zig-zag running, and intermittent bobbing up and down (Barak and Burkholder, 1977).

The black carpet beetle, Attagenus elongatus (Casey) females employ (Z,Z)-3,5-tetradecadienoic acid as the sex pheromone (Fukui et al., 1977). The crude pheromone was extracted using n-hexane from virgin females (1500 individuals) along with filter papers from jars where females were reared. The pheromone was isolated using silicic acid powder column (Mallincrodt) 100-200 mesh. The column was successively eluted with n-hexane, benzene, chloroform and ethanol and the active fraction was purified by silica gel thin-layer plates eluted with a mixture of benzene and acetone (6:1). Then the active
fraction was collected and esterified using diazomethane and bioassayed in the form of methylester which was active as well as the acid. The methylester was purified using another silica gel plate eluted with benzene and the collected methylester (in acetone) was finally purified by injection into the gas chromatograph and trapping the effluent using a splitter. The identification of the purified methylester was performed using GC-MS. In each case the active fraction and the synthetic compound were bioassayed using a small-vial test. The sex pheromone of Anthrenus flavipes (Leconte) females, the closely related genus to Attagenus, was extracted and purified in an identical way as in Attagenus elongatulus and identified as (Z)-3-decenoyl acid (Fukui et al., 1974). The sex pheromones of four Trogoderma species (family Dermestidae) were identified (Cross et al., 1976). The active compound from virgin females maintained on strips of filter paper within glass chambers were collected by aeration on Porapak-Q. After aeration, the Porapak and filter paper samples were extracted into redistilled hexane and concentrated. Identification of the active compound from Porapak extract without purification was performed using GC-MS. Both T. inclusum and T. variabilis females employ (Z)-14-methyl-8-hexadecenal; T. glabrum females employ (E)-14-methyl-8-hexadecenal and T. granarium females employ both (Z)- and (E)-14-methyl-8-hexadecenal.

*Attagenus scalaris (Pic)*

*Attagenus scalaris* is one of the species of the black carpet beetles that belong to the subfamily Attageninae, family Dermestidae, order Coleoptera.

The larvae of carpet beetles are believed to be the most important pests of fabrics in storage. They feed on anything that contains wool or other animal protein fibres, such as fur and
Sex Pheromones of Social Insects

In queens of the honeybee Apis mellifera queens, trans-9-oxodec-2-enoic acid in the mandibular gland secretion (Butler et al., 1961) acts not only as primer pheromone but also as a sex pheromone outside the nest, it stimulates drones to fly towards the virgin queen (Gary, 1962; Butler and Fairy, 1964). Robacker and Hendry (1977) showed that neral and geranial from crude extract of virgin females are components of the sex pheromone of the wasp Itopectis conquistor, eliciting male sexual activity.

In ants, a variety of glands are responsible for sex pheromones secretion; the poison, Dufour and pygidial glands in females and mandibular glands in males. The myrmicine ant Harpagoxenus sublaevis females release a sex pheromone from the poison gland (Buschinger, 1972) in a sexual-calling behaviour when females erect their gasters (Buschinger, 1968; 1983). In Pogonomyrmex females, poison gland secretions stimulate sexual behaviour in males (Holldobler, 1978) and the sympatric species are reproductively isolated in part by their distinct daily nuptial flight rhythms (Holldobler, 1977). The myrmicine species Xenomyrmex floridanus female, produce a sex pheromone in the poison gland (Holldobler, 1971a). The Dufour gland in Monomorium pharaonis queens secretes a sex pheromone (Holldobler and Wust, 1973). Both sexes of the carpenter ant Camponotus herculeanus take off during the nuptial flight and are synchronized by a secretion released from the mandibular glands of males (Holldobler and Maschwitz, 1965; Holldobler, 1978). In the ponerine ants, the wingless virgin females of Rhytidoponera metallica attract males by the release of a pheromone from the pygidial gland (Holldobler and Haskins, 1977; Holldobler, 1978). They demonstrated that ergatoid R.
Metallicas emerge from the nest and group quietly near their nest entrance with the head and thorax lowered to the ground, the gaster raised and arched, and the intersegmental membrane between the last two segments dorsally extended. Males flying out from other nests are attracted by these "calling" females.

Invitation Pheromones

Chemical compounds may be released from a parasite when it has fed on its host, these increase the number of individuals of the same parasite arriving on the host. For example, female mosquitoes find their host primarily by attraction to kairomones released from the host, then they themselves release chemical compounds (invitation pheromones) to attract other female mosquitoes of the same species for a meal (Ahmadi and McClelland, 1985).

Aggregation Pheromones

Aggregation pheromones are chemical compounds which release a behavioural response in members of a species (conspecifics) leading to congregation, i.e. to increase their density in the vicinity of the source of the pheromone. A wide variety of arthropods assemble for various purposes such as feeding, mating, or hibernation. Most aggregations are temporary except in social insect colonies. In many cases, gregarious behaviour functions to bring the sexes together for mating. The bark-beetle Dendroctonus ponderosae (Scolytidae), is a well documented example of temporary aggregation to a food site and increasingly probability of mating. Female D. ponderosae release trans-verbenol after finding a suitable host tree (Vite and Pitman, 1968; Pitman and Vite, 1969). Trans-verbenol attracts males and the attractiveness is synergized by a host-tree monoterpene (myrcene) which is the most potent synergist (Conn et al., 1983; Borden et al., 1983). The arriving males secrete (+)-exo-brevicomin (Pitman et al.,
1969; Borden et al., 1983; Conn et al., 1983) which attracts mainly female beetles. The combined action of myrcene, trans-verbenol and exo-brevicomin results in a mass attraction of both sexes.

Of all the types of pheromonally induced insect aggregation, perhaps the most dramatic is exhibited by the fertilized social insect queen, continuously surrounded by a retinue of crowding, licking, food-offering attendants. If a colony of honeybees (Apis mellifera) is deprived of its hive and combs it behaves like a reproductive swarm, and will cluster on any convenient support. When the queen is taken from the swarm soon after it has settled, the bees usually become disturbed within 10-15 minutes and return to the old hive (Simpson, 1963). The queen mandibular gland secretion, trans-9-hydroxy-dec-2-enolic acid is responsible for re-forming and stabilizing the dispersing clusters (Butler et al., 1964).

**Dispersal or Spacing Pheromones**

Dispersal or spacing pheromones stimulate behaviour leading to increasing space between individuals (dispersion), consequently it gains a reduction in intraspecific competition. The compounds may be considered to act as deterrents or repellents.

The presence of an oviposition-deterring mechanism among insects was suggested by field work done by Chiang et al. (1960) with the European corn borer (Ostrinia nubilalis), which showed that heavily infested corn plants deter second-brood moths from oviposition. Dittrick et al. (1983), demonstrated that a methanol extract of frass of fifth-instar larvae of Ostrinia nubilalis was deterrent for oviposition by adults and the effect lasted for at least 72 hours under laboratory conditions. Prokopy (1972) noted that female apple maggot flies Rhagoletis pomonella mark the fruit following oviposition with a pheromone by dragging the ovipositor across the surface, that
deters subsequent oviposition and mediates accommodation of larval density relative to food availability. Renwick and Radke (1980, 1981) demonstrated that gravid Trichoplusia ni were deterred from oviposition up to three days by first-instar larvae feeding on cabbage and by aqueous extracts of larval frass. The mandibular gland secretion of larvae of the flour moth, Anagasta kuhniella induces dispersion between larvae individuals, increasing life span and lowering fecundity of females crowded as larvae (Corbet, 1971) that leads to regulation of larval density in this stored product pest. The hymenopterous parasite, Trichogramma evanescens deposits dispersion pheromones on lepidopteran eggs in which she has oviposited, resulting in a uniformity of egg deposition among the available oviposition sites (Salt, 1937).

Alarm Pheromones

The original meaning of alarm was to call to arms during danger (Pasteels, 1975) which seems an apt description of some insect behaviour. Alarm and defensive behaviour often go side by side. Chemical releasers of alarm behaviour are considered to be volatile compounds and produce stereotyped reactions among social insects which accelerate movement (or rapid flight in flying insects) and attack (Brown, 1960). An increased rate of locomotion, characterized by zig-zag or circular movements, often results when workers are exposed to alarm pheromones (Maschwitz, 1964). Lofqvist (1976) studied alarm pheromones of Formica rufa and pointed out that the alarm behaviour includes many steps: 1) ants raise and wave their antennae around in the air, 2) opening the mandibles, 3) slow movements towards the odour source with opened mandibles and raised antennae, 4) fast movements if ants are exposed to a strong stimulus and they are looking for an enemy (fast-running phase), 5) attack behaviour when ants meet an
intruder, 6) cleaning of the antennae and the abdominal tip. Alarm pheromones are known to occur in hymenopterous, isopterous and heteropterous insects.

The green peach aphid, *Myzus persicae* secretes trans-farnesene as an alarm pheromone from its cornicles (Edwards et al., 1973). Ant-associated (*Myrmecophilus*) aphid species when attacked by predators, secrete sticky droplets, composed largely of triglycerides, which can hinder an attacking predator and result in the release of the aphid prey; the droplets also contain trans-B-farnesene, as alarm pheromone causing aphids to fall, jump, or walk away (Nault et al., 1976).

In the termite *Macrotermes subhyalinus*, the soldier's frontal gland secretes n-tricosane, n-pentacosane, 3- and 5-methylpentacosane, 5-methylheptacosane, (Z)-9-heptacosene and (Z)-9-nonacosene as major compounds and the secretion appears to impair the healing of wounds in test ants and thus could represent a valuable supplement to the mechanical defense mechanism (Prestwich et al., 1977).

In social wasp, *Polistes exclamans* and *P. fuscatus*, the venom elicits alarm and attraction and heterospecific venom elicits an alarm response (Post et al., 1984). In *Apis mellifera*, the Koshevinkov's gland which, along with the setose membrane adjacent to the sting organ, produces a collection of aliphatic acetates including the main alarm pheromone isopentyl acetate (Boch et al., 1962; Gunnison and Morse, 1968), and n-butyl acetate (Free et al., 1983; Al-Sa'ad et al., 1985).

In myrmicine ants, the mandibular and pygidial gland secretions act as alarm pheromones. Maschwitz (1964) reported that crushed heads of *T. caespitum* released an alarm behaviour. 4-Methyl-3-hexanol and 4-methyl-3-hexanone act as attractants for workers of *T. impurum*.
(Pasteels et al., 1980). In Atta texana, 4-methyl-3-heptanone acts as an alarm pheromone (Riley et al., 1974b). McGurk et al. (1966) isolated 4-methyl-3-heptanone as the mandibular gland secretion of Pogonomyrmex barbatus, P. californicus, P. desertorum, and P. occidentalis. It appears to function as an alarm pheromone, and apparently releases alarm behaviour in P. barbatus. In P. barbatus (McGurk et al., 1966) and P. badius (Wilson, 1958), the ants when exposed for a few minutes to the ketone, carry pebbles in their mandibles and infrequently make digging movements with their legs. Crew and Blum (1970a, b) identified 6-methyl-3-octanone, 3-nonanone, 3-octanone and 3-octanol as alarm pheromones in ten Myrmica species. Another study with Myrmica species indicated that the mandibular gland secretions act as an alarm pheromone; 3-octanol is an attractant for workers, 3-octanone increases the effect of 3-octanol, and 3-nonanone increase the linear speed of the ants (Morgan et al., 1978; Cammaerts, et al., 1981b, 1983, 1985a). In Manica mutica (Fales et al., 1972), 4,6-dimethyl-4-octene-3-one (manicone) functions as a powerful releaser of alarm behaviour; a weaker response was obtained to 4-methyl-3-hexanone, 4-methyl-3-heptanone, 3-octanone and 3-decanone; the mixture of the five ketones made up in the same proportion as they were determined to be present in the mandibular gland did not appear to be any more active as an alarm releaser than manicone alone.

Hayashi and Komae (1977) indicated that B-pinene, limonene, O-pinene and camphene from the whole extract of Pristomyrmex pungens may act as alarm pheromone. In Pheidole biconstricta, the pygidial gland produce an alarm-defense secretion (Kugler, 1979). In Novomessor cockerelli and N. albatisosus, the pygidial gland secretes a strong smelling secretion which causes "panic alarm" response in workers (Holldobler, 1982).

In formicine ants alarm releasers appear to originate from
mandibular, poison and Dufour glands. In the African weaver ant, *Oecophylla longinoda* (Bradshaw et al., 1979a) the mandibular gland of major workers contains multicomponent alarm pheromones, which release behavioural responses including components for alerting, attraction and biting. The gland contains more than 30 different components. Hexanal and 1-hexanol, release alerting and attraction respectively; 2-butyl-2-octenal and 3-undecanone act as makers for attack (Bradshaw et al., 1979a). In addition to the mandibular gland, major workers use the poison and Dufour gland as sources of alarm pheromones (Bradshaw et al., 1979b), they release formic acid from the poison gland and n-undecane, 4-tridecane, 8-heptadecene and 4,7-heptadecadiene from the Dufour gland. Formic acid elicits a 'mass attack' response in other major workers; formic acid and Dufour gland secretions are considerably more effective than either secretion tested separately. These compounds act in combination with the mandibular gland secretions to form a complex alarm/defense system.

In the slave-keeping ants, *Formica sanguinea*, *F. subintegra* and *F. pergandi* Dufour and poison gland secretions are used against the slave species *Formica subsericea* and *F. fusca*; formic acid from the poison gland and decyl, dodecyl and tetradecyl acetate from the Dufour gland (Regnier and Wilson, 1971) act as defensive and offensive weapons. In *Lasius niger*, only undecane releases an alarm behaviour in the ants while decyl acetate or dodecyl acetate cause the ants to move their antennae only and open their jaws but they do not show any excitement (Bergstrom and Lofqvist, 1970). Dumpert (1972) indicated that n-undecane functions as an alarm substance for workers of *Lasius fuliginosus*. It has variously been claimed that the undecane acts as an alarm substance (Regnier and Wilson, 1968, 1969; Ayre and Blum, 1971; Bergstrom and Lofqvist, 1970, Lofqvist, 1976) or as a spreading agent for the formic acid released from the poison gland (Regnier and
Wilson, 1968; Lofqvist, 1976). The mandibular gland secretion of Polyrhachis simplex contains a mixture of 4-heptanone, 6-methyl-5-hepten-2-one and 6-methyl-5-hepten-2-ol and acts as an alarm pheromone (Hefetz and Lloyd, 1982). In Cataglyphis bombycina the mandibular gland secretion consists of citronellol and geraniol while C. nigra contains only geraniol and the secretion in both species act as alarm pheromones (Hefetz and Lloyd, 1985).

In the new world army ants (Dorylinae, tribe Ecitonini) Eciton hamatum, Nomaomyrmex esenbecki and Labidus praedator, the detached heads of workers or soldiers or objects smeared with the substance from the crushed heads of soldiers were attacked by their nestmates when placed in the foraging column, while the remainder of the body evoked little or no response (Brown, 1960).

In Aneuretus simoni, the only living representative of the subfamily Aneuretinae, pygidial gland secretion caused aggressive alarm without having a repellent function (Traniello and Jayasuriya, 1981a).

In ponerine ants, Odontomachus troglodytes, the mandibular gland secretion, 2,6-dimethyl-3-n-butyl, n-pentyl and n-hexylpyrazine components released the full range of behaviour in workers (alerting, approach and attack) whereas the fragile males retreat (Longhurst et al., 1978). In Gnamptogenys pleurodon, methyl 6-methylsalicylate functions as an alarm pheromone (Duffield and Blum, 1975). In Neoponera villosa, 4-methyl-3-heptanone acts as alarm pheromone (Duffield and Blum, 1973).

The dolichoderine ant Iridomyrmex pruinosus contain an anal gland (better known as pygidial gland) as the source of an alarm pheromone methyl-n-amyl ketone (Blum et al., 1963).
Trail Pheromones

A trail pheromone is a chemical substance or mixture of substances applied to a surface by a first worker which is detected and followed by other individuals. When a foraging worker finds a food source, she returns to the nest laying broken streaks of scent trail to direct nestmates to follow the pathway to the food source, and as they return to the nest, reinforce the chemical deposit. When the food source is exhausted, the returning workers do not reinforce the trail so that it evaporates away.

Trail pheromones are widely used by ants, some termites and some stingless bees.

In termites, the sternal glands, found in a number of species of several families, situated on the 4th or the 5th sternite, is the only source of trail pheromones (Luscher and Muller, 1960; Stuart, 1961b, 1963). Birch et al. (1972) identified nasutene (cembrene-A) as the trail pheromone from the workers of the Australian nasute termite *Nasutitermes exitiosus*. The trail pheromone of the grass-feeding termite *Trinervitermes bettonianus* has been identified as the diterpene hydrocarbon cembrene-A (McDowell and Olooloo, 1984); and workers, male alates and female alates produce the pheromone in the ratio, 1:62:1090 (12ug) respectively, and the compound is active at 0.5ng/cm. Soldiers of *T. bettonianus* do not take part in active trail laying (Leuthold and Luscher, 1974), while soldiers of *Nasutitermes costalis* lay trails and are responsible for the organisation of foraging in this species (Traniello, 1981).

The aerial odour trail is used by flying insects and has been demonstrated in some stingless bees (von Frisch, 1967; Lindauer and Kerr, 1960). The mandibular gland secretion is used as a trail pheromone, the scout bee that has located a food source, deposits drops of the trail pheromone at specific intervals on foliage, twigs and
other substrates on its way back to the nest. Blum et al. (1970) identified (E)- and (Z)-citra1 as major components of the mandibular gland secretion used as the trail pheromone of Trigona subterranea.

In ants, the poison gland, Dufour gland, meta-tibial gland, hind-gut gland, rectal gland, Pavan's gland, pygidial gland or sternal gland, may be the source of trail pheromones. The location of the general exocrine glands of a typical ant is shown in Fig. 1. It is well known that foragers in ants lay scent trails on the ground which can be followed by other individuals (Forel, 1908; Goetsch, 1934; Carthy, 1950; 1951; 1952). When an ant is following such a trail it is guided primarily by the scent marks, although other orienting factors including visual stimuli, are sometimes involved (Goetsch, 1934; Sudd, 1959). Trail pheromones may be applied in mass-foraging recruitment, colony migration (Wilson, 1962) or initiation and guidance of slave-raids (Regnler and Wilson, 1971).

In myrmicine ants, the poison, Dufour, and tibial glands are the source of trail pheromones. Tandem running is a very primitive form of recruitment as observed in Leptothorax acervorum (Wilson, 1971) in which a scout ant, after finding a food source or a new nest site, leads nestmates, one at a time, by keeping the recruited ant in close antennal contact with the leader ant. The recruited worker may invite nestmates to tandem following by "tandem calling behaviour" in which it extrudes the sting and releases a secretion from the poison gland (Moglilch et al., 1974). Moglich (1979) demonstrated that there is a subgenus-specificity of the tandem calling pheromone between the subgenus Leptothorax and Mycothorax. Tandem running was also investigated in Harpagoxenus sublaevis (Buschinger and Winter, 1977). The poison gland is involved in tandem running. The chemical and tactile signals involved in tandem running seem to be the same for Harpagoxenus and the slave species L. acervorum, since mixed tandems
Fig. 1: Idealized section through a typical ant showing the location of known exocrine glands.
were noticed with a Harpagoxenus as leader and vice versa.

Transposition studies of the trail pheromones among myrmicine ants, indicated that there is a variability of specificity. Blum et al. (1964), demonstrated that the poison glands are the source of the trail pheromone in the attine species, Cyphomyrmex rimosus, Trachymyrmex septentrionalis, Acromyrmex octospinosus, Atta cephalotes and Atta texana and were found to be non-species-specific (Table 1). In a cross trail-following test among Sericomyrmex urichi, Trachymyrmex septentrionalis and Atta texana (Blum and Portocarrero, 1966), it was indicated that S. urichi did not follow odour trails of A. texana, T. septentrionalis or D. armigerum; and S. urichi trail did not release trail-following in workers of T. septentrionalis and only slightly in workers of A. texana (Table 2). Daceton armigerum a myrmicine ant and a primitive member of the tribe Dacetonini, does not lay odour trails, but its venom elicits a trail-following activity in some attine ants including, T. septentrionalis, Acromyrmex coronatus, Acromyrmex nr. coronatus, Atta texana and Atta cephalotes (Blum and Portocarrero, 1966 (Table 2).

In the myrmicine genera Myrmica, Manica, Pogonomyrmex and Veromessor, the poison gland is the source of the trail pheromone (Blum, 1974). The same author demonstrated that Aphaenogaster fulva and Novomessor cockerelli workers did not follow artificial extracts of their own sting-associated glands or hind guts, but Holldobler et al. (1978) demonstrated that Novomessor cockerelli workers lay a chemical trail from the poison gland. In a cross trail-following test among Myrmica, Manica and Pogonomyrmex species (Blum, 1974), it was found there was no intra- and inter-generic specificity (Table 3) while Pogonomyrmex barbatus elicited a trail-following activity in Myrmica brevinodis and Manica bradleyi but did not do so in Pogonomyrmex badius. Aphaenogaster fulva, Novomessor cockerelli and
TABLE 1
Responses of some Attine Ants to artificial trails laid from their poison glands

<table>
<thead>
<tr>
<th>Source Species</th>
<th>Test species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. rimosus</td>
</tr>
<tr>
<td>Cyphomyrmex rimosus</td>
<td>+++</td>
</tr>
<tr>
<td>Trachymyrmex septentrionalis</td>
<td>++</td>
</tr>
<tr>
<td>Acromyrmex octospinosus</td>
<td>++</td>
</tr>
<tr>
<td>Atta cephalotes</td>
<td>+</td>
</tr>
<tr>
<td>Atta texana</td>
<td>+++</td>
</tr>
</tbody>
</table>

a Response Code:  o - no trail-following activity;  +++ - high trail-following activity.
Reference: Blum et al. (1964).

TABLE 2
Responses of some Myrmicine ants to artificial trails laid from their poison glands

<table>
<thead>
<tr>
<th>Source species</th>
<th>Test species</th>
</tr>
</thead>
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<tr>
<td></td>
<td>S. urichi</td>
</tr>
<tr>
<td>Sericomyrmex urichi</td>
<td>+++</td>
</tr>
<tr>
<td>Trachymyrmex septentrionalis</td>
<td>0</td>
</tr>
<tr>
<td>Atta texana</td>
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<tr>
<td>Dacetan armigerum</td>
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</tr>
</tbody>
</table>

a Response Code:  o - no trail-following activity;  +++ - high trail-following activity.
TABLE 3

Responses of some Myrmicine ants to artificial trails laid from their poison glands\textsuperscript{a}

<table>
<thead>
<tr>
<th>Source species</th>
<th>M. bradleyi</th>
<th>M. hunteri</th>
<th>M. mutica</th>
<th>M. americana</th>
<th>M. brevinodis</th>
<th>M. brevispinosa</th>
<th>M. emeryana</th>
<th>M. fracticornis</th>
<th>M. monticola</th>
<th>M. rubra</th>
<th>P. badius</th>
</tr>
</thead>
<tbody>
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<td>+++</td>
<td>+++</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Manica hunteri</td>
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<td></td>
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<td>+++</td>
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<td></td>
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</tr>
<tr>
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<td>+++</td>
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<td></td>
<td>+++</td>
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</tr>
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<td>Myrmica brevispinosa</td>
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<td></td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Myrmica emeryana</td>
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<td>+++</td>
<td></td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Myrmica fracticornis</td>
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<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td></td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Myrmica monticola</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myrmica rubra</td>
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<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>Pogonomymex badius</td>
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<td>+++</td>
<td>+++</td>
<td>+++</td>
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<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Pogonomymex barbatus</td>
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<td></td>
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</tr>
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<td>Aphaenogaster fulva</td>
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<td></td>
<td></td>
<td></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Novomessor cockerelli</td>
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<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veromessor pergandei</td>
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<td>0</td>
<td>0</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Response code: 0 - no trail-following activity; +++ high trail-following activity

Veromessor pergandei did not elicit trail-following activity in Myrmica or Manica workers. In eight species of Myrmica, the poison gland is the source of the trail pheromone and there is no species-specificity in trail-following behaviour, since they employ one compound as the trail pheromone (Evershed et al., 1981, 1982). Attygalle et al. (1986) showed that Manica rubida employs the trail pheromone of Myrmica species consequently it was found that Manica rubida workers follow the trail pheromone of Myrmica rubra and vice versa.

Tetramorium guineense and T. caespitum produce their trail pheromones from their poison gland and both species showed species-specificity in a trail-following test (Blum and Ross, 1965). T. guineense workers followed artificial trails prepared from the poison glands of the two attines, Trachymyrmex septentrionalis and Atta texana and vice versa; the Dufour gland of Solenopsis saevissima ("invicta") was totally inactive as a releaser of trail-following when tested with the other myrmicine. Attygalle and Morgan (1984a) found that T. caespitum and Myrmica ruginodis follow each other's trail but less well than its own, where T. caespitum employs 2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine, while M. ruginodis employs 3-ethyl-2,5-dimethylypyrazine alone and there it is produced in higher quantity than in T. caespitum.

Novomessor cockerelli and N. albisetosus employ the poison glands secretions for chemical communication as a short-range recruitment, in which the scout releases the poison gland secretion into the air after locating a prey, the nestmates already in the vicinity are attracted from as far away as 2m and move upwind toward the prey; the same two species also employ the poison gland secretions for long-range recruitment, in which, if the scout failed to attract enough foragers in a short range recruitment, she lays a chemical trail from the prey
to the nest to be followed by the nestmates toward the prey (Holldobler et al., 1978). In a cross-test of the recruitment trail pheromone between *M. cockerelli* and *M. albisetosus*, it was found that *M. cockerelli* responds only to its conspecific, while *M. albisetosus* does not discriminate between its own trail substance and that of the second species; neither of the two species responded to poison gland trails of several *Pogonomyrmex* species (Holldobler et al., 1978).

The trail pheromone of *Monomorium minimum*, *M. floricola*, *M. pharaonis* and *Hubera striata* originates in the poison gland (Blum, 1966). Holldobler (1973) investigated the Dufour gland of *M. pharaonis* as the source of the trail pheromone. In transposition tests among the species, it was demonstrated that a species-specificity was present among the poison gland extracts of the species, but *M. minimum* followed *M. pharaonis*’ trail while *M. pharaonis* did not follow *M. minimum*’s trail (Blum, 1966). The poison gland extract of *Cardiocondyla nuda minutor* a myrmicine ant that does not lay trails, is somewhat active as a releaser of trail-following for *M. minimum* (Blum, 1966).

Wilson (1959) showed that the Dufour gland is the source of the trail pheromones of *Solenopsis* species, which carry out mass-foraging. The secretion is a powerful attractant, it excites workers, draws them out of the nest and directs them toward the trail, while workers unable to reach the mass turn back without laying trails (Wilson, 1962). The trail substances are somewhat species-specific where in transposition experiments utilizing *Solenopsis invicta* (saevissima), *S. geminata* and *S. xyloni*, it was found that *S. invicta* did not elicit a trail-following in *S. geminata* and *S. geminata* did not elicit a trail-following in *S. invicta* and *S. xyloni*, while *S. xyloni* elicits a trail-following in *S. geminata* and *S. invicta* (Table 4) (Wilson, 1962). *S. richteri* and *S. invicta* follow each other’s trails and they
### TABLE 4

Species-specificity of trails laid with Dufour gland extracts of some fire ants\(^a\)

<table>
<thead>
<tr>
<th>Source species</th>
<th>Test Species</th>
<th>(S.) richteri</th>
<th>(S.) invicta</th>
<th>(S.) geminata</th>
<th>(S.) xyloni</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S.) richteri</td>
<td>(+++^2)</td>
<td>(+++^2)</td>
<td>(o^2)</td>
<td>(+^2)</td>
<td></td>
</tr>
<tr>
<td>(S.) invicta</td>
<td>(+++^2)</td>
<td>(+^1,2)</td>
<td>(o^1,2)</td>
<td>(+^2)</td>
<td></td>
</tr>
<tr>
<td>(S.) geminata</td>
<td>(+^2)</td>
<td>(o^1,2)</td>
<td>(+^1,2)</td>
<td>(+^2, o^1)</td>
<td></td>
</tr>
<tr>
<td>(S.) xyloni</td>
<td>(+++^2)</td>
<td>(o^2; +++^1)</td>
<td>(+^1,2)</td>
<td>(+^1,2)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Response code:  
- o - no trail-following activity;  
- +++ - high trail-following activity.

Reference:  
1. Wilson (1962)  
2. Barlin et al. (1976a)
elicit a little activity in S. xylophora; S. geminata and S. xyloni; follow each other's trails and they elicit activity in S. richteri, but S. geminata elicit less activity (Table 4) (Barlin et al., 1976a). There are some contradictory result between Wilson's and Barlin's investigations as shown in Table 4. Vander Meers (1986) explained that for a successful foraging ant exploring a new food source, its trail must first attract or recruit workers to the trail and secondly induce or initiate the workers to follow the trail, so the successful foraging for S. invicta involves steps of behaviour starting with a) recruitment, followed by b) an orientation primer, followed by c) orientation. In S. invicta (Z,E)-oc-farnesene is the major component and it solely elicits the most sensitive response in the trail orientation and it is responsible for the recruitment only in combination with two isomeric tricycle homosesquiterpenes (C1/C2). The orientation primer pheromone alters a worker's physiological state which maximizes the behavioural releaser effects of the orientation trail pheromone (Vander Meers, 1986). The two isomeric tricyclic homosesquiterpenes are responsible for recruitment and orientation for S. richteri (Vander Meers, 1986); the orientation primer pheromone has not been identified in either species. Detrain (1984) indicated that the poison gland of Pheidole pallidula is a source of trail pheromone while the Dufour gland is a source of trail pheromone in Pheidole Fallax (Wilson, 1963b).

The trail pheromone of Crematogaster species originates from the tibial gland of the hind legs (Leuthold, 1968; Fletcher and Brand, 1968). A tube passes from the gland down to the foot, and the trail is laid from the feet, with the ant walking in a strange manner with its hind legs close together.

In formicine ants, the trail pheromones originate from the hind gut. In Camponotus sericeus a recruitment step is initiated by
mechanical signals and motor patterns involving tandem running, and
the chemical trails secreted from the hind gut function only as
orientation cues and do not release any recruitment effect (Holldobler
et al., 1974; Holldobler, 1978). In Camponotus socius, the trail
pheromone alone, does not release a recruitment effect; the recruiting
ant performs a "waggle" display when facing nestmates, after locating
a food source, subsequently the recruited ants follow the recruiting
ant and follow the trail to the food source by the guidance of the
recruiting ant; while "jerking" movements replace the waggle display
in the case of recruitment to a new site nest, and males respond to
the signals and move to the new site (Holldobler, 1971b; 1978). In
Formica fusca, the trail pheromone laid from the hind gut does not
alone release a recruitment behaviour, unless the recruiting ant show
a waggle display inside the nest, to induce nestmates to follow the
trail without being guided by the recruiting ant (Moglich and
Holldobler, 1975). In C. pennsylvanicus a scout ant recruits
nestmates to new food sources with alerting motor display, then
nestmates follow the recruitment trail (Traniello, 1977). The trail
appears to be composed of hind gut material which functions as a
long-lasting olfactory orientation cue, and formic acid from the
poison gland secretion makes the recruitment trail highly attractive
to stimulated ants (Traniello, 1977); the leader ant is not necessary
to guide a recruited ant to a food source. In species-specificity
tests with C. pennsylvanicus, Barin et al. (1976b) found that C.
pennsylvanicus workers followed the hind gut extracts of C. americanus
and C. socius, but did not follow C. vasilis, C. castaneus, C.
impressus, C. floridanus, or C. nearcticus; only one trail-following
experiment was undertaken with workers of C. americanus, it was found
that workers follow the hind gut extracts of C. pennsylvanicus
workers.
Carthy (1950, 1951) investigated the trail pheromone of Lasius fuliginosus and found that it originates from the hind gut. Lasius fuliginosus trail pheromone did not elicit a trail-following activity from workers of L. emerginatus, L. niger and L. flavus, but L. fuliginosus workers were able to follow the trails of L. emerginatus, L. niger and also trails of Formica species such as Formica rufa (Hangartner, 1967).

Oecophylla longinoda workers use multiple or stereotyped recruitment systems in which they use the rectal and sternal gland (Holldobler and Wilson, 1978). The rectal gland secretes odour trails which function as 1) recruitment to new food sources, 2) recruitment to new terrain (foraging area), 3) emigration to new sites, 4) long-range recruitment to repel intruders. In such cases, the trail pheromone is aided with tactile stimuli (mouth-opening, antennation and head wagging). The sternal gland secretion performs the short-range recruitment to territorial intruders.

Dolichoderine ants employ Pavan's gland (sternal gland) as the source of the trail pheromone (Wilson and Pavan, 1959). In a cross trail-following test among Iridomyrmex, Tapinoma, Liometopum, and Monacis genera, a species-specificity of the trail-following activity was noted (Wilson and Pavan, 1959).

Ponerine ants utilize pygidial, poison and sternal gland as the source of the trail pheromone. For example Leptogenys chinensis which feeds mainly on termites, lays trails from the pygidial and poison glands, the pygidial gland is responsible for recruitment and orientation, while the poison gland is responsible for the orientation reaction (Maschwitz and Schonegge, 1977). In Pachycondyla (+Termitopone) laevigata, the pygidial gland serves as a powerful chemical recruitment signal and orientation cue in trail communication and not from the hind gut as Blum (1966) assumed (Holldobler and...
Traniello, 1980a). Pachycondyla obscuricornis employs the pygidial gland secretion as a tandem running pheromone (Holldobler and Traniello, 1980b). The secretion appears to be transferred to the hind legs by a series of self-grooming behaviours and the tandem running is used only during nest emigration whereas the species has no food recruitment behaviour (Traniello and Holldobler, 1984). 

Onychomyrmex, a genus belonging to the primitive ponerine tribe Amblyoponini, is the only genus that employs the sternal gland as the source of trail pheromones (Holldobler et al., 1982). The gland is absent in the two genera, Cerapachys and Sphinctomyrmex, tribe Cerapachyini which utilize the poison and pygidial glands for trail communication. In species-specific tests for evaluating the trail-following activity among Onychomyrmex hedleyi, O. sp.1 and O. sp.2, it was found that O. hedleyi and sp.2 do not readily follow each other’s trails and both follow O. sp.1. O. sp.1 responded to O. hedleyi, but with a weaker response to O. sp.2 (Holldobler et al., 1982). Paltothyreus tarsatus utilizes the sternal gland secretion as recruitment and trail pheromone (Holldobler, 1982).

Ecitonine ants employ the hind gut and the pygidial gland as the source of trail pheromones. Blum and Portocarrero (1964) identified the hind gut as the source of trail pheromones in Eciton ants, but Holldobler and Engel (1978) state that the pygidial gland is the source of the trail pheromone of Eciton hamatum. Chadab and Rettenmeyer (1975) found that mass recruitment in the army ants Eciton hamatum, Eciton rapax and Eciton burchelli is based primarily on chemical and tactile signals, and the recruitment trail contains the essential information necessary for recruitment, but the response is lower than when combined with recruiter activity. The army ant Nelvamyrmex nigrescens workers employ the hind gut as the source of the trail pheromone (Watkins, 1964). Trails of the army ants are relatively non-volatile
and can persist after deposition on the substrate for several days (Torgerson and Akre, 1970b). Neivamyrmex nigrescens workers utilize not only non-volatile and volatile components of the chemical trail deposited from the hind gut, but other chemicals secreted from the surface of the ants bodies (Topoff and Mirenda, 1975). Neivamyrmex nigrescens workers use not only chemical trails but also tactile stimuli (Topoff and Lawson, 1979). No species-specificity in trail-following behaviour has been found among ecitonine ants, however, Neivamyrmex pilosus did not follow any trail other than its own (Watkins, 1964; Watkins et al., 1967). Torgerson and Akre (1970a) indicated that ecitonine trails might be composed of 2 components: a general one responded to by all species of army ants and an additional component that is genus- or even species-specific.

Aneuretus simoni, the only representative of the subfamily Aneuretinae, utilize the sternal gland as the source of the trail pheromone (Traniello and Jayasuriya, 1981a, b). The scout ants employ a motor display for recruiting nestmates to trail laying. The trail pheromone is employed by workers to food sources and during nest migration.

Chemical Nature of Trail Pheromones

The first trail pheromone identified as methyl 4-methylpyrrole-2-carboxylate (II) which originates from the poison gland of the leaf-cutting ant Atta texana (Tumlinson et al., 1971, 1972). The pheromone was isolated from the whole bodies of workers (3.7kg), macerated in methylene chloride, the extract distilled and further fractionated by gas-liquid chromatography. The synthesized pheromone was tested in the laboratory using a circle 50cm in circumference (cardboard sheet) and strong responses were obtained from 0.8, 8 and 80pg/cm. Methyl 4-methylpyrrole-2-carboxylate was also identified as
the trail pheromone of *Atta cephalotes* (Riley *et al.*, 1974) and *Acromyrmex octospinosus* (Cross *et al.*, 1982).

The second trail pheromone was 3-ethyl-2,5-dimethylpyrazine (IIIa) from the poison gland of the leaf-cutting ant, *Atta sexdens rubropilosa* (Cross *et al.*, 1979). Then later Evershed *et al.* (1981, 1982) found it in eight species of *Myrmica* using solid sample injection and trapping. Quantification showed it was present in nanogram quantities in the poison gland. Attygalle *et al.* (1986) found that 3-ethyl-2,5-dimethylpyrazine is also the trail pheromone of *Manica rubida*. Attygalle and Morgan (1983) indicated that the trail pheromone of *Tetramorium caespitum* contains two pyrazine compounds. In 1984, the same author gave a full identification of the two pyrazines. They are 2,5-dimethylpyrazine (IV) and 3-ethyl-2,5-dimethylpyrazine (IIIa) in the ratio 70:30 (2.7 and 1.15ng) respectively. When the two pyrazines were tested separately in the same concentration as they are present in the gland, they elicited a trail-following activity less than a mixture of the two at the same concentration (2.7 and 1.15ng).

Monomorine I (5-methyl-3-butyloctahydroindolizine) and monomorine II (2-(5-hexenyl)-5-pentylpyrrolidine) are found in the poison gland secretion of the Pharaoh's ant, *Monomorium pharaonis*, and release some activity as trail pheromones at high concentration in workers (Ritter *et al.*, 1973, 1975). Later Ritter *et al.* (1977a, b) investigated the Dufour gland and found it is the true source of the trail pheromone of *M. pharaonis*; the pheromone was isolated from about $10^5$ ants by benzene extraction, liquid and gas chromatography, and bioassayed using a circular trail. They found the substance was a sesquiterpenoid compound (V) which they called faranal (3,4,7,11-tetramethyltrideca-6E, 10Z-dienal). It is present only in trace quantity ($10^5$ workers=70ug); workers as well as queens and males
followed the trail (<1pg/cm).

Bärnith et al. (1976a) indicated that the main trail pheromone of Solenopsis richteri has a molecular weight of 218 and empirical formula of C\textsubscript{16}H\textsubscript{26}. Williams et al. (1981) identified the trail pheromone of S. invicta as Z,Z,Z-allofarnesene ((2Z,6Z)-3,7,11-trimethyl-2,4,6,10-dodecatetraene) (VI) which is active at high concentration (100-500pg/cm). Another group identified the pheromone as a mixture of Z,E-\alpha-farnesene ((3Z,6E)-3,7,11-trimethyl-2,4,6,10-dodecatetraene) (VII); E,E-\alpha-farnesene ((3E,6E)-3,7,11-trimethyl-2,4,6,10-dodecatetraene) (VIII); Z.Z-homofarnesene ((3Z,6Z)-3,4,7,11-tetramethyl-2,4,6,10-dodecatetraene) (IX); and Z,E-homofarnesene ((3Z,6E)-3,4,7,11-tetramethyl-2,4,6,10-dodecatetraene) (X) (Vander Meer et al., 1981). Vander Meer et al. (1984, 1985) found very small quantities of two isomeric tricyclic homosesquiterpenes (75pg/worker) which act as a trail pheromone of S. richteri. S. richteri trail pheromone also contains the two isomeric tricyclic homosesquiterpenes (4000pg/worker) (Vander Meer et al., 1984, 1985).

In Pristomyrmex pungens, nine fatty acids were identified from the total extract of the ant (C\textsubscript{14}, C\textsubscript{16}, C\textsubscript{16:1}, C\textsubscript{18}, C\textsubscript{18:1}, C\textsubscript{18:2}, C\textsubscript{18:3}, C\textsubscript{20:4}, C\textsubscript{20:5}) that act as the trail pheromones, using 5-tailed shape trail and circular trail (Hayashi and Komaie, 1977); the source of the trail pheromone was not known.

The trail pheromones of the formicine ant Lasius fuliginosus were isolated from the hind gut by dissecting ants (5 workers), then the dissected hind guts were washed successively with water, alcohol and ether; blotted dry after each washing and squeezed out (1.6ul); a preliminary experiment indicated that the active material to be composed of acidic and non-acidic fractions; rectal fluid (obtained from 20 ants) was allowed to stand until dry and the residue was twice
extracted with two drops of ether; the ether extract was reacted with diazomethane and submitted to gas chromatographic analysis; six fatty acids were identified (hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, decanoic acid and dodecanoic acid) and bioassayed using S-tailed shape trail (Huwyler et al., 1973, 1975). The trail pheromone of L. niger was found to be non-acidic by analysing the rectal fluid (Huwyler et al., 1975).

(Z)-9-hexadecenal was isolated and identified from Pavan's gland of the Argentine ant, Iridomyrmex humilis and acted as a trail pheromone (Cavill et al., 1979, 1980). Van Vorhis Key and Baker (1982) tested different concentrations of synthetic (Z)-9-hexadecenal (0.01, 0.1, 1.0, 10, 100 and 1000ng/50.7cm trails) as trail-following activities for Iridomyrmex humilis workers, it was found that 0.01, 0.1, and 1.0ng/trail elicit trail-following activities than controls, while 10, 100 and 1000ng/trail elicit nearly the same highest activity and 100 times more than a gaster extract. Among analogs of (Z)-9-hexadecenal tested, only (Z)-7-hexadecenal elicited significant trail-following activity but this analog is not present in the Pavan’s gland secretion (Van Vorhis Key and Baker, 1982).

Territorial Pheromones (Home Range)

Chemicals by which animals mark and recognize their own territories (home range) for food gathering or defense (Cammaerts et al., 1977a; Holldobler and Wilson, 1977) are called home range marking pheromones. When the marked territory is defended against intrusion by other colonies or other species, it is called a territorial pheromone.

In bees and wasps, particularly social bees, the use of chemical cues to mark foraging areas is not accurate, consequently, it is not significant where the insect forages over a vast area. Honey bee Apis mellifera workers crawl and deposit a "footprint" substance which
attracts other workers and stimulates them to enter the hive.

Homecoming honeybees are also attracted by an odour in the hive atmosphere (Butler, et al., 1969). The same authors demonstrated the behaviour with workers of the wasp Vespa vulgaris. In the Costa Rican solitary bees Centris nitida and C. trigonoides subtarsata males use a tibial gland secretion to mark the boundaries of defended territories (not more than a metre and half in diameter in the flowering corms of Casia grandis) (Williams et al., 1984). Other Centris species mark their territories by the mandibular glands (Vinson et al., 1982).


Surface pheromone

Surface pheromones are found on all members of the social insect colony. Such substances seem to be mainly absorbed on the body surface, and perceived by direct contact or, at most over a very short distance (Shorey, 1973). They include recognition pheromones (caste-recognition; brood recognition); releasers of grooming behaviour, courtship behaviour and the secretions that stimulate food exchange. They are thought to be composed of a combination of species-specific odours and food sources. Rocca et al. (1983) identified α-pyrones ((E)-6-(1-pentenyl)-2H-pyran-2-one) from the whole extract of queens of Solenopsis invicta which acts as queen recognition pheromone. Edwards and Chambers (1984) identified neocembrene
(E,E,E)-1-isopropenyl-4,8,12-trimethylcyclooctadeca-3,7,11-triene) as the Dufour gland secretions of fertile queens of Monomorium pharaonis and it may serve as a queen-recognition pheromone.

Funeral Pheromones

Chemical compounds are produced from dead ants that stimulate a live ant to remove a dead ant to a refuse pile outside the nest. The funeral pheromones are said to include saturated fatty acids, their esters and unsaturated fatty acids. When any object or living worker is daubed with these substances, they are treated as dead ants (Wilson et al., 1958, Wilson, 1963a; Matthews and Matthews, 1978).

Dufour Gland Secretions

The accessory gland of the poison apparatus, found in all aculeate Hymenoptera, first described by Dufour (1841) and generally known by his name, is part of the exocrine system of this insect order. It has been described as having an opening into the duct of the poison vesicle (Forel, 1878), and is emptied together with the poison vesicle in formicine ants (Hermann and Blum, 1968; Regnier and Wilson, 1968), although more recent ultrastructural studies on ants (Billen, 1986) indicate that the Dufour gland has its own valve and duct leading to the sting lance in myrmicines, or to the opening of the poison duct in formicines. The Dufour gland in most social Hymenoptera is an elongated sac-like structure, lined with a unicellular layer of epithelial cells varying from squamous (Billen, 1986) to a cuboidal form (Hermann and Blum, 1981; Billen, 1986). In formicine ants, the Dufour gland is bilobed.

In myrmicine ants, the Dufour glands contain more volatile oxygenated compounds and less volatile straight chain saturated and unsaturated and branched chain hydrocarbons and sesquiterpenes.
compounds.

In formicine ants, the Dufour gland contains a wide variety of substances ranging among alkanes, methylbranched alkanes, alkenes, ketones, alcohols, aldehyde, acetates, formates, farnesenes, all-trans-geranylgeraniol, all-trans-geranylgeranyl acetates, farnesyl acetate, farnesyl propionate, lactones and fatty acids.

The primary function of the Dufour gland is unknown, although it persists in all the aculeate Hymenoptera. What are probably secondary functions are, however, recognized in a number of species and genera.

Myrmicine ants’ Dufour gland secretions act as territorial pheromones (home range), recruitment, trail pheromones, sex pheromones, and queen recognition pheromones. Cammaerts et al. (1977) found that the Dufour gland secretion of Myrmica rubra acts as marking territory and home range pheromones. The Dufour gland acts as the source of the trail pheromone in some myrmicine ants such as Monomorium pharaonis (Holldobler, 1973) and Solenopsis species (Wilson, 1959). The Dufour gland of queens of M. pharaonis secretes a sex pheromone (Holldobler and Wust, 1973). The Dufour gland of fertile queens of M. pharaonis secretes neocembrene (E,E,E)-1-isopropenyl-4,8,12-trimethylcyclooctadeca-3,7,11-triene) which is not present in workers or in young alate (virgin) queens and may act as a queen-recognition pheromone (Edward and Chamber, 1984).

The oxygenated compounds, methanol, acetaldehyde, ethanol, acetone, butanone, 2-methylpropanol and 1-butanol were investigated in Myrmica species (Cammaerts et al., 1976, 1978, 1981a; Morgan et al., 1977; Attygalle et al., 1983a,b) and found to have a short-lived recruiting activity.

Linear saturated and unsaturated hydrocarbons predominate in the Dufour gland secretions of some Myrmica species (Morgan and Wadhams, 1972a; Morgan et al., 1977; Cammaerts et al., 1978, 1981a; Attygalle
et al., 1983a, b), Tetramorium (Billen et al., 1986), Atta (Evershed and Morgan, 1980, 1981) and Harpagoxenus (Ollett et al., 1987).

Pentadecane was the major compound in Tetramorium; heptadecene was the major compound in Myrmica and Harpagoxenus. The major compound in Attine ants differs from one species to another, in Atta cephalotes, heptadecane is the major compound and (Z)-9-nonadecene is the major compound in Atta sexdens rubropilosa while (Z)-9-tricosene is the major compound in Atta sexdens sexdens.

Branched chain hydrocarbons were only investigated in the Dufour glands of Pogonomyrmex species (Regnier et al., 1973; Billen et al., 1987). Dodecane and 6-methylidodecane were the major components in P. barbatus while 6-methylundecane and 5-methylundecane were the major components in P. rugosus. In P. occidentalis the major compound was pentadecane. Sesquiterpene compounds dominate the Dufour gland secretions in other Myrmica species (Morgan et al., 1979; Cammaerts et al., 1978, 1981a; Attygalle et al., 1983a, b); Aphaenogasterdfniceps (Cavill et al., 1967); and Acromyrmex octospinosus (Evershed and Morgan, 1980). Faranal was identified as the trail pheromone of Monomorium pharaonis (Ritter et al., 1977a, b). Allofarnesene was identified as a Dufour gland secretion of S. invicta and showed a trail-following activity at high concentration (Williams et al., 1981). Farnesene, homofarnesene and two isomeric tricyclic homosesquiterpenes were identified as the true trail pheromones of S. invicta and the two isomeric tricyclic homosesquiterpenes as the trail pheromone of S. richteri (Vander Meer et al., 1981, 1984, 1985).

In formicine ants, the Dufour glands act as an alarm as well as a propaganda substance in slave-raiding ants.

Alkanes are present in all members of formicine ants while methylbranched alkanes and alkenes sometimes are present. Undecane or tridecane is the major compound. Undecane is the major compound in
Formica and Lasius species (Bergstrom and Lofqvist, 1968, 1970, 1973; Regnier and Wilson, 1971; Billen et al., 1983; Attygalle et al., 1987); Polyrhachis lamellidens (Hayashi and Komae, 1980); Camponotus japonicus and C. obscursipes (Hayashi and Komae, 1980). Tridecane is the major compound in Polyrhachis simplex (Hefetz and Lloyd, 1982); Polyrhachis species (Brophy et al., 1982). Camponotus ligniperda and C. herculeanus (Bergstrom and Lofqvist, 1972a,b).

Farnesene was detected in Formica sanguinea and Polyergus rufescens (Bergstrom and Lofqvist, 1968) and it is the major compound in Polyergus rufescens. Also farnesene was found in Formica fusca in small quantities (Bergstrom and Lofqvist, 1968), and in Camponotus ligniperda and C. herculeanus in a trace amount (Bergstrom and Lofqvist, 1972a,b).

The oxygenated compounds were investigated in some Formica species (Bergstrom and Lofqvist, 1968, 1973; Regnier and Wilson, 1971; Francke et al., 1985); Lasius species (Bernardi et al., 1968; Bergstrom and Lofqvist, 1970; Attygalle et al., 1987); Notoncus ecatamoides (Brophy et al., 1982) and Camponotus species (Bergstrom and Lofqvist, 1972a,b; Brophy et al., 1973).

The gasters of workers of the slave-keeping ant Formica sanguinea contain alkyl acetates as the major compounds (C_{10}-C_{12}) (Bergstrom and Lofqvist, 1968); also the slave-maker ant Formica subintegra and F. pergandi contain mainly decyl, dodecyl, and tetradecyl acetate which are said to be used in slave-raiding (Regnier and Wilson, 1971).

Ketones have been reported in some species of Formica species (Bergstrom and Lofqvist, 1968; Regnier and Wilson, 1971), Lasius (Bernardi et al., 1967; Bergstrom and Lofqvist, 1970), Notoncus (Brophy et al., 1982) and Camponotus (Bergstrom and Lofqvist, 1972a,b). Notoncus has 2-tridecanone as the major compound.

Alcohols were identified in Formica (Bergstrom and Lofqvist,
Formica nigricans, rufa and polyctena were distinctive by having two isoprenoids, all-trans-geranylgeraniol and the corresponding acetate all-trans-geranylgeranyl acetate (Bergstrom and Lofqvist, 1973). One aldehyde (nonanal) and formate were investigated from a hexane extract of F. rufa by Francke et al. (1905) which had not been found before in Dufour gland secretion. They did not find the geranylgeraniol and geranylgeranyl acetates described by Bergstrom and Lofqvist (1973). Lasius flavus gasters contain two lactones (4-hydroxyhexa- and octadec-9-enoide) and 4-hydroxyoctadec-9-enoic acid among its secretion which make the species distinctive from the others (Bergstrom and Lofqvist, 1970). L. niger is distinctive in having trans-farnesyl acetate (Bergstrom and Lofqvist, 1970); the latter compound was also found in Camponotus ligniperda and C. herculeanus (Bergstrom and Lofqvist, 1972a,b).

Attygalle et al. (1987) identified more new compounds from the Dufour gland of Lasius niger than Bergstrom and Lofqvist (1970) found, which include methylbranched alkanes, methylbranched acetates, terpenoid, and propionate; the propionate-decyl, dodecyl, octadecyl, and farnesyl propionate have not been previously reported from ants secretions. Traces of cis and trans-citral were found in L. fuliginosus (Bernardi et al., 1967). Fatty acids, myristic, pentadecanoic, palmitic and stearic acid were found only in C. intrepidus which make the species unique among formicine ants (Brophy et al., 1973).

Mandibular Gland Secretions

Mandibular glands are found in many insect species (Blum and Hermann, 1978). They are present in the mesal side of the mandibular
A variety of compounds have been identified in ants. The mandibular gland secretions have defensive and pheromonal functions (alarm and sex pheromones).

A variety of ethyl and methyl ketones are present in myrmicine ants, 3-octanone and 3-octanol were the major compounds in Myrmica species (Crewe and Blum, 1970a, b; Morgan et al., 1978; Cammaerts et al., 1981b, 1982, 1983). 3-octanone was the major compound in Tetramorium species including *T. caespitum* (Longhurst et al., 1980) while *T. angulinode* is the only species that has pergolen present as the major compound. 4-methyl-3-hexanone and 4-methyl-3-hexanol were investigated in both males and alate females of *T. caespitum* while workers contain only 4-methyl-3-hexanol (Pasteels et al., 1980). Later they corrected this when they discovered they had examined the morphologically almost identical *T. impurum* (Foerster). Pasteels et al. (1981) by a re-examination of *T. impurum* found the ketone also in workers. Manicone (4,6-dimethyl-4-octene-3-one) was found in *Manica mutica* and *M. bradleyi* as the major compound (Fales et al., 1972). 4-methyl-3-heptanone was found in *Pogonomyrmex* species (McGurk et al., 1966; Benthuyesen and Blum, 1974), *Atta texana* and *A. cephalotes* (Riley et al., 1974).

Citronellol and geranial were found in *Atta* species (Blum et al., 1968). Benzaldehyde was found in *Veromessor pergandei* (Blum et al., 1969). Some myrmicines are able to synthesize aromatic compounds such as o-aminoacetophenone which is produced by *Mycocepurus goeldii* (Hermann and Blum, 1981). Alkylpyrazine compounds only were found in *Aphaenogaster rudis* (Wheeler et al., 1982).

The mandibular gland of formicine ants contains ketones (Bernardi et al., 1967; Bergstrom and Lofqvist, 1970; Hefetz and Lloyd, 1982), alcohols (Bergstrom and Lofqvist, 1970; Lloyd et al., 1975; Brashaw et al., 1979a; Hefetz and Lloyd, 1982; Francke et al., 1985), aldehyde
(Bergstrom and Lofqvist, 1970; Bradshaw et al., 1979a) and a variety of compounds (Bernardi et al., 1967; Bergstrom and Lofqvist, 1970; Brand et al., 1973; Lloyd et al., 1975; Bradshaw et al., 1979a, Francke et al., 1985; Hefetz and Lloyd, 1985) including, citral, dendroasarin, farnesal, citronellal, 2,3-dihydrofarnesal, geranylgeraniol, geranlycitronellal, citronellol, nerol, geraniol, acids (e.g. n-nonanoic acid, 2,4-dimethyl-2-hexanoic acid, citronelic acid, geranic acid), esters (e.g. 2,6-dimethyl-5-heptenyloctanoate, 2-phenylethyl nonanoate, methyl 6-methylsalicylate, methyl-anthraniilate) perilien, mellein or ochracin (3,4-dihydro-8-hydroxy-3-methylisocoumarin) and hydrocarbons (Francke et al., 1985) which were found for the first time in mandibular gland secretions.

The present work was undertaken to study some of the pheromones and glandular secretions of some ant species to try to shed more light on function of these substances from various aspects. The work includes a study on the sex pheromone of the coleopteran Attagenus scalaris that could not be pursued as intended because of the shortage of insect materials.
RESULTS AND DISCUSSION

Trail Pheromone Investigation of Pheidole pallidula (Nylander)

The principal characteristic of Pheidole pallidula is the possession of two subcastes of workers, distinct in their morphology (Bernard, 1968). The minor workers are very small (about 2-2.5 mm long), the major workers are larger (3 mm) with very large heads and powerful mandibles. There is a high diversity of polyethism during food recruitment between majors and minors in different Pheidole species. For example, majors of several New World Pheidole species are never involved in food retrieval and do not lay chemical trails, but in P. rugulosa and P. sciophila, both castes participate in foraging and trail laying (Holldobler and Moglich, 1980). Pheidole pallidula minors are responsible for food discovery and nestmates’ recruitment. Only a few majors follow the chemical trail, sometimes carrying food (Detrain, 1984). Morphological and chemical differences also exist in the abdominal glands (poison, Dufour and pygidial glands) of the two subcastes (Detrain et al., in press, personal communication). Detrain (1984) had shown, using a different test with straight line trails on paper, that the poison gland of minor workers is the source of trail pheromone. We confirmed this with gasters using the circular test of Pasteels and Verhegge (1974) which can give a quantitative measure of effectiveness of the substance under test.

We found in this work that the trail pheromone is present only in the minor workers, however, a few major workers were always found in
the foraging area and followed the trail.

The trail following effect of an extract of *P. pallidula* worker gaster was compared with similar extracts of gasters of *Myrmica rubra* and *Tetramorium caespitum* in inducing trail-following in the *P. pallidula* workers. Pheidole workers followed the trail made from *Myrmica* and *Tetramorium*, but less well than the extract obtained from its own gaster. The activity of Pheidole on a *Myrmica* trail was stronger than on a *Tetramorium* trail (Table 5a).

**Micro-column fractionation**

Microfractionation was performed with a micro-column of silica using an extract of five gasters of *Pheidole pallidula* minor workers using a series of solvents of increasing polarity for elution, namely hexane, hexane-ether (1:1), ether, ether-methanol (99:1) and ether-methanol (90:10). Each fraction was examined separately using circular trail-following tests with the ants on the foraging area, to quantify the trail-following activity. It was found that hexane-ether (1:1) evoked the highest activity as shown in Table 5b, for which the median number of arcs which the workers ran along the trail was 7cm, while the other fractions evoked no activity.

In another experiment to separate the pheromone by micro-column chromatography, using hexane, hexane-acetone (1:1) and acetone as eluting solvents, the activity was eluted with the hexane-acetone fraction which recorded the highest activity (27.5cm) (Table 5c). Some individuals occasionally responded to the other fraction with extreme values of 7 and 9cm for the hexane and acetone fractions respectively but the values were much lower.

The microfractionation of the hexane extract of whole Pheidole gasters indicated that the active fraction was moderately polar in nature.
TABLE 5
Response of Pheidole pallidula workers to various extracts of poison glands or whole gasters, using the circular trail-following test of Pasteris and Verhaege (1974). The median no. of arcs run by the group of workers observed, together with the highest number of arcs followed by a single worker are recorded.

<table>
<thead>
<tr>
<th>Test extract</th>
<th>No. of individuals observed</th>
<th>Median no. of cm arcs run</th>
<th>Highest no. of cm arcs run</th>
<th>Test extract</th>
<th>No. of individuals observed</th>
<th>Median no. of cm arcs run</th>
<th>Highest no. of cm arcs run</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. P. pallidula minor worker gaster</td>
<td>36</td>
<td>22</td>
<td>247</td>
<td>f. Programmed g.c. retention times</td>
<td>20</td>
<td>0.76</td>
<td>4</td>
</tr>
<tr>
<td>M. rubra worker gaster</td>
<td>43</td>
<td>8.5</td>
<td>280</td>
<td>3-8 min</td>
<td>38</td>
<td>5.0</td>
<td>45</td>
</tr>
<tr>
<td>I. caespitum worker gaster</td>
<td>62</td>
<td>5.0</td>
<td>35</td>
<td>8-13</td>
<td>20</td>
<td>1.0</td>
<td>5</td>
</tr>
<tr>
<td>b. Micro-column of P. pallidula minor gaster</td>
<td>18-23</td>
<td>15</td>
<td>0.5</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hexane</td>
<td>44</td>
<td>1.1</td>
<td>35</td>
<td>g. Programmed g.c.</td>
<td>31</td>
<td>1.0</td>
<td>6</td>
</tr>
<tr>
<td>hexane-ether (1:1)</td>
<td>47</td>
<td>7.25</td>
<td>270</td>
<td>8-10 min</td>
<td>28</td>
<td>11.0</td>
<td>35</td>
</tr>
<tr>
<td>ether</td>
<td>42</td>
<td>1.0</td>
<td>7</td>
<td>10-12</td>
<td>41</td>
<td>3.25</td>
<td>22</td>
</tr>
<tr>
<td>ether-methanol (99:1)</td>
<td>24</td>
<td>1.0</td>
<td>5</td>
<td>12-14</td>
<td>9-11</td>
<td>30</td>
<td>0.75</td>
</tr>
<tr>
<td>ether-methanol (9:1)</td>
<td>40</td>
<td>0.5</td>
<td>7</td>
<td>h. Solid sampling g.c.</td>
<td>3-4 min</td>
<td>40</td>
<td>0.75</td>
</tr>
<tr>
<td>c. Micro-column of P. pallidula minor gaster</td>
<td>9-11</td>
<td>30</td>
<td>0.75</td>
<td>5-6</td>
<td>5-13</td>
<td>43</td>
<td>3.0</td>
</tr>
<tr>
<td>hexane</td>
<td>15</td>
<td>0.75</td>
<td>7</td>
<td>11-13</td>
<td>27</td>
<td>12.5</td>
<td>95</td>
</tr>
<tr>
<td>hexane-acetone (1:1)</td>
<td>21</td>
<td>27.5</td>
<td>180</td>
<td>13-15</td>
<td>43</td>
<td>3.0</td>
<td>40</td>
</tr>
<tr>
<td>acetone</td>
<td>15</td>
<td>2.25</td>
<td>9</td>
<td>i. Isothermal g.c.</td>
<td>3-4 min</td>
<td>40</td>
<td>0.75</td>
</tr>
<tr>
<td>d. t.l.c. of hexane-acetone fraction</td>
<td>4-5</td>
<td>59</td>
<td>6.75</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rf 0-0.3</td>
<td>16</td>
<td>0.74</td>
<td>4</td>
<td>5-6</td>
<td>54</td>
<td>0.75</td>
<td>10</td>
</tr>
<tr>
<td>0.3-0.6</td>
<td>33</td>
<td>10.75</td>
<td>70</td>
<td>j. OV-101 g.c.</td>
<td>2-5 min</td>
<td>54</td>
<td>3.75</td>
</tr>
<tr>
<td>0.6-1.0</td>
<td>16</td>
<td>1.0</td>
<td>4</td>
<td>5-8</td>
<td>55</td>
<td>1.25</td>
<td>10</td>
</tr>
<tr>
<td>e. t.l.c. of hexane-acetone fraction</td>
<td>8-12</td>
<td>46</td>
<td>2.0</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rf 0.3-0.4</td>
<td>19</td>
<td>2.0</td>
<td>10</td>
<td>k. OV-101 g.c.</td>
<td>1-2 min</td>
<td>33</td>
<td>14.5</td>
</tr>
<tr>
<td>0.4-0.5</td>
<td>21</td>
<td>2.25</td>
<td>23</td>
<td>2-3</td>
<td>39</td>
<td>0.75</td>
<td>10</td>
</tr>
<tr>
<td>0.5-0.6</td>
<td>30</td>
<td>16.75</td>
<td>130</td>
<td>3-5</td>
<td>36</td>
<td>1.75</td>
<td>25</td>
</tr>
</tbody>
</table>
Micro-column and thin-layer chromatography

Following the results obtained in the above experiment, the active fraction was obtained by eluting an extract of ten gasters through a micro-column, discarding the first (hexane) fraction, then applying the hexane-acetone (1:1) fraction on the origin of a thin layer plate and developing it, with hexane-acetone (1:1) for 10cm. The silica was cut into three Rf bands, which were washed with acetone and the extracts tested separately for trail following. From the experiment it was found that the activity recorded its highest value (10.75cm) from the band of Rf value 0.3-0.6 while the Rf bands 0-0.3 and 0.6-1 evoked only 0.75 and 1cm respectively (Table 5d).

By repeating the same experiment and dividing the Rf band of activity from the thin layer plate into three narrower regions, the activity was found only in the Rf bands 0.5-0.6. The Rf bands 0.3-0.4 and 0.4-0.5 showed a little activity, perhaps from overlap or diffusion of the active compound (Table 5e).

Because of the overlap in activity between P. pallidula and M. rubra, we thought that possibly 3-ethyl-2,5-dimethylpyrazine (EDMP) (IIIa) the trail pheromone of M. rubra and other Myrmica species (Evershed et al. 1982) might be the active substance here too, since EDMP has similar chromatographic behaviour to that of the P. pallidula compound so far encountered. Experiments with synthetic EDMP showed that it also was eluted on thin layer plate by hexane-acetone (1:1) and had Rf 0.5-0.6.

Micro-column and gas chromatography trapping

The active fraction from gasters of minor workers, eluted from a micro-column with hexane-acetone (1:1) was injected directly onto a PEGA packed column, using the temperature programme conditions described in the Experimental section. Fractions were collected using
the effluent splitter and bioassayed. The trail-following behaviour showed its highest activity from the fraction at the period between 8-13 min as is shown in Table 5f.

Under the same chromatographic conditions as above, the effluent was collected at short periods started from 8-10, 10-12 and 12-14 min., to narrow down the active fraction. The fraction between 10-12 min. recorded the highest activity where workers ran a median distance of 11cm (Table 5g).

**Solid sample injection and G.C. trapping**

Using a different approach, ten gasters of minor workers were directly injected into the gas chromatograph without solvent, by the method of Morgan and Wadhams (1972b) using the same conditions as above, the effluent was split and fractions collected for bioassay. Again only one active fraction was found (Table 5h) and, with the previous experiment, narrowed down the elution time of the active substance to between 11-12 min., corresponding to a Kovats index of approximately 1600 (i.e. elution time of n-hexadecane). ECMP had Kovats index of 1577 on this column. Changing to the isothermal conditions described for this column, activity was found between 4 and 5 min. (Table 5i) which again corresponds to a Kovats index of 1600.

Another experiment was performed using a non-polar column of OV-101 under isothermal conditions at 120°C. The effluent was collected at different periods and bioassayed. The fraction between 2 and 5 min. evoked highest activity comparing with the other periods of collection (Table 5j) but weaker than that found in earlier experiments, therefore, it was predicted that the activity could be before 2 min. The experiment was repeated with the same conditions, but the time of collection was started earlier from 1-2, 2-3 and 3-5 min. The activity recorded the highest value at the period between 1
and 2 min. (Table 5k). EDMP had retention time of 1.5 min. and Kovats
index of 1070 under these conditions.

Identification of the active compound

Gas chromatography of three cleanly dissected poison glands of
minor workers on a capillary column with a flame detector gave several
very small peaks of short retention, one of which corresponded to
EDMP. A similar experiment using whole gasters of minor workers with a
nitrogen specific detector showed only one peak (Fig. 2a)
corresponding to EDMP. Using synthetic EDMP as a standard, with the
nitrogen detector, the average amount of EDMP present per single
worker gland was calculated to be 0.2 ng.

Similar gas chromatography experiments with major worker gland
or gasters gave no evidence for the EDMP peak (Fig. 2b).

Selective ion monitoring by gas chromatography-mass spectrometry
of the ions at m/z 178, 164, 150, 136, 122 and 108 (the molecular ions
of a homologous series of alkyl pyrazines) showed a peak only at m/z
136 (corresponding to EDMP) with a very weak ion at m/z 108, both of
which had retention times appropriate to the EDMP and a weak peak at
m/z 122 at much longer retention, which must correspond to the
fragment ion from butyldimethyl pyrazine.

The total ion chromatogram from the gas chromatography-mass
spectrometry experiment showed four weak peaks (Fig. 3a). Scanning for
m/z 136, the molecular ion of an ethyldimethylpyrazine gave one clear
peak (Fig. 3a) which had the mass spectrum corresponding to
3-ethyl-2,5-dimethylpyrazine (EDMP). Scanning for m/z 122, an ion
common to this compound and higher 3-alkyl-2,5 dimethylpyrazines
showed two peaks (Fig. 3a) that of EDMP and one at longer retention
corresponding to a butyldimethylpyrazine. Similarly scanning for m/z
108, the molecular ion of dimethylpyrazines, a small peak was detected
Fig 2: Gas chromatogram of the gasters of (a) a single minor worker and (b) a single major worker of *P. pallidula*, using a capillary column and a nitrogen specific detector. The peak at 9.107 min corresponds in retention to EDMP.
Figure 3: Single ion monitoring of a sample of 10 poison glands of (a) minor workers and (b) major workers of *P. pallidula* by combined gas chromatography mass spectrometry.
(Fig. 3a). The two other peaks gave no recognizable mass spectra.

Repeating the work with ten poison glands of minor workers and selectively scanning at high sensitivity for m/z 107, 108, 122, 135, 136, 43 again revealed the EDMP and butyldimethylpyrazine but no other substances. Neither of these substances was found in the glands of major workers (Fig. 3b).

Bioassay of synthetic compounds

Solutions of various amounts of EDMP in hexane were tested on the circular trails for trail-following activity with P. pallidula workers. The results in Table 6a showed poor median activity except at unnaturally high concentrations, although a few individual workers walked many arcs on the trail where they recorded 3, 5, 10, 12 and 30 cm with 0.1, 1, 4, 10 and 100 ng respectively.

The maximum value for activity was expected to be found near or below one gland equivalent, as was found for Myrmica (Evershed et al., 1982) and Tetramorium caespitum (Attygalle and Morgan, 1984a) trail pheromones. It was thought that the low values obtained here for 0.1 and 1 ng might be due to inhibition by impurities in the EDMP. Therefore, the synthetic substance was purified by thin layer chromatography under the conditions used for separating the gland extracts and re-tested in the bioassay. Slightly higher activity at lower concentrations was found (Table 6b). The extreme values of trail following by individual minors were 6, 35 and 22 cm with concentrations of 1, 10 and 100 ng respectively.

When purified EDMP still did not give high values in the trail following tests, compared to a single worker gland, the possibility was considered that another isomeric ethyldimethylpyrazine might be the active compound because the three isomeric ethyldimethylpyrazines all give similar mass spectra. There are two other possible isomers
Responses to *P. pallidula* workers to various synthetic pyrazines in the trail-following test of Pasteels and Verhaege (1974).

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>No. of individuals observed</th>
<th>Median no. of cm arcs run</th>
<th>Highest no. of cm arcs run</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 3-Ethyl-2,5-dimethylpyrazine (EDMP)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1ng</td>
<td>13</td>
<td>0.5</td>
<td>3</td>
</tr>
<tr>
<td>1.0</td>
<td>13</td>
<td>1.0</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>0.75</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>1.75</td>
<td>12</td>
</tr>
<tr>
<td>100</td>
<td>27</td>
<td>6.25</td>
<td>30</td>
</tr>
<tr>
<td>b. Purified EDMP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1ng</td>
<td>32</td>
<td>0.75</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>38</td>
<td>3.5</td>
<td>35</td>
</tr>
<tr>
<td>100</td>
<td>41</td>
<td>2.5</td>
<td>22</td>
</tr>
<tr>
<td>c. Isomeric pyrazines at 4ng trail⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Ethyl-2,5-dimethylpyrazine</td>
<td>18</td>
<td>4.25</td>
<td>17</td>
</tr>
<tr>
<td>2-Ethyl-3,5-dimethylpyrazine</td>
<td>13</td>
<td>0.75</td>
<td>3</td>
</tr>
<tr>
<td>5-Ethyl-2,3-dimethylpyrazine</td>
<td>13</td>
<td>1.25</td>
<td>5</td>
</tr>
<tr>
<td>d. Mixed ethylidimethylpyrazines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4ng EDMP + 1ng 2-ethyl- + 1ng 5-ethyl-</td>
<td>15</td>
<td>4.5</td>
<td>23</td>
</tr>
<tr>
<td>4ng EDMP alone</td>
<td>13</td>
<td>4.5</td>
<td>20</td>
</tr>
<tr>
<td>e. Lower alkylpyrazines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,5-Dimethylpyrazine 1ng</td>
<td>15</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Trimethylpyrazine 1ng</td>
<td>11</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>EDMP 4ng</td>
<td>12</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>f. Mixed alkylpyrazines; EDMP 2ng +</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,5-trimethylpyrazine 0.5ng +</td>
<td>16</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>2,5-dimethylpyrazine 0.5ng</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(2-ethyl-3,5-dimethylpyrazine (IIIB) and 5-ethyl-2,3-dimethylpyrazine (IIIC)). Thanks to the cooperation of David G. Ollett, who synthesized 3-ethyl-2,5-dimethyl and 2-ethyl-3,5-dimethylpyrazine and Professor W. Francke, who sent a sample of 5-ethyl-2,3-dimethylpyrazine, all the structures were separately tested in the trail following test, in which the EDHP (the 3-ethyl isomer) showed the strongest activity (Table 6c). The possibility was also considered that a mixture of two or all three isomers might act synergistically but no enhanced activity of the mixtures was detected (Table 6d). Synergism and activity were also sought in the lower alkylpyrazines (2,5-dimethylpyrazine, and trimethylpyrazine) but again no increased activity was observed (Table 6e.f).

We have the apparent dilemma that all the evidence from thin layer chromatography, and gas chromatography on two columns and different temperature conditions, all point to one substance, or a group of substances eluting very closely together are the trail pheromone for this species. No secondary substance is required, nor is it necessary to mix fractions or to use material collected over broad intervals. The elution properties of EDMP, and the demonstration that this substance is present in minor workers and absent from major workers also points to this substance as the pheromone. The cross activity with M. rubra and T. caespitum is also an indication that EDMP is the substance, since it is present in workers of both these species. Yet the pure synthetic substance shows activity far inferior to the glandular extract. To claim the pheromone has been identified, this discrepancy must be explained.

We must presume that the trail pheromone is a complex mixture of different chemical compounds each responsible for distinct behaviours. Vander Meer (1986) distinguish three broad subcategories of response from the trail pheromone complex of Solenopsis invicta and S.
recruitment, orientation primer and orientation pheromones. The lack of an orientation primer pheromone which acts as a switcher to induce the trail orientation response, could explain the weak trail following response of most *P. pallidula* minors to pure EDMP but currently, we have no evidence of any other active compound than EDMP present in the poison gland apparatus. In *P. pallidula* and *P. teneriffana*, the trail pheromone alone has a weak recruitment and its effect is enhanced in conjunction with motor display. The quiet scent trail recruitment in *P. pallidula* and *P. teneriffana* drew 3.3 and 5.6 foragers respectively while trail-laying accompanied by motor display recruited 15.6 and 16.4 foragers respectively (Szep, 1970). Since the gland extract has a strong effect in trail-following activity comparing with the synthetic EDMP, therefore the suppression of activity with EDMP does not belong to motor display, so the absence of an orientation primer pheromone makes the activity too weak.

Another experiment was attempted, to synergize the synthetic EDMP by adding a small fraction of the gland (as orientation primer) to a solution of synthetic EDMP at one ng per trail. However, the colony had become rather old, was queenless and reduced in numbers and had lost the ability to follow trails of its own poison glands. Any further work to discover the primer pheromone, if this hypothesis is correct, will have to wait until fresh colonies are available.
Dufour gland investigations of myrmicine ants

Leptothorax species

*Leptothorax acervorum* Fabr. is a myrmicine ant found throughout Britain and the whole of Europe except the extreme south. *Leptothorax nylanderi* Forst. is found throughout central and southern Europe and occasionally in southern England. *L. acervorum* is preyed upon in different ways by two related species, *Harpagoxenus sublaevis* (Nyl.) and *Leptothorax kutteri* (Buschinger). Workers of *L. acervorum* are always found in the nests of *H. sublaevis*. Workers of the latter raid colonies of *L. acervorum* apparently by using volatile chemicals, they overcome the defenses of the former, steal the brood of the slave species and carry them back to their nest, where they emerged as adults and are then accepted as slaves in the new colony. These worker slaves perform all the duties of food gathering, brood rearing and nest maintenance for their *H. sublaevis* mistresses (Wilson, 1971; Buschinger, 1966, 1968). This kind of enslavement is called dulosis. The raiding party starts with a single worker of *H. sublaevis* which acts as a scout, then by tandem running (Buschinger and Winter, 1977) it collects some workers of the same species to start an attack on the *L. acervorum* colony. The *L. acervorum* colony is also preyed on by *L. kutteri* queens in a different way from that of *H. sublaevis*. The *L. acervorum* colony is parasitized by *L. kutteri* queens. *L. kutteri* queens enter the nest of *L. acervorum* singly, overcome the natural reaction of the *L. acervorum* workers to eject or kill them, and settle there to lay their eggs to be nourished and reared by the *L. acervorum* workers (Buschinger, 1965).

It is reported that *H. sublaevis* workers smear the captured brood of *L. acervorum* with their Dufour gland secretion, making the brood unattractive to the *Leptothorax* defenders (Buschinger et al., 1980; Buschinger, 1983). The "propaganda substance" that the workerless *L.
kutteri use to disrupt the defense of L. acervorum workers, causing them to attack each other, and permitting the entry of the L. kutteri queens, is also located in the Dufour gland (Allies et al., 1986).

L. nylanderi is closely similar to L. acervorum in anatomy and organisation but it differs from L. acervorum in that workers of L. nylanderi forage and live independently and are not enslaved or parasitized.

Ollett et al. (1987) examined the contents of the very large Dufour glands of H. sublaevis which contains a mixture of hydrocarbons but also (E)-β-farnesene (XI), a compound not previously found in ants, but commonly encountered as an alarm pheromone of aphids (Edwards et al., 1973). We have examined the much smaller Dufour glands in its potential slave L. acervorum and its close relative L. nylanderi to see if a comparison of their compositions shed light on the dulotic relationship between H. sublaevis and L. acervorum.

Analysis of the Dufour glands of both workers and queens of L. acervorum was first carried out by our sampling methods using single glands. The analysis revealed no difference between the Dufour gland secretions of L. acervorum workers and queens, both contain mainly a mixture of simple linear alkanes, alkenes and one alkadiene (C\textsubscript{15} to C\textsubscript{19}) and one sesquiterpenoid substance (Fig. 4). β-Heptadecene is the major compound, followed by heptadecadiene. The position of the double bond of heptadecene was investigated by g.c. trapping of heptadecene, then micro-ozonolysis and re-injection which yielded a mixture of n-octanal and n-nonanal, hence the double bond was between the 8th and 9th carbon atoms. The mean amounts of the substances found in the gland were calculated from ten replicate determinations. The mean quantity and percentage of each component is given in Table 7. Although the mean quantity of each component differs in queens from those in workers, the percentage composition remains nearly the
Fig. 4. An example of a gas chromatogram of a single Dufour gland of a worker of L. acer- vorum. C17:1 indicates pentadecene, etc. T indicates the acqueufenoid substance tetra- morene-2. The chromatogram obtained from queen Dufour glands was very similar. Chromatographic conditions are as described in the text.
TABLE 7

Chemical composition of the contents of the Dufour gland of workers and mature queens of Leptothorax acervorum determined by gas chromatography and mass spectrometry.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Queens Mean quantity ng±SD</th>
<th>%±SD</th>
<th>Mean quantity ng±SD</th>
<th>%±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng±SD</td>
<td></td>
<td>ng±SD</td>
<td></td>
</tr>
<tr>
<td>Pentadecene</td>
<td>4.21 ± 2.12</td>
<td>2.6 ± 0.5</td>
<td>3.0 ± 2.0</td>
<td>3.0 ± 1.2</td>
</tr>
<tr>
<td>n-Pentadecane</td>
<td>5.21 ± 2.02</td>
<td>3.6 ± 1.4</td>
<td>3.0 ± 2.0</td>
<td>3.9 ± 2.0</td>
</tr>
<tr>
<td>Hexadecene</td>
<td>1.77 ± 1.13</td>
<td>0.95 ± 0.5</td>
<td>0.6 ± 0.8</td>
<td>0.5 ± 0.7</td>
</tr>
<tr>
<td>n-Hexadecane</td>
<td>t</td>
<td>t</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>Heptadecadiene</td>
<td>28.9 ± 12.5</td>
<td>18.2 ± 2.9</td>
<td>20 ± 10</td>
<td>19.6 ± 4.6</td>
</tr>
<tr>
<td>8-Heptadecene</td>
<td>113.4 ± 47.6</td>
<td>71.1 ± 3.0</td>
<td>60 ± 20</td>
<td>68.6 ± 3.7</td>
</tr>
<tr>
<td>n-Heptadecane</td>
<td>4.86 ± 2.24</td>
<td>3.2 ± 0.9</td>
<td>4 ± 1</td>
<td>4.3 ± 0.5</td>
</tr>
<tr>
<td>Tetramorene-2</td>
<td>t</td>
<td>t</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>Octadecene</td>
<td>t</td>
<td>t</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>n-Octadecane</td>
<td>t</td>
<td>t</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>n-Nonadecane</td>
<td>t</td>
<td>t</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>total amount</td>
<td>159 ± 66</td>
<td></td>
<td>91 ± 36</td>
<td></td>
</tr>
</tbody>
</table>

*t = trace
same in both two castes as shown in Table 7. The total amount of the Dufour glands in queens was greater than in workers, 159+/-66ng and 91+/-36ng respectively. The amount of material in the Dufour gland can vary considerably from one individual ant to another, and the mean value for one nest can be different from that of another, the difference observed here is, however, real because the queens and workers came from the same nests. A sesquiterpenoid compound was found in trace quantities in both queens and workers. The substance was readily identified by its mass spectrum (Fig. 5), and it is called tetramorene-2. Tetramorene was first found in the Dufour glands of Tetramorium caespitum and T. impurum (Billen et al., 1986), but its structure has not yet been studied.

The Dufour glands of L. nylanderi workers contained a simple mixture of linear hydrocarbons (C_{14}-C_{17}) with pentadecane the major component (Fig. 6). The glands of this species were extremely small and contained a mean of only 20ng per worker (Table 8). The second most abundant compound is the same sesquiterpenoid. There were no queens available for analysis.

The secretions of the Dufour glands of Leptothorax species studied here have different major components, but are similar in having simple hydrocarbons and tetramorene-2, and agreed with the general pattern of the Dufour glands of myrmicine ants except that in Pogonomyrmex in which the Dufour glands contain branched hydrocarbons (Regnier et al., 1973; Billen et al., 1987). The hypothesis that the Dufour gland substances from a species-specific mixture, already substantiated in many species is further confirmed by this example.

The (E)-β-farnesene found in H. sublaevis (Ollett et al., 1987) is not present in either species of Leptothorax. Selective scanning in the GC-MS experiment at high sensitivity for m/z 204, the molecular ion of the farnesenes, showed none present. It is possible that this
Fig 5. Mass spectrum of tetramorene-2, a sesquiterpenoid found in the Dufour glands of *Tetramorium caespitum*, *T. impurum*, *L. acervorum* and *L. nylanderi*. 
Fig. 6. A gas chromatogram of the single Dufour gland of a worker of L. nylanderi. T indicates tetramorene-2.
Chemical composition of the content of the Dufour gland of workers of *Leptothorax nylanderi* determined by gas chromatography and mass spectometry.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean quantity ng ± SD</th>
<th>% ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Tetradecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>Pentadecene</td>
<td>3 ± 2</td>
<td>13.9 ± 7.0</td>
</tr>
<tr>
<td>n-Pentadecane</td>
<td>10 ± 7</td>
<td>57.1 ± 13.3</td>
</tr>
<tr>
<td>n-Hexadecane</td>
<td>0.1 ± 0.2</td>
<td>0.2 ± 0.6</td>
</tr>
<tr>
<td>Heptadecene</td>
<td>0.04 ± 0.1</td>
<td>0.2 ± 0.8</td>
</tr>
<tr>
<td>n-Heptadecane</td>
<td>0.1 ± 0.3</td>
<td>0.2 ± 0.7</td>
</tr>
<tr>
<td>Tetramorene-2</td>
<td>6 ± 8</td>
<td>26 ± 15.4</td>
</tr>
<tr>
<td>n-Octadecane</td>
<td>0.7 ± 2</td>
<td>1.5 ± 2.7</td>
</tr>
<tr>
<td><strong>Total amount</strong></td>
<td><strong>21 ± 20</strong></td>
<td></td>
</tr>
</tbody>
</table>

* t = trace
substance is responsible for the disruptive effect of raiding *H. sublaevis* workers in the *L. acervorum* nest. However preliminary attempts to identify the same substance in *L. kutteri* queens have been unsuccessful.

The Dufour glands of the workerless *L. kutteri* queens are remarkably larger in size than those in *L. acervorum* queens, although the body size of *L. kutteri* is smaller than in *L. acervorum* queens. Analysis of the Dufour glands of *L. kutteri* queens by the same techniques revealed no substances similar to those in *L. acervorum, L. nylanderi* or *H. sublaevis*. In the first samples, sent from Bath, no material at all was present. We considered the possibility that the queens had emptied their glands in the disturbance. For further samples I went to Bath and dissected and sealed the Dufour glands there. The samples were brought back to Keele for analysis. A group of about 5 peaks in the $C_{19}$-$C_{25}$ region were observed, which did not correspond to simple hydrocarbons. The shortage of experimental material prevented any further investigation.

The mixture of hydrocarbons in *H. sublaevis* (Ollett et al., 1987) is close in comparison to that in *L. acervorum*, but quite unlike that in *L. nylanderi*. In both *H. sublaevis* and *L. acervorum* heptadecene is the major substance, with heptadecadiene second. The chief differences are (1) there is no detectable (E)-$\beta$-farnesene in either *Leptothorax* species, (2) there are small amounts of $C_{19}$-$C_{23}$ hydrocarbons in *H. sublaevis* not present in *L. acervorum*. These would render the *Harpagoxenus* secretion somewhat less volatile than that of *Leptothorax*. (3) Tetramorene-2 has not been found in *H. sublaevis*.

If *L. kutteri* queens gain access to *L. acervorum* nests with the aid of their Dufour gland secretion, the substance responsible is not (E)-$\beta$-farnesene, and this subject requires further investigation, when material is available.
Harvester ants are those which collect and store seeds to feed their brood and provide food for the winter. The *Messor* species common in the Mediterranean area take the corresponding place of the *Pogonomyrmex* harvester ants of central and southern U.S.A. Some studies have already been carried out on *Pogonomyrmex* Dufour glands. Therefore it was interesting to examine those of two *Messor* species which became available (collected by J.P.J. Billen in Corsica).

Moreover in the case of *Messor minor* (Andre), both queens and workers were collected. There have been relatively few opportunities to compare queens' and workers' Dufour glands. We have described earlier an examination of *Leptothorax acervorum* which showed little difference between the castes, and later describe the contents of glands of workers and queens of *Camponotus aethiops*.

The Dufour gland secretion of *M. minor* workers and queens are closely similar. The glands are filled with branched and saturated and unsaturated hydrocarbons ranging from C_{11} to C_{19} in workers as shown in Fig. 7. Tridecane is the major compound followed by pentadecane, then nonadecene, 3-methyl-tridecane, heptadecane, 5-methyltridecane, undecane, 7-methyl-tridecane and tetradecane respectively (33.4, 16.7, 9.87, 5.19, 4.88, 4.6, 4.01, 3.83, and 2.34% respectively) (Table 9). In queens the hydrocarbon compounds ranged from C_{11} to C_{17} as shown in Fig. 8. The pentadecene is the major compound followed by tridecane, pentadecane, tetradecene and heptadecane respectively (56.5, 14.4, 13.5, 2.46 and 2.27% respectively) (Table 10). The overall difference between the Dufour gland secretion of workers and queens is that the workers also contain C_{18} and C_{19} hydrocarbons not present in queens and the workers have mostly saturated compounds while unsaturated compounds are dominant in queens. This is the largest difference between the gland contents of
Fig. 7: A gas chromatogram of a single Dufour gland from a worker of *Messor minor*, analysed on an OV-1 capillary column. No. of peaks refer to Table 9.
TABLE 9
Mean amounts and percentages of the Dufour gland compounds of *Messor minor* workers determined by gas chromatography and mass spectrometry.

<table>
<thead>
<tr>
<th>Number</th>
<th>Compound</th>
<th>% ± SD</th>
<th>Mean quantity (µg ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-Undecane</td>
<td>4.01 ± 1.19</td>
<td>0.09 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>5-Methylundecane</td>
<td>1.19 ± 1.56</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>3</td>
<td>3-Methylundecane</td>
<td>1.46 ± 0.53</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>n-Dodecane</td>
<td>1.70 ± 0.22</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>Tridecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>6</td>
<td>n-Tridecane</td>
<td>33.4 ± 3.63</td>
<td>0.85 ± 0.33</td>
</tr>
<tr>
<td>7</td>
<td>7-Methyltridecane</td>
<td>3.83 ± 0.72</td>
<td>0.09 ± 0.08</td>
</tr>
<tr>
<td>8</td>
<td>5-Methyltridecane</td>
<td>4.6 ± 0.44</td>
<td>0.11 ± 0.05</td>
</tr>
<tr>
<td>9</td>
<td>3-Methyltridecane</td>
<td>5.19 ± 0.44</td>
<td>0.12 ± 0.06</td>
</tr>
<tr>
<td>10</td>
<td>Tetradecane</td>
<td>0.84 ± 0.20</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>11</td>
<td>n-Tetradecane</td>
<td>2.34 ± 0.36</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>12</td>
<td>n-Pentadecane</td>
<td>16.7 ± 1.54</td>
<td>0.40 ± 0.19</td>
</tr>
<tr>
<td>13</td>
<td>7-Methylpentadecane</td>
<td>0.34 ± 0.18</td>
<td>0.008 ± 0.005</td>
</tr>
<tr>
<td>14</td>
<td>5-Methylpentadecane</td>
<td>0.86 ± 0.19</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>15</td>
<td>3-Methylpentadecane</td>
<td>1.92 ± 0.39</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>16</td>
<td>Hexadecene</td>
<td>0.56 ± 0.14</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>17</td>
<td>n-Hexadecane</td>
<td>1.30 ± 0.21</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>18</td>
<td>Heptadecadiene</td>
<td>1.24 ± 0.25</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>19</td>
<td>Heptadecene</td>
<td>0.54 ± 0.29</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>20</td>
<td>n-Heptadecane</td>
<td>4.88 ± 0.44</td>
<td>0.12 ± 0.05</td>
</tr>
<tr>
<td>21</td>
<td>Octadecene</td>
<td>0.69 ± 0.11</td>
<td>0.02 ± 0.007</td>
</tr>
<tr>
<td>22</td>
<td>Nonadecadiene</td>
<td>0.87 ± 0.49</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>23</td>
<td>Nonadecene</td>
<td>9.87 ± 2.35</td>
<td>0.23 ± 0.11</td>
</tr>
<tr>
<td>24</td>
<td>n-Nonadecane</td>
<td>0.21 ± 0.35</td>
<td>0.003 ± 0.004</td>
</tr>
</tbody>
</table>

Total amount in µg: 2.34 ± 1.0

* t = trace
Fig. 8: A gas chromatogram of a single Dufour gland of a mouse, separated using a given capillary column. No. of peaks refer to Table 10.
Mean amounts and percentages of the Dufour gland compounds of *Hessor minor* queens determined by gas chromatography and mass spectrometry.

<table>
<thead>
<tr>
<th>Number</th>
<th>Compound</th>
<th>% ± SD</th>
<th>Mean quantity (ug ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-Undecane</td>
<td>1.21 ± 0.69</td>
<td>0.09 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td>5-Methylundecane</td>
<td>0.11 ± 0.10</td>
<td>0.006 ± 0.005</td>
</tr>
<tr>
<td>3</td>
<td>3-Methylundecane</td>
<td>0.14 ± 0.14</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>n-Dodecane</td>
<td>0.30 ± 0.09</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>Tridecane</td>
<td>1.03 ± 0.32</td>
<td>0.08 ± 0.04</td>
</tr>
<tr>
<td>6</td>
<td>n-Tridecane</td>
<td>14.4 ± 2.36</td>
<td>1.05 ± 0.36</td>
</tr>
<tr>
<td>7</td>
<td>5-Methyltridecane</td>
<td>0.73 ± 0.31</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>8</td>
<td>3-Methyltridecane</td>
<td>0.81 ± 0.39</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>9</td>
<td>Tetradecene</td>
<td>2.46 ± 0.43</td>
<td>0.18 ± 0.07</td>
</tr>
<tr>
<td>10</td>
<td>n-Tetradecane</td>
<td>1.23 ± 0.26</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>11</td>
<td>Pentadecadiene</td>
<td>0.4 ± 0.1</td>
<td>0.03 ± 0.007</td>
</tr>
<tr>
<td>12</td>
<td>Pentadecene</td>
<td>56.5 ± 7.23</td>
<td>4.02 ± 0.89</td>
</tr>
<tr>
<td>13</td>
<td>n-Pentadecane</td>
<td>13.5 ± 2.52</td>
<td>0.97 ± 0.25</td>
</tr>
<tr>
<td>14</td>
<td>7-Methylpentadecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>15</td>
<td>5-Methylpentadecane</td>
<td>0.41 ± 0.20</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>16</td>
<td>3-Methylpentadecane</td>
<td>1.37 ± 0.31</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td>17</td>
<td>Hexadecene</td>
<td>0.32 ± 0.10</td>
<td>0.02 ± 0.009</td>
</tr>
<tr>
<td>18</td>
<td>n-Hexadecane</td>
<td>0.73 ± 0.20</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>19</td>
<td>Heptadecene</td>
<td>0.55 ± 0.10</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>20</td>
<td>n-Heptadecane</td>
<td>2.27 ± 0.70</td>
<td>0.16 ± 0.06</td>
</tr>
</tbody>
</table>

Total amount in pg: 7.16 ± 1.54

*t = trace*
the two castes yet encountered. The total amount of the Dufour gland secretion of queens is more than workers (7.16 and 2.34ug respectively).

In Messor capitatus (Latr.) workers, the Dufour gland secretion contains only saturated and unsaturated hydrocarbons \( \{C_{11} \text{ to } C_{21}\} \) as shown in Fig. 9. Tridecane is the major component (27.6%) followed by nonadecane (25.8%) (Table 11). No branched chain hydrocarbons were found except traces of two compounds which agreed in retention times with 5-methyltridecane and 3-methyltridecane but they were not detected by mass spectrometry.

M. capitatus workers differ in their secretion from both M. minor workers and queens in that the hydrocarbon chain was extended by two and four carbon atoms more than the M. minor workers and queens respectively and the branched compounds were not detected in M. capitatus workers.

The Dufour gland secretion of both M. minor workers and queens differ from the general pattern of myrmicine ants studied so far, except those in Pogonomyrmex species. Pogonomyrmex barbatus and P. rugosus contain straight and branched chain hydrocarbons \( \{C_{12} \text{ to } C_{15}\} \) with n-dodecane and 6-methyldodecane as major components in P. barbatus while 6-methylundecane and 5-methylundecane were the major components in P. rugosus (Regnier et al., 1973). Billen et al. (1987) studied P. occidentalis Dufour gland which follows the general pattern of M. minor and P. barbatus and P. rugosus in having branched chain hydrocarbons in addition to linear hydrocarbons \( \{C_{12} \text{ to } C_{19}\} \) the 4- and 6-methyl branched hydrocarbons present in Pogonomyrmex species were not detected in M. minor. The similarities between Pogonomyrmex species and M. minor may indicate a closer phylogenetic connection between these two groups of harvester ants than has been recognized.
Fig. 9: A gas chromatogram of a single Dufour gland from a worker of *Messor capitatus*, analysed on an OV-1 capillary column. No. of peaks refer to Table 11.
Mean amounts and percentages of the DuFour gland compounds of *Messor capitatus* workers determined by gas chromatography and mass spectrometry.

<table>
<thead>
<tr>
<th>Number</th>
<th>Compound</th>
<th>% ± SD</th>
<th>Mean quantity (ug ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-Undecane</td>
<td>0.41 ± 0.33</td>
<td>0.009 ± 0.009</td>
</tr>
<tr>
<td>2</td>
<td>n-Dodecane</td>
<td>0.37 ± 0.30</td>
<td>0.008 ± 0.009</td>
</tr>
<tr>
<td>3</td>
<td>Tridecane</td>
<td>1.86 ± 0.38</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>4</td>
<td>n-Tridecane</td>
<td>27.6 ± 5.9</td>
<td>0.58 ± 0.47</td>
</tr>
<tr>
<td>5</td>
<td>Pentadecene</td>
<td>6.5 ± 1.3</td>
<td>0.14 ± 0.12</td>
</tr>
<tr>
<td>6</td>
<td>n-Pentadecane</td>
<td>3.26 ± 0.94</td>
<td>0.07 ± 0.06</td>
</tr>
<tr>
<td>7</td>
<td>n-Hexadecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>8</td>
<td>Heptadecene</td>
<td>3.62 ± 1.03</td>
<td>0.08 ± 0.10</td>
</tr>
<tr>
<td>9</td>
<td>n-Heptadecane</td>
<td>3.72 ± 1.38</td>
<td>0.08 ± 0.11</td>
</tr>
<tr>
<td>10</td>
<td>Octadecene</td>
<td>0.28 ± 0.16</td>
<td>0.006 ± 0.008</td>
</tr>
<tr>
<td>11</td>
<td>n-Octadecane</td>
<td>1.40 ± 0.29</td>
<td>0.03 ± 0.03</td>
</tr>
<tr>
<td>12</td>
<td>Nonadecene</td>
<td>8.78 ± 2.9</td>
<td>0.21 ± 0.22</td>
</tr>
<tr>
<td>13</td>
<td>n-Nonadecane</td>
<td>25.8 ± 6.5</td>
<td>0.53 ± 0.44</td>
</tr>
<tr>
<td>14</td>
<td>Eicosene</td>
<td>0.47 ± 0.33</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>15</td>
<td>n-Eicosane</td>
<td>0.47 ± 0.22</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>16</td>
<td>Heneicosene</td>
<td>9.63 ± 4.6</td>
<td>0.20 ± 0.18</td>
</tr>
<tr>
<td>17</td>
<td>n-Heneicosane</td>
<td>4.06 ± 1.20</td>
<td>0.10 ± 0.10</td>
</tr>
</tbody>
</table>

Total amount in µg: 2.15 ± 1.93

*t = trace*
No sesquiterpene compounds were detected in Messor species. The presence of undecane and the high proportion of tridecane in their glands places these species closer to the formicines than any other Myrmicine ant studied so far.

**Tetramorium caespitum (L.)**

The contents of the Dufour gland of *T. caespitum* has already been investigated (Billen et al., 1986). They found a series of alkanes and alkenes ranging from $C_{13}$ to $C_{17}$ in addition to sesquiterpenes with pentadecane as the major compound (Fig. 10).

It was thought by R. Cammaerts (University of Brussels) that there might be different races of this species in different areas, because of the contradictory results on mandibular glands and also from a difference in workers size. We were therefore encouraged to examine Dufour glands of workers of *T. caespitum* from 17 widely distributed nests collected from England, France, Belgium, Luxembourg and Denmark (Table 12). The location of these nests and some indication of the terrain where they were collected is given in Table 12. A visual comparison of the Dufour glands of workers from all of these colonies did not show any marked difference. In every case pentadecane was the major substance, followed by pentadecene. A typical example is given in Table 13. The greatest difference discernable was in two colonies from central France, a somewhat higher value of heptadecene was found than elsewhere. However, within the wide variation from one worker to another it was felt that much more data would be necessary to see if this difference was statistically significant.

**Myrmica species**

The Dufour gland secretion of some *Myrmica* species are dominated
Fig. 10: A gas chromatogram of a single Dufour gland from a worker of T. caespitum on a 10% PEGA column. The numbers indicate the compounds investigated:
1 = tridecane; 2 = tetradecane; 3 = pentadecane;
4 = pentadecene; 5 = hexadecane; 6 = hexadecene;
7 = heptadecane; 8 = heptadecene; 9 = heptadecadiene;
10 = sesquiterpenoid (M'236); 11 = sesquiterpenoid (M'250).
<table>
<thead>
<tr>
<th>Country</th>
<th>Site</th>
<th>District</th>
<th>Soil</th>
<th>Altitude (m)</th>
<th>Date of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark</td>
<td>Mois</td>
<td>Jutland</td>
<td>Sandy heath</td>
<td>55</td>
<td>10.84</td>
</tr>
<tr>
<td>England</td>
<td>Heartland Moor I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>England</td>
<td>Heartland Moor II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>England</td>
<td>Heartland Moor III</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>England</td>
<td>Dorset</td>
<td></td>
<td>Sandy heath</td>
<td>20</td>
<td>8.82</td>
</tr>
<tr>
<td>Belgium</td>
<td>Adinkerke</td>
<td>N. Sea coast</td>
<td>Old sand dunes</td>
<td>5</td>
<td>5.84</td>
</tr>
<tr>
<td>Belgium</td>
<td>Mol</td>
<td>Kempen</td>
<td>Heath</td>
<td>30</td>
<td>7.83</td>
</tr>
<tr>
<td>Belgium</td>
<td>Dilsen</td>
<td></td>
<td>Heath on coarse sand</td>
<td>80</td>
<td>5.84</td>
</tr>
<tr>
<td>Belgium</td>
<td>Rotselaar</td>
<td>Brabant</td>
<td>Sandy hill</td>
<td>35</td>
<td>9.84</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>Steinfeld</td>
<td>Gutland</td>
<td>Old quarry calcareous sand</td>
<td>350</td>
<td>5.84</td>
</tr>
<tr>
<td>France</td>
<td>Elinghen</td>
<td>Pas-de-Calais</td>
<td>Abandoned colliery dump</td>
<td>80</td>
<td>4.84</td>
</tr>
<tr>
<td>France</td>
<td>Les Moitiers-d’Allonne</td>
<td>Contentin</td>
<td>sea dunes</td>
<td>-</td>
<td>9.84</td>
</tr>
<tr>
<td>France</td>
<td>St. Diery</td>
<td>Auvergne</td>
<td>basaltic soil</td>
<td>700</td>
<td>8.84</td>
</tr>
<tr>
<td>France</td>
<td>Creste</td>
<td></td>
<td>basaltic</td>
<td>1000</td>
<td>8.84</td>
</tr>
<tr>
<td>France</td>
<td>Rochebloine</td>
<td>Vivarais</td>
<td>granitic</td>
<td>1000</td>
<td>8.84</td>
</tr>
<tr>
<td>France</td>
<td>Boucicou-le-Roi</td>
<td>Massif Central</td>
<td>alluvial loam</td>
<td>270</td>
<td>8.84</td>
</tr>
<tr>
<td>France</td>
<td>Causse Noir</td>
<td>Causses</td>
<td>limestone</td>
<td>1100</td>
<td>8.84</td>
</tr>
<tr>
<td>France</td>
<td>Ispagnac</td>
<td></td>
<td>river alluvium</td>
<td>500</td>
<td>8.84</td>
</tr>
</tbody>
</table>
TABLE 13

Amounts of pentadecane and pentadecene in the Dufour glands of two samples of T. caespitum collected in the massif Central of France, at Creste (alt. 1000m), near St. Diery, and Boucieu-le-Roi (alt. 270m) on the eastern slope (Vivarais).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Creste</th>
<th>Boucieu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount ng±SD</td>
<td>% of total ±SD</td>
</tr>
<tr>
<td>pentadecane</td>
<td>150 ± 40</td>
<td>54.1 ± 9.03</td>
</tr>
<tr>
<td>pentadecene</td>
<td>20 ± 10</td>
<td>7.10 ± 2.75</td>
</tr>
<tr>
<td>Heptadecene</td>
<td>50 ± 20</td>
<td>16.51 ± 3.38</td>
</tr>
</tbody>
</table>

Total amount (ng) of volatiles in gland: 290 ± 100 for Creste, 160 ± 70 for Boucieu.
by linear saturated and unsaturated hydrocarbons (Morgan and Wadhams, 1972a; Morgan et al., 1977; Cammaerts et al., 1978, 1981a; Attygalle et al., 1983a,b). In other Myrmica species, the Dufour gland secretion consists chiefly of sesquiterpene compounds, farnesene, homofarnesene, and bishomofarnesene (Morgan et al., 1979; Cammaerts et al., 1978, 1981a; Attygalle et al., 1983a,b). A species-specific mixture of these substances seems to exist in each of the eight Myrmica species studies.

The present work was undertaken in collaboration with a group of taxonomists to see whether this chemotaxonomic test could indicate a difference between different groups of Myrmica, where morphological features of males and workers are unable to make it clear whether they belong to the same species, separated by habitat, or to different species.

Myrmica lonae from the heathlands of the Southern Netherlands is very closely similar to M. sabuleti of Britain and elsewhere in Europe. Analysis of a sample of M. lonae provided by Dr. G. Elmes is given in Table 14a, where it is compared with M. sabuleti determined by Cammaerts et al. (1981a), and a sample of M. sabuleti from Spain provided by C.A. Collingwood. It can be seen that there is no close correlation between these samples, even though bishomofarnesene is the major component in all three. There is a difference between M. sabuleti from Spain and England where homofarnesene is the second major component in the samples from Spain while it is the third major component in the sample from England.

Taxonomically M. scabrinodis of England, should be the same as M. scabrinodis from Spain, and also M. aloba from Spain because the males appear to be the same and M. albuferensis or M. exlandi which live in a specialized habitat of salt marshes at La Albufera on the island of Mallorca. Myrmica aloba from Spain is different from M.
<table>
<thead>
<tr>
<th>Number</th>
<th>Compound</th>
<th>M. ionae (Netherlands)</th>
<th>M. sabuleti (Spain)</th>
<th>M. sabuleti (England)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>Farnesene</td>
<td>0.66</td>
<td>0.54</td>
<td>26.9</td>
</tr>
<tr>
<td>2</td>
<td>Homofarnesene</td>
<td>0.74</td>
<td>0.60</td>
<td>29.6</td>
</tr>
<tr>
<td>3</td>
<td>Bishomofarnesene</td>
<td>0.94</td>
<td>0.77</td>
<td>37.9</td>
</tr>
</tbody>
</table>

Total mean: 2.5±1.93 | 4.30±2.12 | 1.11

* From Cammaerts et al. (1981a)
scabrinodis from Spain, where homofarnesene in the former species is the major component while in the last species is bishomofarnesene. At the same time *M. aloba* from Spain is closely similar to *M. scabrinodis* from England. The two species are different from *M. albuferensis* from Spain where farnesene is the major component followed by homofarnesene then bishomofarnesene (Table 14b).

The patterns of all these species except *M. albuferensis* bear some resemblance to each other, and that the variation in composition from one individual worker to another, makes it impossible at this stage to lay a clear line of distinction between the species. Figure 11 (a,b and c) is an example showing the variation in composition from one individual worker to another in *M. aloba*, where either of farnesene, homofarnesene or bishomofarnesene become the largest one in different samples examined, which indicates no regular pattern among the species. The help of a biostatistician was sought to see if statistical analysis of the data could show significant differences. However the variation between individuals in the small samples available make statistical analysis impossible at this stage.
TABLE 14b

The mean quantity (µg) and percentage of the major compounds contained in the Dufour glands of some Myrmica ants

<table>
<thead>
<tr>
<th>Number</th>
<th>Compound</th>
<th>M. aloba (Spain)</th>
<th>M. scabrinodis (Spain)</th>
<th>M. scabrinodis (England)*</th>
<th>M. albuferensis (Spain)#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>%</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>1</td>
<td>Farnesene</td>
<td>0.65</td>
<td>0.55</td>
<td>37.9</td>
<td>9.6</td>
</tr>
<tr>
<td>2</td>
<td>Homofarnesene</td>
<td>0.77</td>
<td>0.60</td>
<td>42.9</td>
<td>7.4</td>
</tr>
<tr>
<td>3</td>
<td>Bishomofarnesene</td>
<td>0.28</td>
<td>0.25</td>
<td>15.7</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Total mean 1.74±1.40 4.32±3.11 0.91 4.79±0.87

*From Cammaerts et al. (1981a)
#From Attygalle, 1983
Fig. 11a: A gas chromatogram of a single Dufour gland from a worker of Myrmica aloba, analysed on a PEGA 10% packed column. No. of peaks refer to Table 14b.
Fig. 11b: A gas chromatogram of a single Dufour gland from a worker of Myrmica albofasciata, analysed on a PEGA 10% packed column. No. of peaks refer to Table 14b.
Fig. 11c: A gas chromatogram of a single Dufour gland from a worker of Myrmica aloba, analysed on a PEGA 10% packed column. No. of peaks refer to Table 14b.
Dufour gland investigations of formicine ants

Formica species

Formica sanguinea Latr. was interesting to study because it is a slave-making species. The oldest workers of F. sanguinea are active foraging outside the nest and are most aggressive (Otto, 1958; Dobrzanska, 1959). Dobrzanski (1961) stated that there are no special expeditions of F. sanguinea to find the nests of slaves, but the raids are carried out by the same individuals that are actively foraging and bringing back food. Workers raid the nests, mainly of Formica fusca L. and F. rufibarbis Fabr., carrying off the worker pupae, which, when they emerge in the nests of their mistresses, perform the function of slaves. The raiding parties of F. sanguinea are able to repel the defending workers, probably with the aid of a chemical secretion, which is thought to come from the Dufour gland (Regnier and Wilson, 1971).

Bergstrom and Lofqvist (1968) showed that the compositions of the secretion of the Dufour glands of queens and workers of F. sanguinea were similar. The workers contained C_{9} to C_{13} n-alkanes, n-C_{10}-C_{12} acetates and the corresponding C_{10}-C_{12} alcohols, tridecanone and an unidentified isomer of farnesene. Females were similar with a little nonyl acetate. It was pointed out by Bergstrom and Lofqvist (1968), that the odour of F. sanguinea (due to its acetates) was very similar to that of its slave F. rufibarbis which also contains acetates. However, F. fusca, which is the more common slave of F. sanguinea, even in areas where F. fusca and F. rufibarbis are both present, does not contain the acetates and does not have the same characteristic odour (Bergstrom and Lofqvist 1968).

The large colonies of F. sanguinea were available at the University of Leuven, Belgium, with all stages of development; samples were marked and taken at different ages and sent to Keele. We
describe here an investigation of the contents of the Dufour gland of recently emerged imagos of workers of *F. sanguinea*, comparing it with the glands of mature and old workers, to show whether there is a difference between them, to shed light on the chemical compounds which may be used as a slave-raiding weapon. Our present interest in *F. fusca* and *F. jemani* was prompted by the close taxonomic relation between the two species. We were asked whether they were distinguishable by the chemical composition of the Dufour gland secretion.

The contents of the Dufour glands of *F. sanguinea* workers, taken from two large laboratory colonies, using single dissected glands, were analysed. Ten components listed in Table 15 were recognized. Each component has been identified by its mass spectrum and checked by comparing its retention time with that of the pure compound. Table 15 shows a comparison between recently emerged callow workers, mature foragers and very old foragers. The amount in the gland and the proportions were very variable from one worker to another. Nevertheless, trends are observable, chiefly in the fall of the proportion of undecane and the rise in the proportion of farnesene and the higher hydrocarbons with age. Dodecyl acetate rises and then falls again in very old workers.

When the Dufour glands of newly emerged adults were examined in detail even greater variability was encountered. Some workers at 10 days old had no secretion in their glands while others had measurable amounts at only 2 days from emergence. However, when sufficiently large numbers were sampled and averaged, a steady trend of increasing secretion was observed (Table 16), though with large standard deviations. The total amount of secretion increased tenfold in less than two months. At first undecane (65%) is the major component, its proportion decreases with age and the other components increase, with
TABLE 15
The composition of the Dufour gland secretion of workers of
F. sanguinea at different ages by gas chromatography

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Compound</th>
<th>callow 15-24 days</th>
<th>mature foragers</th>
<th>old foragers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ng±SD</td>
<td>%±SD</td>
<td>ng±SD</td>
</tr>
<tr>
<td>1</td>
<td>n-Nonane</td>
<td>4.6±6.9</td>
<td>0.7±1.1</td>
<td>32±21</td>
</tr>
<tr>
<td>2</td>
<td>n-Decane</td>
<td>47.5±157.5</td>
<td>6.8±12.9</td>
<td>48±25</td>
</tr>
<tr>
<td>3</td>
<td>n-Undecane</td>
<td>290±290</td>
<td>51±25</td>
<td>7036±3908</td>
</tr>
<tr>
<td>4</td>
<td>n-Tridecane</td>
<td>10±10</td>
<td>3.3±7.0</td>
<td>171±103</td>
</tr>
<tr>
<td>5</td>
<td>n-Pentadecane</td>
<td>1.9±3.9</td>
<td>0.2±0.6</td>
<td>17±14</td>
</tr>
<tr>
<td>6</td>
<td>n-Heptadecane</td>
<td>15±35</td>
<td>0.8±1.3</td>
<td>10±11</td>
</tr>
<tr>
<td>7</td>
<td>Decyl acetate</td>
<td>170±270</td>
<td>11±13</td>
<td>170±221</td>
</tr>
<tr>
<td>8</td>
<td>(Z,E)-α-farnesene</td>
<td>63±117</td>
<td>9.4±12</td>
<td>511±195</td>
</tr>
<tr>
<td>9</td>
<td>Undecyl acetate</td>
<td>30±50</td>
<td>3±3</td>
<td>313±227</td>
</tr>
<tr>
<td>10</td>
<td>Dodecyl acetate</td>
<td>150±270</td>
<td>12±18</td>
<td>595±1602</td>
</tr>
</tbody>
</table>

Total 800±1040 10,902±4989 4480±2800
No. of samples 27 12 19
t = trace
TABLE 16

The mean quantity of the major components in the Dufour glands of young adult workers of F. sanguinea at different ages.

<table>
<thead>
<tr>
<th>Compound</th>
<th>2-5 days</th>
<th>6-10 days</th>
<th>15-24 days</th>
<th>35-72 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng±SD</td>
<td>%±SD</td>
<td>ng±SD</td>
<td>%±SD</td>
</tr>
<tr>
<td>n-Undecane</td>
<td>80±120</td>
<td>65±31</td>
<td>140±240</td>
<td>48±30</td>
</tr>
<tr>
<td></td>
<td>290±290</td>
<td>51±25</td>
<td>530±600</td>
<td>52±19</td>
</tr>
<tr>
<td>n-Tridecane</td>
<td>2±3</td>
<td>1.2±1.5</td>
<td>6±20</td>
<td>1±1</td>
</tr>
<tr>
<td></td>
<td>10±10</td>
<td>3.3±7.0</td>
<td>30±60</td>
<td>1.0±0.9</td>
</tr>
<tr>
<td>Decylacetate</td>
<td>3±7</td>
<td>2.1±2.9</td>
<td>20±70</td>
<td>2±5</td>
</tr>
<tr>
<td></td>
<td>170±270</td>
<td>11±13</td>
<td>230±410</td>
<td>11±7.5</td>
</tr>
<tr>
<td>Undecyl acetate</td>
<td>3±3</td>
<td>1.3±1.6</td>
<td>20±50</td>
<td>1±2</td>
</tr>
<tr>
<td></td>
<td>30±50</td>
<td>3±2.5</td>
<td>110±210</td>
<td>4.5±3</td>
</tr>
<tr>
<td>Dodecyl acetate</td>
<td>4±10</td>
<td>0.9±2.3</td>
<td>30±60</td>
<td>3±5</td>
</tr>
<tr>
<td></td>
<td>150±270</td>
<td>12±18</td>
<td>400±760</td>
<td>18±13</td>
</tr>
<tr>
<td>(Z,E)-α-farnesene</td>
<td>3±3</td>
<td>14±18</td>
<td>12±14</td>
<td>10±13</td>
</tr>
<tr>
<td></td>
<td>63±117</td>
<td>9.4±12</td>
<td>100±170</td>
<td>4.5±4</td>
</tr>
<tr>
<td>Total secretion</td>
<td>100±145</td>
<td>250±420</td>
<td>800±1040</td>
<td>1510±2310</td>
</tr>
<tr>
<td>No. of samples</td>
<td>9</td>
<td>24</td>
<td>27</td>
<td>22</td>
</tr>
</tbody>
</table>
farnesene and dodecyl acetate increasing most. Decyl and undecyl acetates did not increase in step with dodecyl acetate. The examination shows (1) that the gland fills up very slowly, (2) the initial composition does not resemble the final composition very closely, (3) although the amount of farnesene steadily increases with time, the fraction of the whole that it represents actually falls during this period, (4) the amount of acetates, initially very low, rises rapidly. The young glands of newly emerged adult workers are most efficient at making alkanes and farnesene and only slowly develop their capacity to make or accumulate the alkyl acetates. Undecane is the first substance to appear in the gland. Two workers, analysed on the second day of emergence, contained 30ng and 24ng of undecane and no other detectable substances, but some glands remained empty up to ten days. From the data in Table 16 it is possible to calculate that on average, 25ng of secretion were added to the gland per day for the first two months. At that rate it would require more than a year for the gland to reach its full contents of 10μg. Cammaerts-Tricot (1974) in studying the behaviour of Myrmica rubra found when workers were divided into 5 age groups by degree of pigmentation, the volume of the Dufour gland and the attractive power of the gland increased with age.

When old workers were analysed by combined gas chromatography-mass spectrometry, using a capillary column, a number of minor components were also identified, all of them present as trace components. A typical chromatogram is shown in Figure 12. These additional components have all be identified by their mass spectra, and the alcohols and acetates have been confirmed from the spectra and retention times of the pure standards. The analysis was made most recently after the queen had become inactive and no new brood was being produced, although the colony was still actively foraging and contained hundreds of workers.
Fig. 12: Capillary gas chromatogram showing the minor as well as the major components in five Dufour glands from mature old workers of *F. sanguinea*. (a) at low sensitivity, showing the major components, (b) at higher sensitivity to show the minor components. Symbols indicate the identifications, e.g. $C_{10}$ is n-decane; 5MeC$_{11}$ is 5-methylundecane; C$_{10}$Ac is decyl acetate; C$_{12}$OH, dodecanol; Farnesene, (Z,E)-(c)-farnesene; the other minor components are: a, branched C$_{13}$ acetate; b, heptadecadiene; c, another branched C$_{13}$ acetate; d, heptadecene; e, dodecylpropionate; f, tridecyl acetate; g, n-heptadecane; h, heptadecyl acetate; i, heneicosane; j, an unknown alkene; k, stearic acid; l, an unidentified diterpene acetate. The small peaks of C$_{22}$, C$_{23}$, C$_{24}$ hydrocarbons are probably from the small amount of cuticle in the dissected glands. Column conditions were a temperature programme from 60°C at 4°C min$^{-1}$ to 250°C then isothermal.
The most noticeable change from the mature foragers and young workers was the massive amount of farnesene in these old glands and the relative decline of undecane and decyl acetate. The apparent discrepancy between the amounts and percentages of undecane and farnesene is a result of the variability of individuals. The percentage composition of each component for each individual worker is calculated and the mean of each of these percentages is recorded (Table 15).

The detailed examination of components by linked gas chromatography mass spectrometry revealed a number of minor components that are identified in Fig. 12. These were branched chain alkanes, straight chain alcohols such as undecanol and dodecanol, accompanying the much larger quantities of the corresponding acetates and a tiny amount of dodecyl propionate, free palmitic and stearic acid and an unidentified diterpene acetate.

The farnesene isomer has been identified by its mass spectrum and retention time as \((Z,E)\)-farnesene \((\text{VII})\). This is the same isomer that has been identified in eight species of Myrmica ants (Attygalle and Morgan 1984b) and in other ants.

This analysis was similar to, but not identical with, that described by Bergstrom and Lofqvist (1968) for \(F.\) sanguinea. The chief difference was in the much smaller quantity of decyl acetate in our sample, except in very young workers, and the absence of tridecanone, even in traces. However, Bergstrom and Lofqvist (1968) also note the variability in composition from one sample to another.

According to Regnier and Wilson (1971) the Dufour gland is the source of the pheromone which plays a principle part in the disruption of the raided nest in slave-making raids of species of the Raptiformica sanguinea group. According to these authors, for \(F.\) subintegra Emery and \(F.\) pergandei Emery, two North American species...
closely related to F. sanguinea, it is the alkyl acetates which provide the disorganizing effect for the slaves. According to Czechowski (1977), slave raiding by European F. sanguinea is certainly based on pheromonal secretions, although there is no evidence for a chemical disarming with "propaganda" substances as in the American species. Since it is generally the oldest workers which forage outside the nest and which are the most aggressive (Otto, 1958; Dobrzanska, 1959) we may infer that it is these individuals, with the highest proportion of farnesene in their glands, that perform the slave raids.

Lofqvist (1977) has examined the behavioural effect of the alkyl acetates on F. sanguinea workers and came to the conclusion that they had none, but that their only function was as wetting agents for the penetration of formic acid. We suggest that farnesene is a much more likely substance for colony disruption than alkyl acetates. The latter are relatively stable substances which would remain on the captured brood when it was brought to the raiders' nest. In that nest, the brood would have to be attended by other workers of the slave species which might still be repelled. Farnesene on the other hand is an unstable substance, in the pure state oxidized by air to an involatile and odourless polymeric material. Suitably diluted with oil substances, such as the undecane and dodecyl acetate of the Dufour gland, its half life would be prolonged. The exact length of this time would depend upon concentration, the thinness of the oily film and the odour threshold. Such a substance sprayed on the brood would not persist. The brood might no longer be contaminated by the time it is received by the slave nurses in the F. sanguinea nest. It is noteworthy that the Dufour glands of Polyergus rufescens, another enslaving species, are also rich in farnesene (Bergstrom and Lofqvist, 1967), and that the slave F. fusca also contains it, but the glands of
F. fusca are much smaller (mean of 4.8µg hydrocarbons) with much less farnesene (100ng per individual). On the other hand, F. fusca worker Dufour glands contain no detectable acetates.

The Dufour gland secretion of Formica fusca (L.) consists mainly of saturated straight chain hydrocarbons (Fig. 13). In addition to the saturated hydrocarbons we found only one compound with one double bond represented by tridecane (0.80%). Two sesquiterpenoid compounds, farnesene (1.97%) and homofarnesene, which appeared as a trace, were present. The saturated chain hydrocarbons range from C₉ to C₁₉ with undecane the most abundant hydrocarbon (75% of the total) followed by tridecane (0.3%), then followed by decane, heptadecane, pentadecane, dodecane and nonane. Tetradecane, hexadecane, octadecane and nonadecane were present in trace amounts. The branched chain alkanes contain a methyl group in 3-, 4- or 5-position with 3-methylundecane the most abundant (1.96), followed by 5-methylundecane (1.78%), then 3-methyltridecane (0.98%), while 4-methyldodecane and 5-methyltridecane appeared as traces. No oxygenated compounds (alcohols, ketones or esters), as found in some other formicine species, were present in detectable quantities. Satisfactory mass spectra were obtained for all the compounds listed in Table 17.

The homofarnesene (XII) found in F. fusca is the same (Z,E)-(E)-homofarnesene as was found in Myrmica ants (Attygalle and Morgan, 1984b).

No similarities were found between the Dufour gland secretion of F. fusca workers and F. sanguinea workers except that they agree with the general pattern of formicine ants in having hydrocarbons. No alkyl acetates were found in F. fusca and the (Z,E)-(E)-farnesene was found only in small amount in F. fusca but as a major component in F. sanguinea old workers. It is possible that the presence of large amounts of acetates and the high concentration of farnesene in the F.
Fig. 13: A gas chromatogram of a single Dufour gland from a worker of Formica fusca, analysed on an OV-1 capillary column. No. of peaks refer to Table 17.
Table 17

Mean values for the composition of the contents of the Dufour gland of Formica fusca workers determined by gas chromatography and mass spectrometry.

<table>
<thead>
<tr>
<th>Number</th>
<th>Compound</th>
<th>% ± SD</th>
<th>Mean quantity (μg ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-Nonane</td>
<td>0.31 ± 0.32</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>n-Decane</td>
<td>2.40 ± 1.44</td>
<td>0.13 ± 0.11</td>
</tr>
<tr>
<td>3</td>
<td>n-Undecane</td>
<td>75.0 ± 11.1</td>
<td>3.39 ± 1.36</td>
</tr>
<tr>
<td>4</td>
<td>5-Methylundecane</td>
<td>1.78 ± 1.18</td>
<td>0.11 ± 0.11</td>
</tr>
<tr>
<td>5</td>
<td>3-Methylundecane</td>
<td>1.96 ± 1.64</td>
<td>0.12 ± 0.15</td>
</tr>
<tr>
<td>6</td>
<td>n-Dodecane</td>
<td>1.74 ± 1.91</td>
<td>0.12 ± 0.19</td>
</tr>
<tr>
<td>7</td>
<td>4-Methylidodecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>8</td>
<td>Tridecane</td>
<td>0.80 ± 0.59</td>
<td>0.05 ± 0.05</td>
</tr>
<tr>
<td>9</td>
<td>n-Tridecane</td>
<td>8.28 ± 4.60</td>
<td>0.44 ± 0.42</td>
</tr>
<tr>
<td>10</td>
<td>5-Methyltridecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>11</td>
<td>3-Methyltridecane</td>
<td>0.58 ± 0.54</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>12</td>
<td>n-Tetradecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>13</td>
<td>Farnesene</td>
<td>1.97 ± 0.93</td>
<td>0.11 ± 0.08</td>
</tr>
<tr>
<td>14</td>
<td>n-Pentadecane</td>
<td>1.92 ± 1.06</td>
<td>0.11 ± 0.09</td>
</tr>
<tr>
<td>15</td>
<td>Homofarnesene</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>16</td>
<td>n-Hexadecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>17</td>
<td>n-Heptadecane</td>
<td>2.13 ± 1.14</td>
<td>0.11 ± 0.09</td>
</tr>
<tr>
<td>18</td>
<td>n-Octadecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>19</td>
<td>n-Nonadecane</td>
<td>t</td>
<td>t</td>
</tr>
</tbody>
</table>

Total amount in μg 4.83 ± 2.47

t = trace
sanguine secretion may account for the disruption of the *F. fusca* colony in the slave raid. This idea will have to await behavioural tests on an *F. fusca* colony.

Two samples of *F. fusca*, both collected in summer 1985, were examined, one in midsummer from Belgium and one in late summer from England. Both showed the same compounds and, within the variation shown by individual workers, in the same proportions. The Belgian sample had less material, on average, in the glands (i.e. 2.17 +/- 1.64μg) compared with the English sample (4.9μg). This difference is not considered significant; it may reflect the age of the workers, the season, state of nutrition, or confinement during transport of the Belgian colony. The two samples agreed with that recorded by Bergstrom and Lofqvist (1968) from the Island of Oland in Sweden (though they did not identify the isomers of farnesene and homofarnesene). This would suggest that this species is homogeneous at least over this range of territory. Also our work with colonies of *Tetramorium caespitum* from widely distributed habitats and other work with *Iridomyrmex purpureus sens. strict.* (Cavill et al., 1985) collected over a wide area of south-eastern Australia, suggest that a species can remain homogeneous for this character over a wide area.

The Dufour gland of *Formica lemii* (Bondroit) appeared to be filled with a large number of homologous branched, saturated and unsaturated hydrocarbons ranging from C₉ to C₂₂ and two farnesenes (Fig. 14). Undecane was the major component (57%) followed by tridecane (15.2%), then followed by dodecane, decane, pentadecane, heptadecane, tetradecane and nonane. Hexadecane, octadecane and nonadecane were present in traces. Unsaturated hydrocarbons were present and represented by undecene, dodecane, tridecane and tetradecene with tridecene as the most abundant. Of the branched chain hydrocarbons, 3-methylundecane was the major one (3.47%)
Fig. 14: A gas chromatogram of a single Dufour gland from a worker of Formica lemani, analysed on an OV-1 capillary column. No. of peaks refer to Table 18. The trace is shown at two sensitivities to show the overall proportions and also the minor components.
followed by 5-methylundecane (3.12%), 3-methyltridecane (2.23%),
4-methylundecane (0.63%), 5-methyltridecane (0.57%),
7-methylheptadecane (0.31%), 9-methylnonadecane (0.17%) and
3,7-dimethylundecane (0.14%) while 3-methylnonane, 4-methyldecane,
7-methyltridecane, 5-methylpentadecane and 11-methylhenicosane
appeared as traces. Farnesene and homofarnesene were both present
(0.64% and 2.3% respectively) but in quite different proportions from
that in F. fusca. The mean values in terms of ug per individual worker
and percentage composition are listed in Table 18. The homofarnesene
(XII) found in F. lemani is the same compound as that in F. fusca and
Myrmica species.

Two samples of F. lemani were examined. The above results are
from a colony collected at Skipton in Yorkshire in 1985. Two colonies
collected at Mow Cop in Cheshire in June 1986 had smaller individuals
and less material on average in the Dufour gland. Examination of a
few individuals from both Mow Cop nests showed no consistent
difference between them, therefore 10 samples were taken from the
larger colony and the mean values calculated. The total amount of
hydrocarbons was 3.3 +/- 2.2ug per individual. Undecane represented a
slightly larger proportion at 68.9 +/- 9.8% with tridecane second at
11.1 +/- 3.0%. The amount of farnesene (0.24%) and homofarnesene
(0.88%) were smaller but homofarnesene was the greater of the two.
Otherwise the compositions were very similar and nowhere were they
significantly different.

We have found that F. lemani and F. fusca are easily
distinguishable chemically. Farnesene is more abundant in F. fusca
and homofarnesene is only present as a trace component. In F. lemani
there is only a trace of farnesene while homofarnesene is more
abundant. In addition there is a rather richer mixture of
hydrocarbons in F. lemani (at least 30 identified components while F.
TABLE 1B

Mean value of the composition of the contents of the Dufour gland of Formica lemani workers determined by gas chromatography and mass spectrometry.

<table>
<thead>
<tr>
<th>Number</th>
<th>Compound</th>
<th>% ± SD</th>
<th>Mean quantity (ug ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-Nonane</td>
<td>0.18 ± 0.21</td>
<td>0.009 ± 0.009</td>
</tr>
<tr>
<td>2</td>
<td>3-Methylnonane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>3</td>
<td>n-Decane</td>
<td>2.52 ± 0.95</td>
<td>0.16 ± 0.13</td>
</tr>
<tr>
<td>4</td>
<td>4-Methyldecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>5</td>
<td>Undecene</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>6</td>
<td>n-Undecane</td>
<td>57.0 ± 6.67</td>
<td>2.87 ± 1.29</td>
</tr>
<tr>
<td>7</td>
<td>5-Methylundecane</td>
<td>3.12 ± 0.95</td>
<td>0.18 ± 0.13</td>
</tr>
<tr>
<td>8</td>
<td>3-Methylundecane</td>
<td>3.47 ± 1.16</td>
<td>0.20 ± 0.15</td>
</tr>
<tr>
<td>9</td>
<td>Dodecene</td>
<td>0.66 ± 0.33</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>10</td>
<td>n-Dodecane</td>
<td>4.44 ± 1.37</td>
<td>0.25 ± 0.19</td>
</tr>
<tr>
<td>11</td>
<td>3,7-Dimethylundecane</td>
<td>0.14 ± 0.25</td>
<td>0.008 ± 0.01</td>
</tr>
<tr>
<td>12</td>
<td>4-Methylidodecane</td>
<td>0.63 ± 0.40</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>13</td>
<td>Tridecane</td>
<td>2.45 ± 0.64</td>
<td>0.14 ± 0.11</td>
</tr>
<tr>
<td>14</td>
<td>n-Tridecane</td>
<td>15.2 ± 2.91</td>
<td>0.80 ± 0.50</td>
</tr>
<tr>
<td>15</td>
<td>7-Methyltridecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>16</td>
<td>5-Methyltridecane</td>
<td>0.57 ± 0.23</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>17</td>
<td>3-Methyltridecane</td>
<td>2.23 ± 0.55</td>
<td>0.12 ± 0.10</td>
</tr>
<tr>
<td>18</td>
<td>Tetradecane</td>
<td>0.63 ± 0.94</td>
<td>0.05 ± 0.12</td>
</tr>
<tr>
<td>19</td>
<td>n-Tetradecane</td>
<td>0.42 ± 0.20</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>20</td>
<td>Farnesene</td>
<td>0.64 ± 0.33</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>21</td>
<td>n-Pentadecane</td>
<td>1.91 ± 0.59</td>
<td>0.11 ± 0.06</td>
</tr>
<tr>
<td>22</td>
<td>Homofarnesene</td>
<td>2.30 ± 1.28</td>
<td>0.14 ± 0.10</td>
</tr>
<tr>
<td>23</td>
<td>5-Methylpentadecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>24</td>
<td>n-Hexadecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>25</td>
<td>n-Heptadecane</td>
<td>0.45 ± 0.19</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>26</td>
<td>7-Methylheptadecane</td>
<td>0.31 ± 0.16</td>
<td>0.03 ± 0.03</td>
</tr>
<tr>
<td>27</td>
<td>n-Octadecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>28</td>
<td>n-Nonadecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>29</td>
<td>9-Methylnonadecane</td>
<td>0.17 ± 0.12</td>
<td>0.01 ± 0.009</td>
</tr>
<tr>
<td>30</td>
<td>11-Methylheneicosane</td>
<td>t</td>
<td>t</td>
</tr>
</tbody>
</table>

Total amount in µg 5.27 ± 2.86

t = trace
Lasius fuliginosus Latr.

Bernardi et al. (1967) described the extract of the whole abdomens of L. fuliginosus. They found, in addition to undecane, n-tridecane, n-pentadecane, tridecan-2-one, dendrolasin, pentadecan-2-one and heptadecan-2-one. Traces of cis- and trans-citral were also found. Dendrolasin and citral had not yet been found in Dufour glands and so it was desirable to re-examine L. fuliginosus, using cleanly dissected Dufour glands, avoiding any cross contamination.

The examination revealed that the Dufour gland of L. fuliginosus workers contains straight chain hydrocarbons ranging from C_{10} to C_{17} (Fig. 15) and traces of one unsaturated hydrocarbon, tridecene, not well resolved in Fig. 15 but clearly separated on other columns. Undecane is the most abundant compound representing 76.5% of the total contents, followed by tridecane with only traces of the other hydrocarbons (Table 19). A series of ketones was found ranging from C_{13} to C_{19}, with 2-pentadecanone as the major one (13.5%). Two compounds (peaks 9 and 13, Fig. 15) are present in small quantity, remain unidentified.

As expected, we found no citral or dendrolasin in the Dufour gland, and conclude these substance, found in extracts of whole abdomens by Bernardi et al. (1967) were contaminants introduced from the large mandibular glands during collection.

The contents of L. fuliginosus Dufour glands follows the more common pattern of formicine species with n-undecane the major substance accompanied by a number of other hydrocarbons, and a series of oxygenated compounds, here 2-alkanones, with 2-pentadecanone the second most abundant substance present. No alcohols or methylbranched
Fig. 15: A gas chromatogram of a single Dufour gland from a worker of Lasius fuliginosus analysed on an OV-17 capillary column. No. of peaks refer to Table 19.
Composition of contents of Dufour gland of Lasius fuliginosus determined by gas chromatography and mass spectrometry.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>% ± SD</th>
<th>Mean quantity (μg ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-Decane</td>
<td>t\textsuperscript{a}</td>
<td>t</td>
</tr>
<tr>
<td>2</td>
<td>n-Undecane</td>
<td>76.5 ± 4.6</td>
<td>2.04 ± 0.75</td>
</tr>
<tr>
<td>3</td>
<td>n-Dodecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>4</td>
<td>Tridecane</td>
<td>0.21 ± 0.10</td>
<td>0.006 ± 0.003</td>
</tr>
<tr>
<td>5</td>
<td>n-Tridecane</td>
<td>2.24 ± 0.50</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>6</td>
<td>n-Tetradecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>7</td>
<td>n-Pentadecane</td>
<td>0.37 ± 0.25</td>
<td>0.008 ± 0.003</td>
</tr>
<tr>
<td>8</td>
<td>2-Tridecanone</td>
<td>0.25 ± 0.35</td>
<td>0.009 ± 0.01</td>
</tr>
<tr>
<td>9</td>
<td>2-Tetradecanone</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>10</td>
<td>(2-Pentadecenone\textsuperscript{b})</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>11</td>
<td>2-Pentadecanone</td>
<td>13.5 ± 3.1</td>
<td>0.37 ± 0.20</td>
</tr>
<tr>
<td>12</td>
<td>n-Heptadecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>13</td>
<td>2-Hexadecanone</td>
<td>0.17 ± 0.07</td>
<td>0.005 ± 0.003</td>
</tr>
<tr>
<td>14</td>
<td>(2-Heptadecenone)\textsuperscript{b}</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>15</td>
<td>2-Heptadecanone</td>
<td>4.52 ± 1.03</td>
<td>0.13 ± 0.07</td>
</tr>
<tr>
<td>16</td>
<td>2-Nonadecanone</td>
<td>0.50 ± 0.22</td>
<td>0.01 ± 0.009</td>
</tr>
</tbody>
</table>

Total amount in μg 2.69 ± 1.02

\textsuperscript{a} - trace detected, less than 0.1%
\textsuperscript{b} - tentative identification, see text.
alkanes were detected. The alkene, tridecene is not well resolved from tridecane in Fig. 15, but was well separated on other columns. Four of the alkanes and four 2-alkanones now reported were not found by Bernardi et al. (1967). Various members of the 2-alkanone series from 2-tridecanone to 2-nonadecanone have been previously identified in species of Formica, Lasius, Acanthomyops and Camponotus, (reviewed in Blum and Hermann, 1978) but this is the first time that 2-hexadecanone has been identified. Bergstrom and Lofqvist (1970) reported 3-tetradecanone and 3-hexadecanone in L. carniolicus. It is unusual to find these even-numbered 3-alkanone. They also found the series of 2-alkanones in L. alienus but that species was distinguishable by the presence of hexadecyl acetate and methyl hexadecanoate, neither of which was found here.

Two substances (peaks 9 and 13 in Fig. 15) have not been securely identified, but are probably pentadecenone and heptadecenone respectively. They eluted before pentadecanone and heptadecanone (the two most abundant ketones of the series) on OV-1 and OV-17 capillary columns and after these compounds on an SP2340 column, which specifically retains substances with double bonds.

The Lasius genus appears to produce a relatively rich mixture of alkanes and oxygenated compounds in the Dufour gland, these oxygenated compounds may be esters, alcohols, lactones, or as here, ketones.
Camponotus species

Of the many studies on ant exocrine glands, few or none of them have been on ants of the hot dry areas of Northern Africa. We have therefore carried out some studies on two of the common ants of Egypt. 

Camponotus aegyptiacus (Emery) is a common North-east African species, found inside and outside homes, foraging individually and mainly active at night. It is a species with dimorphic workers.

Moreover, many studies of other species of Camponotus have already been carried out (reviewed in Blum and Hermann, 1978), and we had available also two Mediterranean species C. vagus (Scopoli) and C. aethiops (Latr.), collected in Corsica for study and comparison.

The Dufour gland secretion of major workers of C. aegyptiacus consists mainly of hydrocarbons and acetates in addition to trace compounds of two alcohols, two propionates, farnesy acetate and one terpenoid compound. The gas chromatogram profile of the Dufour gland secretion is shown in Fig. 16. The hydrocarbons comprise branched and saturated and unsaturated compounds ranging from C₁₀ to C₁₉ with undecane (52.4%) as the major compound followed by tridecane (3.89%) then heptadecane (1.46%) and pentadecane (1.07%). The branched chain hydrocarbons were present in traces with 5-methyltridecane the most abundant (0.27). The acetates were the second most abundant compounds (C₁₀-C₂₀) with dodecyl acetate the major compound (28.2%), followed by undecyl acetate (6.80%) then octadecyl acetate (2.4%) and tetradecyl acetate (1.8%). One branched acetate represented by a branched tridecyl acetate was present in a trace amount. Dodecanol and tetradecanol were present in traces. The two propionates were present in traces. The mean values and percentages of the compounds are listed in Table 20.

The Dufour gland secretion of minor workers of C. aegyptiacus
Fig. 16: A gas chromatogram of a single Dufour gland from a major worker of *C. aegyptiacus* analysed on an OV-1 capillary column. No. of peaks refer to Table 20.
Mean values for the composition of the contents of the Dufour gland of Camponotus aegyptiacus major workers determined by gas chromatography and mass spectrometry.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>% ± SD</th>
<th>Mean quantity (ug±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-Decane</td>
<td>0.48 ± 0.30</td>
<td>0.12 ± 0.10</td>
</tr>
<tr>
<td>2</td>
<td>n-Undecane</td>
<td>52.2 ± 16.1</td>
<td>12.3 ± 11.2</td>
</tr>
<tr>
<td>3</td>
<td>5-Methylundecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>4</td>
<td>3-Methylundecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>5</td>
<td>n-Dodecane</td>
<td>0.49 ± 0.87</td>
<td>0.1 ± 0.15</td>
</tr>
<tr>
<td>6</td>
<td>Tridecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>7</td>
<td>n-Tridecane</td>
<td>3.89 ± 1.27</td>
<td>0.93 ± 0.84</td>
</tr>
<tr>
<td>8</td>
<td>Terpenoid</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>9</td>
<td>5-Methyltridecane</td>
<td>0.27 ± 0.40</td>
<td>0.06 ± 0.08</td>
</tr>
<tr>
<td>10</td>
<td>3-Methyltridecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>11</td>
<td>Decyl acetate</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>12</td>
<td>Dodecanol</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>13</td>
<td>Undecyl acetate</td>
<td>6.80 ± 9.5</td>
<td>1.36 ± 1.66</td>
</tr>
<tr>
<td>14</td>
<td>n-Pentadecane</td>
<td>1.07 ± 0.63</td>
<td>0.18 ± 0.17</td>
</tr>
<tr>
<td>15</td>
<td>Dodecyl acetate</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>16</td>
<td>Dodecyl acetate</td>
<td>28.2 ± 10.5</td>
<td>4.8 ± 1.99</td>
</tr>
<tr>
<td>17</td>
<td>Branched tridecyl acetate</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>18</td>
<td>Tetradecanol</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>19</td>
<td>Heptadecene</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>20</td>
<td>Dodecyl propionate</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>21</td>
<td>Tridecyl acetate</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>22</td>
<td>n-Heptadecane</td>
<td>1.46 ± 1.4</td>
<td>0.31 ± 0.28</td>
</tr>
<tr>
<td>23</td>
<td>Tetradecyl acetate</td>
<td>1.18 ± 0.36</td>
<td>0.22 ± 0.12</td>
</tr>
<tr>
<td>24</td>
<td>n-Octadecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>25</td>
<td>Farnesyl acetate</td>
<td>0.16 ± 0.20</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>26</td>
<td>n-Nonadecane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>27</td>
<td>Hexadecyl acetate</td>
<td>0.16 ± 0.23</td>
<td>0.04 ± 0.07</td>
</tr>
<tr>
<td>28</td>
<td>Octadecyl acetate</td>
<td>2.4 ± 1.28</td>
<td>0.44 ± 0.24</td>
</tr>
<tr>
<td>29</td>
<td>Octadecyl propionate</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>30</td>
<td>Nonadecyl acetate</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>31</td>
<td>Elcosyl acetate</td>
<td>t</td>
<td>t</td>
</tr>
</tbody>
</table>

Total amount in μg

21.15 ± 14.15

Average of five samples

* t = trace
contains mainly hydrocarbons in addition to one acetate represented by
dodecyl acetate (Fig.17). The hydrocarbons ranging from \( \text{C}_{10} \) to \( \text{C}_{17} \)
with undecane as the major component (78.4%) followed by tridecane
(8.7%) then decane (2.81%) and dodecane (1.59%). Only two branched
hydrocarbons were identified represented by 5-methylundecane and
3-methylundecane (1.46 and 4.24% respectively). The mean values and
percentages of the compounds are listed in Table 21.

The Dufour gland secretion of *C. aethiops* workers consists mainly
of undecane, tridecane and pentadecane as predominant compounds, while
the virgin queens contain undecane and tridecane only as major
compounds with only traces of pentadecane (Fig. 18). Decane and
dodecane appeared in small quantities. The quantities of all the
components was larger in workers than in queens. The results are
given in Table 22. Undecane is the most abundant compound,
representing 72.1% in workers and 77.1% in queens. Workers of *C.
aethiops* contained, on average, a massive 10\( \mu \)g of these simple
hydrocarbons, the queens contained less (3.83\( \mu \)g). This work was done
at an early stage, with packed columns, with which it was not possible
to separate minor peaks that have been examined in later work with
capillary columns. Very minor peaks, probably due to branched
alkanes, such as 3- and 5-methylundecane could be seen but no mass
spectra were obtained of these components. Identification was made by
comparing retention times on two stationary phases of different
polarity. No other components representing more than 0.2% of the
total were seen.

The Dufour gland secretion of *C. vagus* consists mainly of
hydrocarbons in addition to small quantities of alkyl acetates
\((\text{C}_{12} - \text{C}_{16})\) and one alcohol (Fig. 19). Approximately 99% of the gland
component contain a narrow range of alkanes \((\text{C}_{9} - \text{C}_{15})\) and alkenes
\((\text{C}_{11} - \text{C}_{19})\) with undecane as the major compound (69.6%) followed by
Fig. 17: A gas chromatogram of a single Dufour gland from a minor worker of C. aegyptiacus analyzed on an OV-1 capillary column. No. of peaks refer to Table 21.
**TABLE 21**

Mean values for the composition of the contents of the Dufour gland of *Camponotus aegyptiacus* minor workers determined by gas chromatography and mass spectrometry, on individual samples.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>% ± SD</th>
<th>Mean quantity (µg ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-Decane</td>
<td>2.81 ± 0.88</td>
<td>0.21 ± 0.09</td>
</tr>
<tr>
<td>2</td>
<td>Undecene</td>
<td>0.24 ± 0.29</td>
<td>0.01 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>n-Undecane</td>
<td>78.4 ± 9.14</td>
<td>6.16 ± 2.36</td>
</tr>
<tr>
<td>4</td>
<td>5-Methylundecane</td>
<td>1.46 ± 0.94</td>
<td>0.12 ± 0.11</td>
</tr>
<tr>
<td>5</td>
<td>3-Methylundecane</td>
<td>4.24 ± 2.2</td>
<td>0.34 ± 0.26</td>
</tr>
<tr>
<td>6</td>
<td>Dodecene</td>
<td>0.26 ± 0.27</td>
<td>0.02 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>n-Dodecane</td>
<td>1.59 ± 0.69</td>
<td>0.12 ± 0.07</td>
</tr>
<tr>
<td>8</td>
<td>Tridecane</td>
<td>0.27 ± 0.27</td>
<td>0.02 ± 0.03</td>
</tr>
<tr>
<td>9</td>
<td>n-Tridecane</td>
<td>8.7 ± 3.65</td>
<td>0.63 ± 0.54</td>
</tr>
<tr>
<td>10</td>
<td>Dodecyl acetate</td>
<td>0.16 ± 0.17</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>11</td>
<td>n-Heptadecane</td>
<td>0.49 ± 0.37</td>
<td>0.04 ± 0.04</td>
</tr>
</tbody>
</table>

Total amount in µg | 7.77 ± 2.98
Fig. 10: A gas chromatogram of a single Dufour gland from a queen and worker of C. aethiops analysed on a PEGA 10% packed column. No. of peaks refer to Table 22.
TABLE 22

Composition of the contents of the Dufour gland of both the workers and queens of *Camponotus aethiops* determined by gas chromatography.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>Workers*</th>
<th>Quees /</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% ± SD</td>
<td>Mean quantity ug±SD</td>
</tr>
<tr>
<td>1</td>
<td>n-Decane</td>
<td>1.41 ± 0.82</td>
<td>0.18 ± 0.16</td>
</tr>
<tr>
<td>2</td>
<td>n-Undecane</td>
<td>72.1 ± 9.8</td>
<td>7.65 ± 4.94</td>
</tr>
<tr>
<td>3</td>
<td>n-Dodecane</td>
<td>0.46 ± 0.29</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>n-Tridecane</td>
<td>18.1 ± 4.8</td>
<td>1.89 ± 1.33</td>
</tr>
<tr>
<td>5</td>
<td>n-Pentadecane</td>
<td>6.86 ± 3.91</td>
<td>0.59 ± 0.35</td>
</tr>
</tbody>
</table>

Total amount in µg: 10.45 ± 6.73 (Workers) 3.83 ± 2.92 (Queens)

*Average of 11 samples

/Average of seven samples
Fig. 19:

A gas chromatogram of a single Dufour gland from a worker of C. vagus analysed on an OV-1 capillary column. No. of peaks refer to Table 23.
tridecane (21.8%) (Table 23). The alkenes were represented by undecene, dodecene, tridecene, heptadecene and nonadecene with tridecene the most abundant followed by undecene then nonadecene, dodecene and heptadecene respectively. Tridecene appeared as two peaks which indicates its presence in two isomers with one double bond in different position. Three methylbranched hydrocarbons were found, represented by 5-methylundecane, 3-methylundecane and 5-methyltridecane (0.13, 0.85 and 0.08% respectively). Four acetates were found represented by dodecyl acetate, tridecyl acetate, tetradecyl acetate and hexadecyl acetate (0.4, 1, 0.04 and 0.1% respectively). One alcohol was found in a trace amount (dodecanol).

The Dufour gland contents of a number of species of *Camponotus* have been examined before this work and showed a variety in the nature and number of substances recognized. *C. ligniperda* contains 37 identified and six unidentified substances, consisting of n-alkanes, alkenes, methylbranched alkanes, n-alcohols, 2-alkanones, alkyl and alkenyl acetates, farnesene and farnesyl acetate (Bergstrom and Lofqvist 1972a). *C. herculeanus* contains a mixture of 29 similar substances, with five others unidentified, but with only two alkanols and one alkanone and no farnesyl acetate (Bergstrom and Lofqvist, 1972b). In both these species, tridecane was apparently the major component. In *C. americanus* only four alkanes and two alkenes were found with no oxygenated compound (Ayre and Blum, 1971), and in *C. pennsylvanicus* only alkanes and alkenes were identified, without the pentadecene of *C. americanus* (Ayre and Blum, 1971). Ayre and Blum found in these two species and in *C. herculeanus* from North America, that undecane was the major component. The Australian species *C. intrepidus* showed tridecane as the major substance (30%) with undecane second (25%) (Brophy et al., 1973). It contained 11 n-alkanes and alkenes and ten methylbranched alkanes. In *C. japonicus* and *C.*
TABLE 23

Mean values for the composition of the contents of the Dufour gland of Camponotus vagus workers determined by gas chromatography and mass spectrometry, on individual samples.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>% ± SD</th>
<th>Mean quantity (µg ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-Nonane</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>2</td>
<td>n-Decane</td>
<td>1.78 ± 0.65</td>
<td>0.06 ± 0.05</td>
</tr>
<tr>
<td>3</td>
<td>Undecene</td>
<td>0.58 ± 0.51</td>
<td>0.02 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>n-Undecane</td>
<td>69.6 ± 2.22</td>
<td>2.02 ± 1.27</td>
</tr>
<tr>
<td>5</td>
<td>5-Methylundecane</td>
<td>0.13 ± 0.11</td>
<td>0.005 ± 0.004</td>
</tr>
<tr>
<td>6</td>
<td>3-Methylundecane</td>
<td>0.85 ± 0.59</td>
<td>0.03 ± 0.03</td>
</tr>
<tr>
<td>7</td>
<td>Dodecene</td>
<td>0.06 ± 0.06</td>
<td>0.002 ± 0.003</td>
</tr>
<tr>
<td>8</td>
<td>n-Dodecane</td>
<td>0.76 ± 0.17</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>9</td>
<td>Tridecane</td>
<td>0.37 ± 0.16</td>
<td>0.01 ± 0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.69 ± 0.26</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>10</td>
<td>n-Tridecane</td>
<td>21.8 ± 6.2</td>
<td>0.65 ± 0.52</td>
</tr>
<tr>
<td>11</td>
<td>Terpenoid</td>
<td>0.05 ± 0.04</td>
<td>0.002 ± 0.002</td>
</tr>
<tr>
<td>12</td>
<td>5-Methyltridecane</td>
<td>0.08 ± 0.05</td>
<td>0.003 ± 0.002</td>
</tr>
<tr>
<td>13</td>
<td>n-Tetradecane</td>
<td>0.04 ± 0.30</td>
<td>0.002 ± 0.002</td>
</tr>
<tr>
<td>14</td>
<td>Dodecanol</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>15</td>
<td>n-Pentadecane</td>
<td>1.78 ± 1.1</td>
<td>0.05 ± 0.06</td>
</tr>
<tr>
<td>16</td>
<td>Dodecyl acetate</td>
<td>0.4 ± 0.2</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>17</td>
<td>Heptadecene</td>
<td>0.04 ± 0.05</td>
<td>0.009 ± 0.03</td>
</tr>
<tr>
<td>18</td>
<td>Tridecyl acetate</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>19</td>
<td>Tetradecyl acetate</td>
<td>0.04 ± 0.04</td>
<td>0.002 ± 0.002</td>
</tr>
<tr>
<td>20</td>
<td>Nonadecene</td>
<td>0.17 ± 0.2</td>
<td>0.005 ± 0.009</td>
</tr>
<tr>
<td>21</td>
<td>Unknown</td>
<td>0.1 ± 0.1</td>
<td>0.003 ± 0.006</td>
</tr>
<tr>
<td>22</td>
<td>Hexadecyl acetate</td>
<td>0.1 ± 0.1</td>
<td>0.003 ± 0.006</td>
</tr>
</tbody>
</table>

Total amount in µg: 2.94 ± 1.9

* t = trace
obscuripes only four alkanes and one branched alkane; undecane was the major component (Hayashi and Komae, 1980). In C. aegyptiacaus, the Dufour gland secretion of major workers is quite different from that in minor workers, in that the major workers contain acetates in high quantities, alcohols, propionates and farnesyl acetate which were not found in minors. This may provide a point of departure for some behavioural experiments to find differences in functions of major and minor workers. The 5-methyl and 3-methyl-undecane appeared as traces in majors but in minors as the chief components. C. aegyptiacaus major workers show a similarity in their secretion to those of both C. ligniperda and C. herculeanus which contains branched, saturated and unsaturated hydrocarbons, acetates, aliphatic alcohols, one terpenoid compound and farnesyl acetate. C. ligniperda and C. herculeanus contain ketones which did not exist in C. aegyptiacaus and tridecane was the major compound in both C. ligniperda and C. herculeanus while undecane was the major compound in C. aegyptiacaus. C. aegyptiacaus majors contain two propionates in trace amounts which have not been previously identified in ant secretions except later in Lasius niger (Attygalle et al., 1987). C. aethiops shows a very simple pattern of hydrocarbons like those in the American species C. americanus and C. pennsylvanicus and also like those in C. japonicus and C. obscuripes, the difference between queens and workers was significant and constant. C. vagus shows similarity with C. aegyptiacaus, C. ligniperda and C. herculeanus. Both C. aegyptiacaus major workers and C. vagus are different from both workers and queens of C. aethiops. It is easy to separate the three species by chemical analysis for their Dufour glands in which C. aethiops workers and queens can easily be distinguished from both C. aegyptiacaus and C. vagus since they contain only hydrocarbons while C. aegyptiacaus and C. vagus contain more compounds in addition to hydrocarbons. C. aegyptiacaus can easily
be distinguished from *C. vagus* by having more dodecyl acetate (28.2%) while *C. vagus* contains less (0.4%) and *C. vagus* contains 21.8% tridecane while *C. aegyptiacus* contains 3.89%). In addition two isomers of tridecene were found in *C. vagus* while *C. aegyptiacus* contain only one isomer.

These results on *Camponotus* species are summarized in Table 24.

**Cataglyphis savignyi** (Dufour)

*C. savignyi* is highly adapted to desert conditions and also lives in agricultural areas of the Nile Valley where it is dry and undisturbed. Workers forage individually, run suddenly across hot stones, sand and soil during the heat of the day. Workers returned inside the nest before sunset when day temperature begin to fall. Delye (1968) has shown that *Cataglyphis* may survive temperatures of 50°C for at least one hour.

The Dufour gland of *Cataglyphis savignyi* workers is filled with branched and saturated and unsaturated hydrocarbons in addition to four ketones, three alcohols and one aldehyde as minor components (Fig. 20). The straight chain hydrocarbons range from C_{11} to C_{23} with pentadecane as the major compound (58.2%) followed by tridecane (14.2%) then heptadecane (7.89%). The branched chains contain, methyl groups in 3-, 5- or 7-position. All the branched compounds were present in small and trace amounts with 3-methylpentadecane and 5-methylpentadecane the most abundant. The means values and percentages of the compounds are listed in Table 25.

The desert ant *Cataglyphis savignyi* does not follow the general pattern of formicine ants but is unique in having pentadecane as the major compound, this may reflect its needs for relatively less volatile compounds as it lives in hot regions. One branched aldehyde, represented by 2-methylhexadecanal (0.29%), was found in *Cataglyphis*
### TABLE 24

The major component in some *Camponotus* species with the indication whether acetates are present or not. The species from different origins.

<table>
<thead>
<tr>
<th>Species</th>
<th>Major Compound</th>
<th>Acetates</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Camponotus aegyptiacus</em> minor workers</td>
<td>$C_{11}$</td>
<td>$\pm$</td>
<td></td>
</tr>
<tr>
<td><em>C. aethiops</em></td>
<td>$C_{11}$</td>
<td>-</td>
<td>Ayre and Blum, 1971</td>
</tr>
<tr>
<td><em>C. americanus</em></td>
<td>$C_{11}$</td>
<td>-</td>
<td>Hayashi and Komae, 1980</td>
</tr>
<tr>
<td><em>C. japonicus</em></td>
<td>$C_{11}$</td>
<td>-</td>
<td>Hayashi and Komae, 1980</td>
</tr>
<tr>
<td><em>C. obscruipes</em></td>
<td>$C_{11}$</td>
<td>-</td>
<td>Ayre and Blum, 1971</td>
</tr>
<tr>
<td><em>C. pennsylvanicus</em></td>
<td>$C_{11}$</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>C. aegyptiacus</em> major workers</td>
<td>$C_{11}$</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>C. vagus</em></td>
<td>$C_{11}$</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>C. intrepidus</em></td>
<td>$C_{13}$</td>
<td>-</td>
<td>Brophy et al., 1973.</td>
</tr>
<tr>
<td><em>C. herculeanus</em></td>
<td>$C_{13}$</td>
<td>+</td>
<td>Bergstrom and Lofquist, 1972b</td>
</tr>
<tr>
<td><em>C. ligniperda</em></td>
<td>$C_{13}$</td>
<td>+</td>
<td>Bergstrom and Lofquist, 1972a,b</td>
</tr>
</tbody>
</table>
Fig. 20: A gas chromatogram of a single Dufour gland from a worker of Cataglyphis savignyi analysed on an OV-1 capillary column. No. of peaks refer to Table 24.
Mean values for the composition of the contents of the Dufour gland of *Cataglyphis savignyi* workers determined by gas chromatography and mass spectrometry.

<table>
<thead>
<tr>
<th></th>
<th>Mean quantity (µg ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Undecene</td>
</tr>
<tr>
<td>2</td>
<td>n-Undecane</td>
</tr>
<tr>
<td>3</td>
<td>5-Methylundecane</td>
</tr>
<tr>
<td>4</td>
<td>3-Methylundecane</td>
</tr>
<tr>
<td>5</td>
<td>Dodecene</td>
</tr>
<tr>
<td>6</td>
<td>n-Dodecene</td>
</tr>
<tr>
<td>7</td>
<td>Tridecene</td>
</tr>
<tr>
<td>8</td>
<td>n-Tridecene</td>
</tr>
<tr>
<td>9</td>
<td>7-Methyltridecane</td>
</tr>
<tr>
<td>10</td>
<td>5-Methyltridecane</td>
</tr>
<tr>
<td>11</td>
<td>3-Methyltridecane</td>
</tr>
<tr>
<td>12</td>
<td>Tetradecene</td>
</tr>
<tr>
<td>13</td>
<td>n-Tetradecane</td>
</tr>
<tr>
<td>14</td>
<td>Dodecanol</td>
</tr>
<tr>
<td>15</td>
<td>Pentadecene</td>
</tr>
<tr>
<td>16</td>
<td>n-Pentadecane</td>
</tr>
<tr>
<td>17</td>
<td>7-Methylpentadecane</td>
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<tr>
<td>18</td>
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</tr>
<tr>
<td>19</td>
<td>3-Methylpentadecane</td>
</tr>
<tr>
<td>20</td>
<td>Hexadecene</td>
</tr>
<tr>
<td>21</td>
<td>n-Hexadecane</td>
</tr>
<tr>
<td>22</td>
<td>2-Methyltetradecanone</td>
</tr>
<tr>
<td>23</td>
<td>2-Pentadecanone</td>
</tr>
<tr>
<td>24</td>
<td>Heptadecene</td>
</tr>
<tr>
<td>25</td>
<td>n-Heptadecane</td>
</tr>
<tr>
<td>26</td>
<td>7-Methylheptadecane</td>
</tr>
<tr>
<td>27</td>
<td>5-Methylheptadecane</td>
</tr>
<tr>
<td>28</td>
<td>Pentadecanol</td>
</tr>
<tr>
<td>29</td>
<td>3-Methylheptadecane</td>
</tr>
<tr>
<td>30</td>
<td>2-Hexadecanone</td>
</tr>
<tr>
<td>31</td>
<td>Octadecene</td>
</tr>
<tr>
<td>32</td>
<td>n-Octadecane</td>
</tr>
<tr>
<td>33</td>
<td>2-Methylhexadecanol</td>
</tr>
<tr>
<td>34</td>
<td>Hexadecanol</td>
</tr>
<tr>
<td>35</td>
<td>2-Heptadecanone</td>
</tr>
<tr>
<td>36</td>
<td>Nonadecene</td>
</tr>
<tr>
<td>37</td>
<td>n-Nonadecane</td>
</tr>
<tr>
<td>38</td>
<td>Tricosene</td>
</tr>
</tbody>
</table>

Total amount in µg 10.8 ± 4.4

t = trace
savignyi Dufour glands and has not been detected before in Dufour glands. Aldehydes have not previously been found in Dufour glands of ants. A trace amount of nonanal (possibly from the Dufour gland) was found in a hexane extract of abdomens of F. rufa by Francke et al. (1985). Some long chain aldehydes have recently been found in primitive Australian ants (Jackson, personal communication).
Handibular gland investigations of Tetramorium caespitum (L.)

According to Longhurst et al. (1980) 3-octanone is the major mandibular gland component in the workers of three Tetramorium species, providing 70% of the total in T. caespitum and accompanied by an unidentified component of higher mass. Pasteels and Verhaeghe (1979) and Pasteels et al. (1980) reported that the head of T. caespitum workers contained 100ng of 4-methyl-3-hexanol, while heads of sexuals contained also 4-methyl-3-hexanone (100ng in the females and 1700ng in the males). Later they corrected this when they discovered they had examined the morphologically almost identical T. impurum (Foerster). On re-examination of T. impurum the ketone was also detected in the workers and it was thought that the two Tetramorium species could be distinguished by the different content of the mandibular glands of the males (Pasteels et al., 1981). The disagreement between the results of the Longhurst and Pasteels groups on T. caespitum was thought to be attributable to a possible chemical polymorphism linked to geographical races. This idea was further supported by the report from Japan that whole bodies of T. caespitum contained 2-hexenal, monoterpenes and saturated hydrocarbons (Hayashi and Komae, 1980).

In order to clear up the confusion caused by these conflicting reports, a large number of colonies of the true T. caespitum have been examined and we report our results.

A total of 17 colonies of T. caespitum collected in England, France, Belgium, Luxembourg and Denmark have been examined by gas chromatography for the contents of their mandibular glands. The localities from which the colonies came are listed in Table 12. Although the individual workers varied considerably in the proportions of the substances they contained, there was no pattern evident that indicated different races within the species.
Mass spectrometry was carried out on a sample of 5 heads of workers taken from an English colony. Four substances were identified. These were, in order of elution, 2-pentanone, 4-methyl-3-hexanone, 4-methyl-3-hexanol and decanal, with 4-methyl-3-hexanol as the major substance. The identification was confirmed by the spectrum of a synthetic sample and by the identical retention times of the natural and synthetic compounds of PEGA and OV-1 phases.

Over 200 individual workers' mandibular glands were examined from the 17 colonies by gas chromatography. The most common appearance was as in Fig. 21 with a large peak corresponding to 4-methyl-3-hexanol representing 80% or more of the total substances. However in a significant number, the 4-methyl-3-hexanol was reduced and an equal or larger peak of 4-methyl-3-hexanone appeared, as in Fig. 22. In some of these the 2-pentanone peak was much larger, but all the other peaks of higher retention were always small (each less than 3% of total). The number of these methylhexanone-dominant samples varied from 1 out of 11 analysis from Steinfort to 5 out of 10 from Jutland. Under the conditions used, 3-octanone would elute from the column shortly after methylhexanol. No evidence for it was seen in any of the more than 200 samples. Three of the colonies examined came from Dorset, as did the sample of *T. caespitum* examined by Longhurst et al. (1980). Sampling was made by the same technique (Morgan and Wadhams, 1972b) in both studies. There are two groups of *T. caespitum* in Britain, the one in Dorset and the other in the sandy heathland of Surrey.

The variability from one worker to another in a given sample made quantification of the substance of little value. However in Table 26 are given the absolute amounts and the percentage of the total for two nests, both collected in the Massif Central in France, one at 270m and the other at 1000m altitude. The large standard deviations show the
Fig 21: Gas chromatographic trace showing the more typical profile of substances in the mandibular glands of a single worker *T. caespitum*. A = position of 2-pentanone, not present in this sample; B = 4-methyl-3-hexanone; C = 4-methyl-3-hexanol; F = decanal; D, E and G are unidentified minor components, not always present.
Fig 22. Gas chromatographic trace showing an example of the mandibular glands of a single worker of *T. caespitum* where methylhexanone (B) is the dominant substance, followed by C, 4-methyl-3-hexanol.
Amounts of 4-methyl-3-hexanone and 4-methyl-3-hexanol in the heads of two samples of *T. caespitum* collected in the Massif Central of France, at Creste (alt. 1000m), near St. Diery, and Boucieu-le-Roi (alt. 270m) on the eastern slope (Vivarais).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Creste</th>
<th>Boucieu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount</td>
<td>% of total</td>
</tr>
<tr>
<td></td>
<td>ng ± SD</td>
<td>± SD</td>
</tr>
<tr>
<td>methylhexanone</td>
<td>80 ± 110</td>
<td>12.9 ± 6.4</td>
</tr>
<tr>
<td>methylhexanol</td>
<td>340 ± 130</td>
<td>67.4 ± 9.9</td>
</tr>
<tr>
<td>Total amount (µg) of volatiles in gland</td>
<td>530 ± 220</td>
<td>330 ± 190</td>
</tr>
</tbody>
</table>
poor value of these quantifications, and it has not been continued to
the other samples.
Sex pheromone in *Attagenus scalaris* (Pic)

The aim of the study originally was to identify the sex pheromone of this beetle with the hope that the pheromone could be used to destroy the pest or to reduce the damage it causes by using traps for; (1) monitoring the population of the beetle to select the suitable time for spraying insecticides; (2) using mass trapping to reduce the number of males in the field to decrease the chance of female mating; or (3) possibly to confuse the males to prevent them finding females for mating by spraying the pheromone widely.

Since *Attagenus elongatulus* and *A. megatoma* use fatty acids \((Z,Z)-3,5\text{-tetradecladienoic acid (XIII)}\) (Fukui et al., 1977) and \((E,Z)-3,5\text{-tetradecladienoic acid (XIV)}\) (Silverstein et al., 1967) respectively, as a sex pheromone, we looked specifically for a fatty acid. As fatty acids are difficult to detect as free acids by gas chromatography therefore we analysed extracts of whole abdomens (we do not know exactly the source of the pheromone in the abdomen) that had been treated with diazomethane to convert any acids to methyl esters and mass spectrometric analysis was done in the methyl ester form. Hexadecanoic (XV), octadecanoic (XVI), and octadecenoic acid (XVII) were identified in relatively large quantities in the abdomen. The gas chromatogram profile of methyl esters of the acids is shown in Fig. 23. The octadecenoic acid, by ozonolysis was shown to be 9-octadecenoic acid or oleic acid.

Because no live males were available we were not able to test the identified compounds. Although several attempts were made to transport the beetles from Egypt to England, only a few of them remained alive and of those that did survive none for long enough to lay eggs. The fecundity of the beetle is low, each insect lays one to 27 eggs during their life with an average of 9.5+/- 1.97 eggs per female (Ali et al., 1979). We hope to continue the work on this
Fig. 23:

A gas chromatogram of methyl ester of *Attagus scalaris* abdomens analysed on an OV-1 packed column.
1 = hexadecanoic acid  2 = octadecenoic acid
3 = octadecanoic acid.
beetle in the future.

Overview

This work has generally explored and enlarged a knowledge of the chemical secretions of a number of ants but much more remains to be done on the biological function of these secretions. In most cases, such studies have been delayed or restricted by lack of sufficient live insects.

The orienting component of the trail pheromone of P. pallidula has been identified, but for the first time in this laboratory, we have encountered an example where a further primer component was required. Much time was lost in trying to understand why the synthetic ethyl dimethylpyrazine was not more active, and in that time the colonies aged and decreased in number and further experiments were unsuccessful.

The species-specific nature of Dufour gland secretions has been supported and extended by the many studies described. A better understanding of the differences and similarities within subfamilies and between myrmicines and formicines is now possible, since many earlier studies were on whole abdomen extracts or were incomplete in various ways.

Few clues have emerged from the many studies of Dufour glands that help us to understand the primary function of that gland. The striking difference in content between major and minor workers of C. aegyptiacus gives us a point from which experiments can be planned to see how these two castes respond to the substances found.

The study on the filling of the gland in young workers of F. sanguinea was the first such study to be recorded. Behavioural experiments are planned to test our hypothesis concerning the function of the farnesene in slave raiding with F. fusca.
The large study on the geographical homogeneity of *T. caespitum* has also been a pioneering work, and has been confirmed by that of Cavill et al. (1985) on *Iridomyrmex purpureus*.

The chemotaxonomic aspects of the work on Dufour glands has been very helpful and clear cut with the exception of the confused state of *Myrmica* where it is not known where species dimensions occur.

Most of all the work on the coleopteran *Attagenus scalaris* has been hindered by inability to carry out bioassays. It is hoped that this work will be resumed when I return to Egypt.
General Procedure

Source, collection and identification of the ant colonies

Subfamily: Myrmicinae

Tetramorium caespitum (L.)

Seventeen colonies of T. caespitum were collected from the localities listed in Table 12.

There are no key morphological characters that make it possible to distinguish without doubt between the workers of T. caespitum and its near relative T. imurum, but their trail pheromones are specific (Attygalle and Morgan, 1984a; Cammaerts et al., 1985b; Billen et al., 1986). A few poison glands of workers from the unknown colony were used to make an artificial trail, and workers of a colony of T. caespitum or T. imurum, identified with certainty, were observed on the trail to see whether or not they follow it.

The Danish colony was collected at Mols laboratorlet, field station of the University of Aarhus, and identified by Dr. M.G. Nielsen. The English colonies from Dorset were collected near the Institute of Terrestrial Ecology and identified by the staff of the Institute. That from the Surrey heathland, mentioned briefly, was identified by Dr. A.J. Pontin. All of the other samples were identified with the help of the trail-following test. For four of the nests (Adinkerke, Dilsen, Steinfort and Elinghen) it was possible to
obtain males, from the genitalia of which it is possible to make easy identification (Cammaerts et al., 1985b) (Voucher specimens of all these nests are deposited in Cammaerts' collection).

**Myrmica species**

1) *Myrmica lonae* workers from the Netherlands.

2) *Myrmica aloba* workers from Spain.

3) *Myrmica sabuleti* (Mein.) workers from Spain.

4) *Myrmica scabrinodis* (Nyl.) workers from Spain.

The four species were identified and sent by C.A. Collingwood, City Museum, Leeds, England.

**Leptothorax species**

1) *Leptothorax acervorum* (Fabr.) workers and queens provided by Dr. N. Franks from a culture at Bath University, England.

2) *Leptothorax nylanderi* (Forst.) workers from Dr. J.P.J. Billen, University of Leuven, Belgium. The identification was confirmed by Professor J.V.A. van Boven.

**Messor species**

1) *Messor minor* (Andre), workers and queens from Corsica were identified and sent by Dr. J.P.J. Billen.

2) *Messor capitatus* (Latr.) workers from Corsica were sent by Dr. J.P.J. Billen and identified by C.A. Collingwood.

**Pheidole pallidula** (Nyl.)

Three large colonies were supplied by Mlle Claire Detrain at Laboratoire de Biologie Animale, University of Brussels, Belgium.
Subfamily Formicinae

Formica species

i) Formica sanguinea (Latr.) workers at different ages (2-5, 6-10, 15-24, 35-72 and more than one year old) were provided from the laboratory of Professor J.V.A. van Boven, University of Leuven, Belgium. The Dufour glands were dissected from this colony also, and sent by Dr. J.P.J. Billen.

ii) Formica fusca (L.) workers were collected from two different areas. One sample from England was identified and sent by C.A. Collingwood. The second sample from Belgium was identified and sent by Dr. J.P.J. Billen.

iii) Formica lemani (Bordroit) workers of two samples were collected from England and identified by C.A. Collingwood.

Camponotus species

i) Camponotus aethiops (Latr.) workers and virgin queens from Corsica were identified and sent as ready dissected samples of Dufour glands by Dr. J.P.J. Billen with the assistance of Professor J.V.A. van Boven.

ii) Camponotus vagus (Scopoli) workers from Corsica were identified and sent by Dr. J.P.J. Billen.

iii) Camponotus aegyptiacus (Emery) both major and minor workers from Minia, Egypt, were collected in moist soil at the end of August 1986 by M.F. Ali with the help of Dr. J.P.J. Billen. The species was identified by C.A. Collingwood.

Cataglyphis savignyi (Dufour) workers collected in a dry soil underneath a fence at the University grounds, Minia, Egypt, at the end of August 1986 by M.F. Ali with the help of Dr. J.P.J. Billen. The species was identified by C.A. Collingwood.
Lasius fuliginosus (Latr.) workers were identified and sent by Dr. J.P.J. Billen with the assistance of Professor J.V.A. van Boven.

Order Coleoptera, Family Dermestidae, Subfamily Attageninae, 
Attgenus scalaris (Pic)

Adults of A. scalaris were collected from the field on creamy white flowers of the Bin weed, Convolvulus arvensis at El-Minia Governorate district, Egypt. The flowers were gently shaken in glass vials where the beetles were collected without being harmed. The most convenient time to collect beetles was from 10-12noon during the period from April until September.

Maintenance of the Ant Colonies

Tetramorium caespitum

The ant colonies were reared in nests, each consists of a glass conical flask (250mls) partially filled with moistened plaster of Paris. Seven holes were made in the flask above the plaster layer and each was connected with a short glass tube. Four of the seven holes were connected with test tubes covered with black cardboard to serve as a shelter for the ants. One of the remaining three holes, one, connected below the plaster surface, was a vertical tube for water supply to moisten the flask inside with the required moisture. The remaining two holes were connected with a sloping tube resting on the floor to serve as entrances and exits. The top of the flask was covered with a rubber stopper. The flask was placed in a plastic bowl to serve as a foraging area. The inner vertical walls of the bowl were covered with polytetrafluoroethylene paste (Fluon) to prevent the ants from escaping. The colonies were maintained in the laboratory under natural light conditions and fed a mixture of 10% sucrose
solution, water, fly larvae and mealworm larvae.

Myrmica species

1) Myrmica lonae, M. aloba, M. sabuleti and M. scabrinodis

Workers of Myrmica species were reared in the laboratory in a nest made of a plastic bottle with one hole at the bottom to serve as an entrance for foragers. The bottle was filled with damp soil and covered with a sieved cap. The bottle was covered with black paper to darken the bottle inside. The nest was placed in a plastic bowl to serve as a foraging area. The inner vertical walls of the bowl were covered with polytetrafluoroethylene paste (Fluon) to prevent the ants from escaping. The ants were fed continually with water and sugar solution and at least once a week were given either larvae or mealworm or larvae of dipterous flies.

Leptothorax species

1) Leptothorax acervorum and L. nylanderi

Workers and queens of Leptothorax acervorum and workers of L. nylanderi were reared in artificial nests made from two microscope slides separated by a sheet of cardboard with the centre cut out to make the nest area with one hole for an entrance. The nest was placed in a plastic bowl to serve as foraging area. The inner vertical walls of the bowl were covered with Fluon paste to prevent the ants from escaping. The ants were fed continually with water and sugar solution and at least once a week were given larvae of mealworm.
**Messor species**

**Messor minor** and **M. capitatus**

Workers and queens of *Messor minor* and workers of *M. capitatus* were reared in artificial nests made from plastic bottles, partially filled with moistened plaster of Paris. Some torn tissue paper was placed in the bottle. The nest was placed in a plastic bowl to serve as a foraging area. The inner vertical walls of the bowl were covered with Fluon paste to prevent the ants from escaping. The ants were kept in the laboratory on a diet of sugar solution, grass seeds and larvae of mealworm.

**Pheidole pallidula**

The ant colonies were reared in nests, each consists of a glass conical flask (250mls), partially filled with moistened plaster of Paris. They were fed as described under *T. caespitum*. The colonies were kept in the animal house at 20°C and photoperiod 16:8 (light:dark).

**Subfamily Formicinae**

**Formica species**

1) **Formica sanguinea**

The ant colony was reared and available at the laboratory of Professor J.V.A. van Boven, University of Leuven, Belgium.

2) **Formica fusa** and **F. lemani**

Workers of *Formica* species were reared in artificial nests, each consists of glass conical flask (250mls), partially filled with moist plaster of Paris and fed as described in *T. caespitum*.

**Camponotus species**
I) Camponotus aethiops. Workers and virgin queens were sent as ready dissected samples of Dufour glands by Dr. J.P.J. Billen.

II) Camponotus vagus and C. aegyptiacus. Workers of C. vagus and major and minor workers of C. aegyptiacus were reared in artificial nests made from plastic bottles, partially filled with moist plaster of Paris as for Messor species. The ants were fed continually with water and sugar solution and at least once a week were given either larvae of mealworm or larvae of dipterous flies. Workers of C. aegyptiacus were kept in the animal house at 26°C and photoperiod 16:8 (light:dark).

Cataglyphis savignyi. Workers were reared, fed and kept as for Camponotus aegyptiacus.

Lasius fuliginosus. Samples of dissected Dufour glands were sent ready for injection by Dr. J.P.J. Billen.

Family Dermestidae

Subfamily Attageninae

Attagenus scalaris. Adult beetles were collected and reared in glass jars. The bottom of each jar was covered by small pieces of woollen cloth for egg laying. Tiny drops of honey were scattered on a piece of paper and used as a food source for the beetles. The jars were covered by muslin cloth held in place by rubber bands, and kept in the animal house at 28°C and photoperiod 16:8 (light:dark).

Preparation of glands for analysis

The samples for injection were prepared by anaesthetizing worker ants by momentarily immersing them in the cold vapour above liquid nitrogen, then dissecting out the gland in distilled water under a
Vickers Zoomax binocular microscope using two sharp tweezers (Idealtek No.4, Trady’s, Switzerland). In myrmicine ants, the Dufour gland was removed by gently pulling off the sting apparatus with one tweezer while holding the ant at the point between the abdomen and petiole and then separating the Dufour gland from the sting apparatus. While in formicine ants, the Dufour gland was removed by gently pulling off the sternite of the abdominal tip. Then the Dufour gland was cleaned from the viscera. Excess water was removed by touching it with a fragment of filter or tissue paper and the Dufour glands were attached to a fragment of glass and placed individually in short soda glass capillaries (3.5mm x 1.8mm) sealed at one end, the other end was then sealed in a flame.

Gas Chromatography (GC)

Instrumentation

A) A Pye Unicam 4500 gas chromatograph equipped with a flame ionization detector and Spectra Physics DPIOI computing integrator were used initially for the analysis and quantification. The glands were introduced in the sealed soda glass capillaries into the heated injector via the solid sampler (Morgan and Wadhams, 1972b) and left there for 2 to 3 minutes. The sample was then crushed with the plunger and the volatiles analysed on the column. Nitrogen was used as a carrier gas at a flow rate of 50 to 60ml min⁻¹.

B) A Pye Unicam 104 gas chromatograph equipped with a flame ionization detector using the same injection technique and supplied with an effluent splitter was used for collection of fractions. Nitrogen was used as a carrier gas at a flow rate of 50 to 60ml min⁻¹.
C) A Carlo Erba 4160 gas chromatograph equipped with a flame ionization detector and Shimadzu C-R3A Chromatopac computer integrator were used in later work for the analysis and quantification, with the same kind of solid injection system. The instrument was also equipped with a nitrogen-specific detector. Helium was used as a carrier gas at a flow rate of 2 ml min⁻¹.

Columns

I. Packed Columns

1) 10% PEGA on Chromosorb W (100-120 mesh; 2.75 m x 4 mm i.d.)
2) 10% PEG 20M on Chromosorb W (100-120 mesh; 2.75 m x 4 mm i.d.)
3) 3% OV-101 on Chromosorb W (100-120 mesh; 1.5 m x 4 mm i.d.)
4) 3% OV-17 on Chromosorb W (80-100 mesh; 1.5 m x 4 mm i.d.)

II. Capillary Column

A fused silica (25 m x 0.4 mm) coated with bonded OV-1.

Packing of G.C. Column

Before packing, the inside wall of the column was cleaned by washing with chloroform followed by acetone and drying the column in an oven (100-200°C) for an hour. To deactivate the inside wall of the glass column, it was washed with a 20% solution of hexamethyldisilazane in dry toluene, then washed by methanol and dried by a current of nitrogen. Typically the packing materials were prepared by weighting 25 gram Chromosorb W-HP (100-200 mesh) as the supporting material and, for example, 2.5 g polyethylene glycol adipate as the stationary phase to give the required percentage (10%) of the stationary phase on the supporting material. The supporting material was dissolved in chloroform (just a volume to cover the supporting material to give a slurry and the stationary phase was dissolved in the same solvent using a magnetic stirrer. The two solutions were
mixed together in a ribbed "rotavapor" (Buchi, Switzerland) flask (500ml) placed in a warm water avoiding bubbling and the solvent evaporated by a rotavapor under a vacuum with a slow motion by hand turning of the flask to avoid breaking the particles of the supporting material, and to ensure even coating of the stationary phase on the supporting material. The coated support was transferred to a hot fluidised bed drier (100°C) and dried with a steady stream of nitrogen (one hour) to remove the remaining solvent and any fine particles. The column for packing was sealed at one end with a silanised glass wool plug, and packed under vacuum, tapping gently as the material poured in. Once the column was full, the very minimum of vibration from a Pifco body massager was applied to it, ensuring a tight, even packing of the particles and sealed with another glass wool plug. The first 7cm of the column was left without packing to introduce the solid sampler. The column was conditioned by connecting it with the injector while the other end was left disconnected, and left overnight with low nitrogen flow and temperature below the maximum limit of the stationary phase.

Quantification of the Gland Components

The mean quantity of each component in a glandular mixture and the total amount per gland were calculated by injection of 10 glands separately using either of a Pye Unicam 4500 gas chromatograph equipped with a Spectra Physics DPI01 computing integrator, or a Carlo Erba 4160 gas chromatograph equipped with a Shimadzu C-R3A Chromatopac computer integrator. The mean was taken for the ten injections by comparing the peak area for each gland with the peak area given by a standard solution of suitable substances, often hydrocarbons.
Linked Gas Chromatograph-Mass Spectrometry (GC-MS)

GC-MS was performed on:
A) A Finnigan 3200 E quadrupole spectrometer with a Finnigan 6000 Data System. A fused silica column (CP-19, 38m x 0.22mm) was directly coupled to the mass spectrometer. Helium was used as the carrier gas at a flow rate of 1 ml min⁻¹. Spectra at 70eV EI were recorded at a rate of 2 sec scan⁻¹.
B) A Hewlett-Packard 5970 B Mass Selective Detector, using a fused silica column (12m x 0.32mm) coated with OV-1 silicone gum. The carrier gas and ionization condition were as for the other instrument. Spectra were scanned at 0.5 sec scan⁻¹.
C) A VG-70F mass spectrometer with a Finnigan INCOS Data System. A 10% PEGA column on Chromosorb W (100-120 mesh; 2.75 x 4mm i.d.) was directly coupled to the mass spectrometer. Helium was used as the carrier gas. Spectra were obtained at an ionization potential of 70eV, accelerating voltage of 4kV scan range m/z 20-500 (at low resolution, ca 1000).

Trapping of Nanogram Quantities

A Pye Unicam 104 gas chromatograph was used. The effluent was split using an all-glass splitter (Baker et al., 1976) (95:5, trap: FID). The outlet (collector) end of the splitter was sealed with a 6mm metal screw and a metal capillary fitted to the detector. Sterilized glass capillaries (kept at 200°C for an hour) (5cm x 0.5mm o.d. x 0.45mm i.d.) were inserted individually at one end of a silicone rubber septum lining a hexagonal metal nut and the other end inserted in a polyethylene specimen tube cap for cooling with liquid nitrogen. The substance under investigation was trapped in the glass capillary by screwing the collector onto the outlet (collector) end of the splitter. Soon after the peak had eluted, the collector was unscrewed.
and the capillary was immediately removed.

Ozonolysis

1) Ozonolysis without a solvent.

Ozonolysis was undertaken to determine the position of a double bond in a compound. After the compound had been trapped, the glass capillary containing the compound was carefully broken into three parts and placed in a soda glass capillary sealed at one end and ozonized in the glass collecting capillary using a micro-ozone generator by passing a fine stream of ozone (10 ml/min) for 20 seconds, through the drawn-out end of a melting point tube extended in the bottom of soda glass capillary cooled in ice containing the trapped compound. After ozonolysis, the soda glass was sealed at the other end and was left in the injector (200°C) in a solid sampler for 5 minutes for the pyrolytic cleavage of the ozonides before crushing. The resultant compounds were separated and identified from their retention times comparing them with the retention times of authentic standard compounds.

ii) Ozonolysis with a solvent

Ozonolysis of a compound with a solvent was performed in a small specimen tube using the micro-ozone generator. The stream of ozone was passed for one to two minutes according to the volume of the solvent. Then triphenyl phosphine \((\text{C}_6\text{H}_5)_3\text{P}\) was added for reduction of the ozonides. The solution was injected \((170°C)\) and separated and identified as mentioned above.

Micropipette

For handling very small quantities of solutions or extracts for reaction or analysis, a micropipette \((100 \mu l)\) (Eppendorf, W. Germany) was used. The micropipette was connected with an Eppendorf standartip or a
rubber silicon tube which connected with a melting point tube drawn out at one end to a finer capillary. Solutions were transferred by sucking up gently with the micropipette.

Esterification

A known weight of N-methyl-N-nitro-N-nitroguanidene (MNNG) (1mg) was placed in a clean Reacti-vial (1ml) cooled in ice. Water (100ul) was added to cover the MNNG using a micropipette. Then distilled, anhydrous tetrahydrofuran (THF 100ul) was added followed by 50% Potassium hydroxide (50ul). A yellow layer of $\text{CH}_2\text{N}_2$ in THF was produced, this was sucked up gently using a micropipette and added to the solution of acids to be esterified, which was then warmed on a water bath.
Trail Pheromone Investigation of Pheidole pallidula

Preparation of Gland Extracts for Bioassay

A tissue grinder was made from a pyrex test tube (3cm x 8mm i.d.) and a piece of glass rod with a spherical knob at one end. The abrasive surface on the bottom of the test tube and on the spherical base of the rod were produced by grinding the two surfaces together with a slurry of carborundum and Teepol (detergent). The tissue grinder was kept in an oven at 230°C for 2 hours before use. The gasters from minor workers were extracted in 100ul hexane by grinding the gasters with the spherical knob of the glass rod against the test tube. The extract for microfractionation or thin-layer chromatography analysis was removed from the grinder by a micropipette.

Trail-Following Bioassay

For measuring trail-following activity, a circle of 5cm radius was drawn on white paper with a pencil using a geometric compass. The circumference of the circle was divided into arcs of 1cm each (Pasteels and Verhaeghe, 1974). The solution in hexane of the substance being studied, was placed in the reservoir of a Standardgraph pen (Blundel Harling, Dorset) and then transferred to the circumference of the circle by tracing out the line drawn on the paper with a steady motion of the arm. After two minutes to allow the solvent to evaporate, the paper was placed on the foraging area of the colony. Each ant reaching the paper was observed. The number of 1cm arcs along the circular trail walked by each ant was counted. At least 10 individuals were observed, or else the number of workers examining the paper within twenty minutes. From these values, the median number of arcs run on the trail was calculated from each test.
Microcolumn for microfractionation

A "microcolumn" for microfractionation by means of a syringe (Bestman et al., 1985) was used. It was made of silica gel (average grain size 5 μm) between two glass wool plugs at the bottom of a 100μl syringe. The length of the column was 5 mm (volume 5μl). Five gasters from minor workers were extracted in 100μl hexane. The extract was charged on the top of the microcolumn by a micropipette and eluted through the microcolumn for bioassay and subsequently eluted with 100μl hexane-ether (1:1), 100μl ether, 100μl ether-methanol (99:1) and 100μl ether-methanol (90:10) directly into a glass specimen tube and the solution used for bioassay as described above. Another experiment was performed using 100μl hexane, 100μl hexane-acetone (1:1) and 100μl acetone as elution solvents.

Microcolumn and thin-layer chromatography (t.l.c.)

Ten gasters from minor workers were extracted in 100μl hexane and eluted through the microcolumn and the eluted hexane was thrown away, the column was then eluted with 100μl hexane-acetone (1:1) and applied directly drop by drop on the origin of a glass t.l.c. plate developed with hexane-acetone (1:1). The solvent front was allowed to move 10 cm. The silica plate was dried, then the width of the silica gel on the plate was shortened to 3 cm by scraping away 1 cm on each side with a microspatula. Then the remaining silica was cut into 3 bands (0-3, 3-6 and 6-10 cm). Each band was scraped off with a microspatula and the silica gel transferred into Pasteur pipettes, plugged with glass wool, and absorbed material was eluted with 200μl acetone directly into a glass specimen tube and used for bioassay as described above.

Microcolumn and Gas Chromatography Trapping

Gas chromatography was performed with a packed column of 3m x 4mm
1.5 mm I.D., 10% PEGA on Chromosorb W (100-120 mesh) with nitrogen (40-60 ml min⁻¹) as a carrier gas, isothermal at 70°C for 2 minutes then programmed at 12°C min⁻¹ to 225°C.

Ten gasters were extracted into 100ul of hexane and eluted with hexane through a microcolumn and the eluted solution was discarded. Then the microcolumn was eluted with 100ul hexane-acetone (1:1) and injected directly onto the column. The effluent was split using an all-glass splitter (Baker et al., 1976) (95:5, trap: FID). For collecting the effluent, the outlet of the splitter was opened and a new nut with a 25cm x 1.5mm o.d., 1mm i.d. U-shaped stainless steel tube going through the silicone rubber septum was screwed in and the U-tube was cooled with a mixture of liquid nitrogen and ethyl acetate in a small Dewar flask. The effluent was collected at periods started from 3 to 8, 8-13, 13-18 and 18 to 23 minutes. The metal U-tube was washed with 100ul hexane after each trapping period and stored in glass specimen tubes until bioassayed.

In the light of the result obtained from the above experiment, another experiment was performed at periods starting from 8 to 10, 10-12 and 12-14 minutes, and bioassayed in the same way.

Solid Sampling and G.C. Trapping

A) Using a PEGA 10% column.

i) Isothermal at 70°C for 2 minutes then programmed at 12°C min⁻¹ to 225°C. The effluent was collected at periods starting from 9 to 11, 11-13 and 13-15 minutes.

ii) Isothermal at 150°C. The effluent was collected in 1 minute intervals from 3 to 6 minutes after injection. The fractions were used as before for bioassay.
B) Using OV-101 3% at 120°C isothermal. The effluent was collected at periods started from 2 to 5, 5-8 and 8-12 minutes, and bioassayed.

In the light of the activity obtained, the periods of collection were shortened and collection made from 1 to 2, 2 to 3 and 3 to 5 minutes, and again bioassayed.

Linked Gas Chromatography-Mass Spectrometry (GC-MS)

Identification of poison gland component was performed on two instruments. A Finnigan 3200E quadrupole spectrometer and a Hewlett-Packard 5970B Mass Selective Detector.

Detection of the Pyrazine Compound

Analysis of the poison glands of minor and major workers was performed on a Carlo Erba gas chromatograph with a flame ionization detector or a nitrogen-specific detector. The poison glands in a sealed capillary were placed in the heated injector (170°C) of the chromatograph via the solid sampling method (Morgan and Wadhams, 1972b) crushed and chromatographed. A fused silica capillary column (25m x 0.4mm) coated with OV-1 silicone was used, with helium at 2ml min⁻¹ as carrier gas. The temperature programme was from 60°C to 150°C at 10°C min⁻¹ the 20 minutes at 150°C.

Quantification of the Gland Component

The mean value of the component per gland was calculated by injection of 10 gasters separately using a Carlo Erba gas chromatography equipped with a nitrogen detector and Shimadzu C-R3A chromatopac computer. The mean was taken for the ten injections by comparing the peak area for each gland with the peak area given by a standard solution of 3-ethyl-2,5-dimethylpyrazine.
Chemical Investigation of Dufour Gland Secretion

Subfamily Myrmicinae

Leptothorax species. The Dufour glands of workers and queens of L. acervorum and workers of L. nylanderi were dissected and chromatographed on a fused silica OV-1 capillary column. A temperature programme was used from 120°C to 300°C at 3°C min⁻¹. The injector and detector temperature were 140°C and 300°C respectively. The gland components were quantified.

Trapping of heptadecene from Dufour gland of both queens and workers was performed separately on a PEG 20M packed column at 120°C isothermal.

Ozonolysis and determination of the double bond position of heptadecene of both workers and queens of L. acervorum was carried out. The ozonolysis products were chromatographed on 10% PEGA column. The temperature programme was from 120°C to 200°C at 8°C min⁻¹.

Identification of the gland components was performed using GC-MS.

Messor species. The Dufour gland of workers and queens of Messor minor and workers of M. capitatus were prepared and chromatographed on a fused silica capillary column coated with OV-1. A temperature programme was used from 60°C to 280°C at 6°C min⁻¹, then isothermal for 10 minutes. The injector and detector temperature were 170°C and 250°C respectively. The gland components were quantified.

Identification of the gland components was performed using GC-MS.

Tetramorium caespitum workers. The Dufour glands for injection were prepared and chromatographed on 10% PEGA column. The temperature programme was isothermal at 140°C for 5 minutes then increased at 12°C to 190°C, then isothermal for 10 minutes. The injector and detector
temperature were 200°C and 250°C respectively. The gland components were quantified.

Myrmica species. The Dufour glands of Myrmica lonae, M. aloba, M. sabuleti and M. scabrinodis workers were prepared and chromatographed on 10% PEGA column. A temperature programme was used from 120°C to 170°C at 2°C min⁻¹. The injector and detector temperature were 170°C and 250°C respectively.

Subfamily Formicinae

Formica species. The Dufour glands of workers of Formica sanguinea were received ready for injection from Dr. J.P.J. Billen; while the Dufour glands of F. fusca and F. lemani workers were prepared. The Dufour glands of F. sanguinea were chromatographed on 10% PEGA column. A temperature programme was used from 70°C to 150°C at 6°C, then isothermal for 15 minutes. The injector and detector temperature were 170°C and 250°C respectively. The Dufour glands of F. fusca and F. lemani were chromatographed on a fused silica OV-1 capillary column. A temperature programme was used from 60°C to 260°C at 6°C min⁻¹. The injector and detector temperature were 170°C and 250°C respectively. The gland components were quantified.

Identification of the gland components was performed using GC-MS.

Lasius fuliginosus. The Dufour glands of workers were received ready for injection from Dr. J.P.J. Billen and chromatographed on a fused silica OV-1 capillary column. A temperature programme was used from 60°C to 200°C at 6°C min⁻¹, then isothermal for 20 minutes. The injector and detector temperature as mentioned above. The gland components were quantified.

Identification of the gland components was performed using GC-MS.
Camponotus species. The Dufour glands of both workers and queens of Camponotus aethiops were received ready for injection from Dr. J.P.J. Billen, while the Dufour glands of workers of C. vagus and both major and minor workers of C. aegyptiacus were prepared. The Dufour glands of C. aethiops were chromatographed on 10% PEGA column. A temperature programme was used from 70°C to 150°C at 6°C, then isothermal for 10 minutes. The Dufour glands of C. vagus and C. aegyptiacus were chromatographed on a fused silica OV-1 capillary column. A temperature programme was used from 60°C to 280°C at 6°C min⁻¹. The injector and detector temperature as mentioned in Formica species The gland components were quantified.

Identification of the gland components was performed using GC-MS.

Cataglyphis savignyi. The Dufour glands of workers were prepared and chromatographed on a fused silica OV-1 capillary column. A temperature programme was used from 60°C to 280°C at 6°C min⁻¹. The injector and detector temperature as mentioned above. The gland components were quantified.

Identification of the gland components was performed using GC-MS.
Tetramorium caespitum workers. The heads for injection were removed under a binocular dissecting microscope and sealed individually in a glass capillary and chromatographed on 10% PEGA column. The temperature programmed was isothermal at 100°C for 5 minutes then programmed at 8°C min⁻¹ to 160°C then isothermal for a further 2 minutes. The injector and detector temperature were 170°C and 300°C respectively. The gland components were quantified.

Identification of the gland components was performed using GC-MS.
Chemical Investigation of Sex Pheromone

Family Dermestidae
Subfamily Attagininae

Attagenus scalaris. Abdomen of adult beetles were isolated and crushed with tetrahydrofuran containing diazomethane in a tissue grinder for estrification and chromatographed on an OV-17 column. The temperature was isothermal at 160°C for 5 minutes, then programmed at 6°C min⁻¹ to 220°C.

Trapping of methyl ester of octadecenoic acid was carried out by two methods, that is, with solvent and without solvent as described earlier in the ozonolysis section. The ozonolysis products were chromatographed on 10% PEGA column. The temperature programme was from 130°C to 200°C at 8°C min⁻¹, then isothermal for 20 minutes. The injector and detector temperatures were 170°C and 250°C respectively. Identification of the components was performed using GC-MS.
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