ASPECTS OF CHEMICAL COMMUNICATION
IN PHARAOH'S ANT, *MONOMORIUM PHARAONIS*(L.)

A Thesis submitted to the
University of Southampton
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Doctor of Philosophy.

by

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To my parents.

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ASPECTS OF CHEMICAL COMMUNICATION IN PHARAOH'S ANT, MONOMORIUM PHARAONIS(L.)

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Some morphological, chemical and behavioural aspects of chemical communication were studied in pharaoh's ant, Monomorium pharaonis. The histological study of the sensory and nervous systems showed the presence of a typical formicid structure with two specific adaptations: the increased size and complexity of the organs that are related to olfaction and the decrease in size of the ganglia that control the movements of the sting. The latter can be related to the reduced size of the furcula, the part of the sting apparatus connected with muscles, important for its movement, and with the absence of barbs on the lancets. These observations are related to the use of the sting for emission of pheromones and defensive secretions only. The structure of the exocrine glands is typical for a myrmicine species.

Chemical analysis revealed the presence of the majority of volatiles in the abdomen. The head and the thorax also contained very small amounts. The pheromones monomorine I(all-cis), II and III were present in the poison gland, the alarm and defence secretions and in the trail. The pheromone monomorene was present in the Dufour's gland and in the trail. The trail contained secretions from both Dufour's and the poison glands at a ratio of 15:1. The alarm and defence secretions came only from the poison gland but the former contained 5 times the quantity of volatiles.

Various behaviour patterns of the ant are described. Necrophoric behaviour was studied in detail. The responses of workers to corpses of various ages were observed and a hypothesis is put forward to explain them on the basis of changes in pheromones and other chemical cues after death, taking into account also the role of tactile cues in recognition.

Recruitment of workers was carried out for both food exploitation and new territory exploration. Evidence was found for different types of recruitment in these two cases.

Crushed abdomens, poison and Dufour's glands elicited high recruitment in workers while the crushed heads and thoraces of workers were relatively inactive. Behavioural assays of synthetic pheromones showed that all-cis monomorine I was an attractant and arrestant while its isomers were inactive in these respects. Trans-monomorine III elicited alarm, and a mixture of both elicited both attractancy and alarm in a similar way to the natural alarm-defence secretion. A hypothesis is advanced concerning the function of the pheromones under various circumstances.

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CHAPTER 1

INTRODUCTION

1.1 Chemical communication.

Communication between two organisms has been described as "the phenomenon of one organism producing a signal that when responded to by another organism, confers some advantage (or the statistical probability of it) to the signaler or his group" (Burghardt, 1970). With this definition in mind, communication plays a very important role both in the survival and the maintenance of homeostasis in an individual or a group of individuals and in the evolution of specific adaptive features (morphological or behavioural) in a species. Consequently, in the last twenty years, a lot of attention has been given to its study and a special term, zoosemiotics, (Sebeok, 1965) has been proposed for the branch of science that concerns the ways in which animals communicate with each other, an interdisciplinary approach deriving from "semiotics", the study of signals, and "ethology", the study of behaviour.

Three parameters are absolutely necessary for communication to occur. There must be an organism which emits the signal, there must be a medium, common to both organisms, through which the signal will pass, and there must be an organism with receptors to receive the signal and respond. It is most common to classify communication according to the medium used. Thus we observe acoustic communication, with verbal communication as a sub-category, visual communication, chemical communication etc.

Chemical communication must have been the first way in which primordial organisms interacted with both other organisms and their environment. As evolution proceeded to form multicellulor organisms, chemical communication between individual cells became what we now know as hormone action (a term coined in the beginning of the century to describe those internal secretions which, carried in the blood, act as chemical messengers between different tissues of the body) while individual organisms continued to use chemicals (Wilson, 1970). Today, chemical communication is the main mode of communication in many animals. The insect world especially, provides many examples. Solitary insects use chemicals when seeking mates, in courting and in mating. However, chemical communication is most advanced among social and semi-social insects. Here, it can mediate aggregation and attraction, information about food and its exchange, alarm, defence, grooming and cleaning, recognition of castes as well as among members of a colony or a species, nest construction, care of the young and the sexuals, and other social activities in conjunction with visual, auditory, and tactile signals that build an intricate communication system.

The chemical substances that are used in chemical communication to transmit a message have been termed "semiochemicals"(Law and Regnier, 1971) from the greek semeion: a mark or signal, or "ectohormones" (Bethe, 1932). Semiochemicals can be divided into two categories, those that are used between individuals of different species (interspecific) and those used between individuals of the same species (intraspecific). Interspecific semiochemicals can be allomones (Brown, 1968), chemicals that produce an advantage to the emitter of the signal, such as defensive secretions and repellents, or kairomones (Brown, Eisner and Whittaker, 1970), chemicals that produce an advantage to the receiver of the signal such as chemicals involved in the detection of hosts or prey. However, the latter and part of the former lie outside the concept of communication as defined previously.

1.2 Pheromones.

Intraspecific semiochemicals include the so-called pheromones. The term was first used in 1959 to describe "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, for example a definite behaviour or a developmental process" (Karlson and Butenandt, Karlson and Lüscher, 1959). The word comes from the greek pherein: to transfer and horman : to excite, to stimulate. The term has since been used with various senses, sometimes even including all secretions which convey information from one individual to others and evoke behavioural and physiological reactions in the recipients (Bruce, 1970). Here, it will be used only in its original definition.

Pheromones have been classified in various ways. According to the chemical structure (Jacobson, 1966), according to their origin (surface pheromones, Wilson, 1965), according to the medium they use (olfactory and oral pheromones, Karlson and Butenandt, 1959), according to the behaviour they elicit (alarm pheromones, trail following pheromones, sex pheromones) or according to whether they have a direct effect on behaviour (releaser pheromones) or on the physiology of the animal.(primer pheromones) (Wilson and Bossert, 1963; Wilson,1965). These classifications are not exclusive and some are even misleading. For example, a pheromone at one concentration may act as attractant and at another as alarm (Wilson, 1970). Cephalic and Dufour's gland secretions in fire ants, cause alarm and attraction respectively. When expelled simultaneously, they elicit oriented alarm behaviour (Wilson, 1970). The term releaser can be used for insects where responses appear to be innate, but mammalian behaviour is very modifiable and the term may take an ambiguous behavioural meaning (Wilson, 1970b).

1.3 Structure of pheromones

Most pheromones have to be fairly volatile substances as they must be able to move from one animal to the other and transmit the message in a short time. This excludes compounds such as proteins, lipids and long chain hydrocarbons, leaving however, a very large number of compounds. It has been calculated that alarm pheromones must contain 5-10 carbon atoms and have a molecular weight of 100-200 (Bossert and Wilson, 1963). These simple structures have high volatility and can be ideal for very fast transmission as well as rapid fade out. Sex attractants are more complex, containing 10-17 carbon atoms with molecular weights between 180-300. They are less volatile but show more specificity because they are more complex (Wilson, 1963). Trail pheromones are the most complex, containing both simple small molecules and large complex ones.

The high volatility however, is not a characteristic of all pheromones. Surface or contact pheromones that are on the cuticle and are used in cleaning, recognition and other activities are not very volatile and are not transmitted very far. The alarm substances of termites are of low volatility and are said to be transmitted by body contact (Stuart, 1967).

The pheromones so far identified include acids, esters, alcohols, aldehydes, aliphatic and cyclic hydrocarbons, ketones, terpenes and terpene derivatives and nitrogenous compounds (piperidines, pyrrolidines, indolizines).

1.4 Biosynthesis and emission of pheromones

Not much is known about the biosynthesis of pheromones. Many are related to fatty acid and terpenoid precursors while others are direct by-products of primary biosynthetic routes. Some pheromones are not synthesized by the insects themselves but are either made by symbiotic organisms or by the plants that the insects use as food.

The pheromones are usually produced in specialised glands with external ducts. This is not always the case as cuticular excretions can also be pheromones (Karlson and Butenandt, 1959). The glands can be individual secretory cells irregularly dispersed throughout the epidermis (Orthoptera), modified intersegmental membranes (sex attractants in Lepidoptera, Mecoptera and Coleoptera), modified abdominal sternites (Isoptera, Coleoptera), modified abdominal tergites (Isoptera, Hymenoptera), localised integumentary modifications with disseminating mechanisms (sex pheromones in Dictyoptera, Lepidoptera), internally located glands with cuticular lined reservoirs (Dufour's gland, Pavan's gland, tibial glands, mandibular glands, analgland, poison gland in Hymenoptera, frontal gland in Isoptera) or glandular sources associated with internal organs whose prime function is not pheromone production (hind gut, anal tube) (Percy and Weatherstone, 1974).

Although the morphology of these glands is relatively well known, their ultrastructure and histology, the exact relative proportion of the components of their contents, their synthesis and their mode of release have not been fully studied.

1.5 Transmission of pheromones

According to Bossert and Wilson (1963), pheromones emanating from a given source in air, follow the laws of diffusion of gases. Pheromone concentration at any point is a function of the role of molecular emission, the diffusion coefficient of the pheromone, the distance from the source, and the time from the start of emission. As all animals have a minimum concentration below which they either cannot perceive or do not show a particular response to a pheromone, the identification of this threshold concentration for a particular pheromone and a particular behavioural response, is very important. This concentration (K) can be calculated using a number of equations (Bossert and Wilson, 1963; Wilson Bossert and Regnier, 1969). Using the K value one can also calculate the "active space" of the pheromone, a term used to describe the space in which a pheromone is eliciting a particular response (Bossert and Wilson, 1963) as well as the time needed for the pheromone to reach this space, and the time it takes to fade out, provided no more emission is present. The ratio of the rate of emission Q to the threshold concentration K is fixed for a particular pheromone and a particular behavioural response. Trail pheromones have low Q/K values, alarm pheromones have intermediate values while sex attractants have the highest (Wilson, 1970).

Apart from the modification of Q/K ratios, insects have other ways through which they can increase the information contained in a chemical message. They can use components of different glands at the same time (trail pheromones of M.pharaonis from the Dufour's and poison gland, Ritter et al, 1975), mixtures of compounds from the same gland (multi-component pheromones in Oecophylla longinoda, Bradshaw, Howse and Baker, 1973), the same pheromone in different circumstances (trans-9-keto-2-decenoic acid in honey bees, Gary, 1962) or in different concentrations (mandibular gland pheromone in Pogonomyrmex badius, Wilson, 1958; Bossert & Wilson, 1963), pheromones that produce synergistic effects (Cephalic and Dufour's gland secretion in fire ants, Wilson, 1962) and perhaps frequency and amplitude modulation of a single signal pheromone (Bossert, 1968).

1.6 Perception of pheromones

Insects perceive airborne pheromones through chemoreceptors situated on their antennae. The chemoreceptors are primary neurons consisting of dendrites, cell bodies and axons. The axons continue in the antennal nerve up to the antennal lobe where they join more complex neurons. The receptors are situated in special structures known as sensilla. The basic structure of a sensillum is such that it permits airborne molecules to enter it and excite the neuron. For this, they have a porous cuticular surface, with tubules leading from the pores to the receptor membrane. The lumen of the sensillum is filled with an extracellular fluid, the sensillum liquor. (Ernst, 1969).

There are four basic categories of sensilla: the sensilla trichodea or hairs, the sensilla basiconica or pegs, the sensilla placodea or pore plates and the sensilla coeloconica or pits. There are other less common structures as well. Each sensillum is not sensitive to the same range of chemicals. There are the "odour specialists" which are cells that belong to morphologically recognizable sensilla and all have the same stereotyped reaction spectrum, and there are "odour generalists" which are cells each of which has a unique odour spectrum, but the spectra of different cells overlap to a great degree. (Boeckh et al, 1965).

When the receptor site on a dendrite is stimulated by a pheromone, it produces a slow receptor potential. This induces a slow generator potential at the whole dendrite level. If the generator potential is intense enough, then a series of rapid electric impulses is generated at the proximal end of the cell body and these impulses travel through the axons to the antennal lobe where they are integrated.

There are various theories for the transformation of the olfactory signal to electric response of the receptor site (Receptor protein model, Riddiford, 1970; stereochemical site theory, Amoore, 1965; penetration and puncturing theory, Davies, 1971; molecular vibration theory, Wright, 1964, 1969; three dimensional force model, Kafka, 1974). None of these theories has been conclusively proven and it is possible that more than one may apply.

When stimulated by pheromones, the animal must be able to determine both their quantity and their quality. An important factor for the quantitative determination is the sensitivity of the sense cells. It has been calculated in Bombyx mori that one molecule per sensillum in still air may be sufficient to elicit a nerve impulse. (Kaissling and Priesner, 1970). The more molecules hit the receptor cells, the more impulses are produced, giving a measure of quantity.

The ways in which the animal determines the quality of the pheromone are still not well known. The simplest case can be the use of odour specialist cells that will only respond to a certain kind of chemical. However, such narrow receptors are not always present. Odour generalist cells can be grouped into a number of types with identical chemical sensitivity. It is possible that the identity of a pheromone mixture or a natural odour is perceived as a complicated pattern of action potentials of different cell types. It is also possible that a receptor can be stimulated by compounds of different structure and relay different information (O'Connell, 1972).

The integration of the signals between the receptors and the deutocerebrum (antennal lobe) as well as between the deutocerebrum and the other integrating parts of the brain, is not well known. The main connection between the deutocerebrum and the protocerebrum is a bundle of fibres that terminate in the calyces of the corpora pedunculata (Boeckh, 1975) where further integration and inhibition with neurons from other parts of the brain as well as with olfactory neurons takes place (Howse, 1974, 1975).

The reaction patterns of central olfactory neurons are more complicated than the receptor responses. Inhibition is often observed (Yamada, 1971) as well as spatial integration from different parts of the antenna (Boeckh, 1974) and integration of responses from both chemo- and mechanoreceptors (Waldow, 1975).

1.7 Behavioural responses to pheromones in ants

The last step in the series of events that comprise communication between two animals is the response to the signal that is transmitted. The concept of sociality implies a more varied repertory of responses by the members of a society, that has emerged from the integration of simpler individual patterns with the help of communication (Wilson, 1971). These responses not only vary in number but also in the nature, the duration and the complexity, depending on various factors such as environmental, hormonal or developmental (Blum, 1977). It seems that insect responses to pheromones are not single responses but consist of a series of behavioural steps that so far have received little study (Shorey, 1970).

The responses of social insects to pheromones had originally been grouped into various categories (Wilson, 1968, 1971; Butler, 1970; Shorey, 1973) such as alarm, simple attraction, recruitment, trail laying, sexual attraction, arrestance, dispersion, territory marking, recognition, grooming, trophallaxis, transport, licking and more. It is becoming increasingly evident that because of the multifunctional nature of pheromones, these distinctions are no longer helpful and a new system must soon be devised. However, they are still in use today, accompanied by more precise behavioural terminology such as kineses (non-directed responses) and taxes (directed responses) (Dethier et al, 1960).

The best studied behavioural responses in ants, are those that involve long range volatile pheromones and include alarm, recruitment and trail laying and sex attractance. The relationship of other responses and the surface pheromones is less understood.

Alarm behaviour covers a variety of responses. They can range from retreat and dispersal to attraction and attack on the source of disturbance (*Acanthomyops claviger*, Ghent, 1961; *Atta texana*, Moser et al, 1968; *Crematogaster scutellaris*, Leuthold and Schlunneger, 1973). They may also include jerking movements (A. *claviger*, *Conomyrma pyramicus*, Blum and Warter, 1966) unoriented circular running together with rapid movement of antennae (*C. scutellaris*), transportation of brood (*A. claviger*, *C. pyramicus*), digging behaviour (*Pogonomyrmex badius*, Wilson, 1958), and attack on sister workers (*C. scutellaris*, *A. texana*, *P. badius*, Blum et al, 1971). Finally, alarm behaviour includes the further secretion of substances, either of venom from an elevated gaster (*C. scutellaris*) or from lowered gaster through the legs directed to the source of disturbance (*Formica rufa*, Cosens, 1969) or of the contents of the mandibular glands (*A. claviger*, *C. scutellaris*). The particular response that an ant will follow is related to many factors: the place of emission (inside or outside the nest or on trail, *C. scutellaris*), the concentration and amount of pheromone emitted (*A. texana*), the nature or source of pheromone (mandibular or poison gland), the physiological state (hunger) or the presence of other stimuli such as leaves that are being transported (*Trachymyrmex septentrionalis*, Crewe and Blum, 1972).

Many ants are capable of communicating information about a new food source to other members of the colony. They are also capable of recruiting members of a colony to a new nest. Both these activities can be mediated by chemical signals. Recruitment to a food source is not the product of a single response but can be subdivided into inviting or alerting signals, leading signals and orientation signals (Maschwitz, 1975). These signals can be either tactile or chemical. In the most primitive forms of recruitment, social carrying and tandem running (Wilson, 1959), tactile and chemical signals play an equally important role. (Camponotus socius, Maschwitz, 1975). Chemical signals are used in orientation and leading in tandem, while tactile,in alerting. More developed recruitment strategies employ tactile stimuli for alerting and stimulating the ants inside the nest but use chemical trails for leading and orientation to the food source either in group recruitment (Camponotus socius, Hölldobler, 1971) where a small number of ants leave the nest to forage, or in mass recruitment (Monomorium sps, Tapinoma sps., Szlep and Jacobi, 1907; Formica fusca; Möglich and Hölldobler, 1975) where large numbers are recruited to the food. Finally, there is mass recruitment with chemical signals only (Solenopsis saevissima, Wilson, 1963; Lasius fuliginosus, Hangartner, 1962). Pheromones, sometimes different, act both as alerting substances for the inhabitants of the nest and as leading and orientation workers. Alarm pheromones are also used in recruitment in conjunction with other compounds (S. saevissima, Camponotus pennsylvanicus, Ayre & Blum, 1971; C. socius, Hölldobler, 1971).

The behavioural mechanism of orientation during trail following is believed to be chemo- or osmotropotaxis (Hangartner, 1967). In this, the ant turns one antenna to one direction until it leaves the odour field and then it alternately turns the other antenna to the other side. The course is regulated as if to achieve balanced olfactory stimuli.

Sex attractants have not been well studied in ants (Buschinger, 1975). In some genera (Camponotus, Hölldobler and Maschwitz, 1965; Lasius, Acanthomyops, Law, Wilson and McCluskey, 1965) swarming males produce mandibular gland secretions that attract females from their nest and synchronize swarming. In other species (M. pharaonis, Hölldobler & Wüst, 1973; Formica sp., Kannowski, 1963; Harpagoxenus sublaevis, Buschinger, 1968), it is the females that produce attractants from various glands when calling. No ant sex pheromone has yet been identified.

There is a number of other behavioural patterns whose activity is believed to be mediated by surface pheromones (Wilson, 1971). These are less volatile substances that produce small active spaces and are perceived only by contact chemoreception. One of the most important social aspects they mediate is the recognition of nest mates at the colony, the caste or the developmental level. Recognition of colony members is almost certainly carried out mainly through chemical signals. Members of alien colonies are usually greeted with degrees of "aggression" varying from a reduced rate of exchange of food (Lange, 1967) to attack. The basic problem that has not yet been fully explained is the source of the colony odour, whether it is genetically or environmentally controlled.

Chemical signals are used in the recognition of castes and developmental stages, in conjunction with other tactile and acoustic stimuli (Wilson, 1971). Although no specific chemical has been identified, there exists substantial evidence for the presence of brood pheromones in *Solenopsis invicta* (Walsh & Tschinkel, 1974) and in *Myrmica rubra* (Brian, 1975) and it is also known that the volatiles of queens and workers differ in *M. pharaonis* (Ritter et al, 1975) as do those of major and minor workers in *O. longinoda* (Bradshaw, 1976).

There is also evidence that dead ants are chemically recognised by members of their own colony and transported to specific or non-specific refuge piles (Wilson, Durlach and Roth, 1958; Howard & Tschinkel, 1976).

1.8 Use of pheromones in pest control

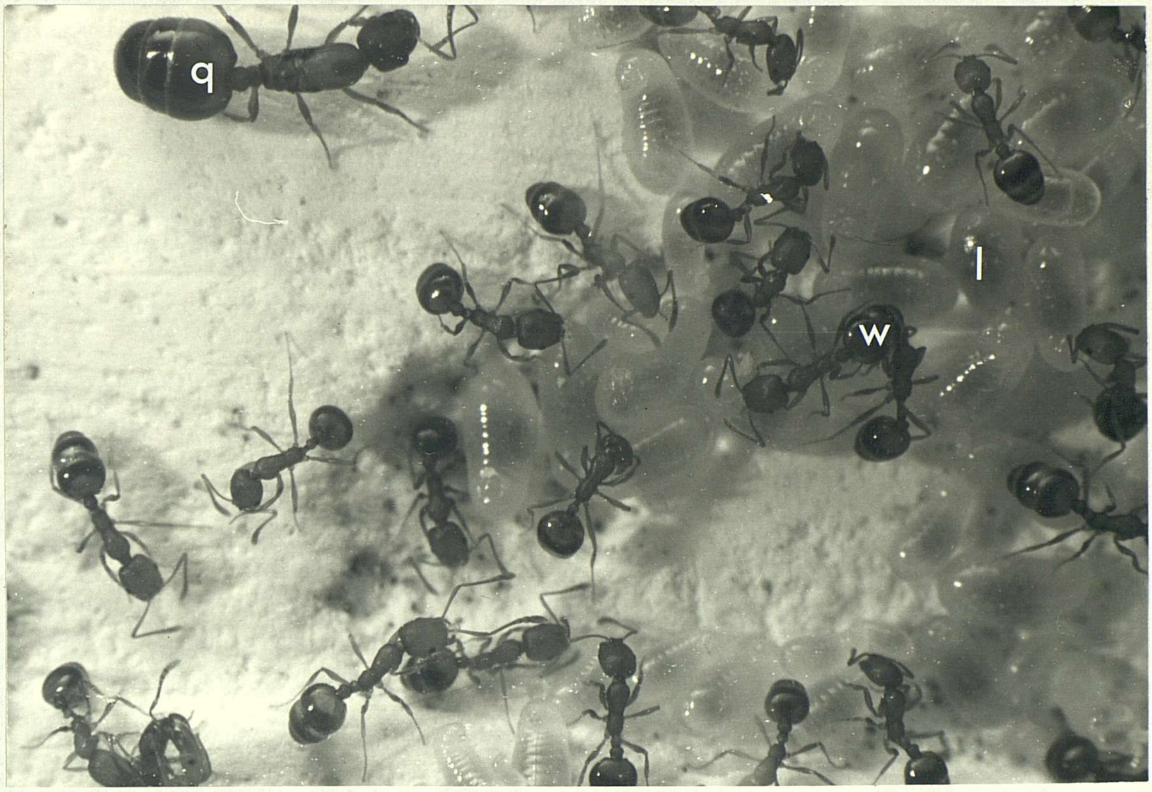
Pheromones have already been used in insect integrated control systems sometimes in conjunction with chemical insecticides. There are three basic ways in which pheromones can be used in pest control. One technique involves the use of attractant pheromones, usually sex attractants, in baits together with some toxic insecticides or means of trapping the insects. Baited traps can be used as monitoring systems for determining the timing of insecticide applications. Insects that have been dealt with these two methods include the gypsy moth (Stevens and Beroza, 1972), the boll-weevil (Boyt, 1973), the redbanded leafroller, Argyrotaenia velutinana (Trammel, Roelofs and Glass, 1974), the western pine beetle Dendroctonus brevicornis (Bedard and Wood, 1974) the elm bark beetle (Peacock) and others. A third use in control is that of the confusion technique in which the air is saturated with a sex attractant or inhibitor in order to disrupt the orientation of males towards females (Beroza, 1960).

1.9 Monomorium pharaonis : its importance

Monomorium pharaonis (L), (pl 1.1, 1.2), pharaoh's ant, as it is commonly called, is a tropical species which originally comes from Africa. Lately, it has moved to the mild temperate climates of Europe and the U.S. It has established itself in well heated and humid places such as kitchens, bakeries, hospitals, toilets and foodstores (Stawarski, 1963). In its native habitat, it is both harmful and beneficial. It can cause damage to crops and foodstuffs but it is also known to be the major pollinator of certain crops (Bambarra groundnut, Doku, 1968; Doku and Karikari, 1972) and also a serious predator of important pests. (Thrips in Ficus nitida, Tawfik, 1970). In the mild, temperate climates, this insect and the german cockroach (Blattella germanica) are pests whose importance has increased together with the increase in housing facilities and the standard of living (Berndt and Nitschmann, 1977). It has been shown to carry pathogenic agents such as Pasteurella (plague vector)(Alekseev et al, 1972), Salmonella, Pseudomonas (Cartwright and Clifford, 1973) Staphylococcus, Streptococcus and Clostridium in its vestitutes, mouthparts and legs for 2-3 days after contacting them, and therefore hospital infestations are highly dangerous (Beatson, 1972). Because of its small size (2-3 mm), it is able to penetrate bandages or sterile packs, feeding on wound discharges and baby slobber (Wintringham, 1973). It has also been reported to bite infants around eyelids and has also been found in operation rooms, sterile laundry, among surgical instruments, between the toes of postoperative patients, possibly spreading digital mycoses, in surgical incubation equipment, central humidifiers, computers, drug storage units, and refrigeration units (Eichler, 1974). Outside hospitals, it can infest foodstuffs, such as powdered milk and coffee (Eichler & Kleinsorge, 1973). It has even been reported to attack beehives and cause high mortality. (Gottschalk, 1963).

pl. 1.1. Inside of the nest of M. pharaonis
(l: larvae, q: queen, w: workers)

pl. 1.2. Queen, worker and brood of M. pharaonis
(l: larva, q: queen, w: worker)



q
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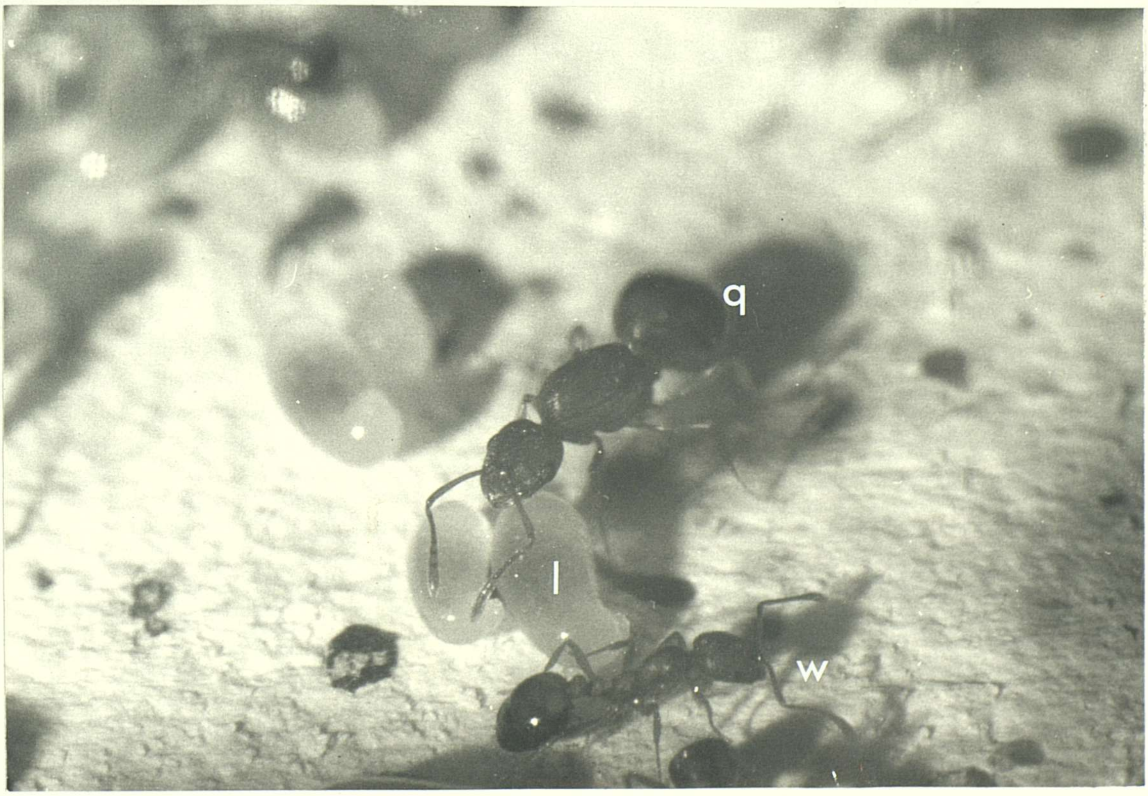

q
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w

M. pharaonis is not an easily controllable pest, especially in view of its small size. The use of common insecticides diminishes its activity only temporarily because the nests are well protected and inaccessible and also because the ants usually move away from adverse conditions. At first, attempts were made to control it with insecticides such as thallium sulfate, sodium benzoate (Davidson, 1950) and sodium arsenate (Berndt, 1974) which proved very effective but difficult to apply and dangerous to other forms of life, especially in hospitals. Later control methods included the use of diazinon (Sy, 1961, 1970) borax (Berndt et al, 1974; Vorbrazkova et al, 1976) trichlorphon, aprocarb, DDT (Sy, 1961) dieldrin, chlordane, dichlorvos and primarily chlordecone (kepone) (Berndt & Nitschmann, 1976), which is considered the most effective so far, in sprays, oils, lacquers or food baits. The only effective control method would be the destruction of the queen and the brood. Unfortunately, the queens do not leave the nest and search for mates or food but are fed by the workers. Also, because they lie at the end of the food chain, they receive sublethal doses of insecticides especially when fast acting contact insecticides are used (Berndt, 1974). For these reasons, finding the nest is a very hard and time consuming work.

The ants multiply very rapidly. Every colony may contain several thousand workers including up to 100 queens at the same time, each of which can produce approximately 400 eggs during her lifetime (Peacock, 1950)/max. lifetime for queens, 40 weeks (Peacock & Baxter, 1950)/. In addition, the ants can feed on any kind of foodstuff, preferably raw meat and sweet items.

All these factors show that the problem cannot be tackled with the ordinary control methods which are highly toxic to mammals and are especially dangerous to hospital patients since most formulations contain chlorine, arsenic, borax and other substances of high toxicity. Furthermore, bearing in mind the great ability of insects to develop resistance to insecticides, new control methods must be found which take into account the biology of the species.

One area towards which research has turned, is the destruction of the ants with γ - radiation (Cole et al, 1958). Another area is the use of pathogen vectors such as Bacillus thuringiensis (Berndt et al, 1974; Vankova et al, 1975) Although positive results have been obtained with this method, it is difficult to evaluate them because the vector has always been used with borax which is itself a slow acting toxin (Berndt and Nitschmann, 1977). Further study has been carried out with the queen technique, which is distinguished from other methods by acting selectively on the queens by inhibiting their reproduction capacity (Berndt et al, 1973). The sterilization is effected by chemo-sterilants such as TEPA (triethylene phosphoramide) (Berndt & Nitschmann, 1974).

The juvenile hormone analogue Altosid (Methoprene)(Isopropy l-11-methoxy - 3,7,11, trimethyl-dodeca - 2,4, dienoate) can also be used for the sterilization of queens as well as for the disruption of normal metamorphosis and the subsequent death of larvae and pupae. (Edwards, 1975, 1977). Another means, is the use of synthetic trail pheromones in baits for the increase of their attractivity (Ritter & Stein, 1974, 1976). Future possibilities may include cases in which pheromones or other chemical secretions can play a role in recruitment to a food source, alarm behaviour or colony recognition.

1.10 The biology of M. pharaonis

Monomorium belongs to the family Formicidae, subfamily Myrmicinae, tribe Myrmicini (Bolton, 1973; Bolton & Collingwood, 1975; Collingwood, 1964). It is a large specialized genus, occuring in both tropical and warm temperate regions (Ettershank, 1966). Monomorium pharaonis is an African species that has been spread by man almost everywhere. The ability to adapt to various climates is due to two basic characterisitcs. The first is the abandonment of the nuptial flight. The queens lose their wings and are unable to fly. They are ready to mate 48-60 hrs after eclosion and up to the age of 14 weeks (Petersen-Braun & Buschinger, 1971). Copulation takes place in the nest or nearby. There are indications that a sex pheromone originates in the Dufour's gland and the bursa pouches of M. pharaonis (in the female) (Hölldobler & Wüst, 1973). The queens mate once whereas the males mate up to 4 times.

The second characteristic is the abandonment of territorial boundaries. The colony multiplies by budding. When it becomes crowded, scout ants start searching for a new nest site. When this is found, odour trails are laid and a part of the colony consisting of a minimum of 50 workers together with brood and some queens, moves to the new nest (O'Toole, 1973). New queens are not necessary for budding. If the new colony has no sexuals, they are reared from the existing brood. M. pharaonis is the only ant species so far recorded that can make this immediate response and ensure its own supply of sexuals. The workers seem to be able to distinguish brood which will become queens or males from brood destined to become workers. It seems that the differentiation and production of sexuals is induced by the absence of queens or by the excessive number of workers over queens. Experimental results have shown that fertilized queens possess an inhibitory pheromone on the cuticle that supresses the production of other sexuals. Fertilization must play a role because unfertilized queens do not supress the sexual forms (Berndt, 1975; Berndt & Nitschmann in prep.) Other results have shown that queens, shortly before the end of their lives, stop producing glandular secretions. This is coupled with the appearance of sexual brood (Buschinger & Kloft, 1974; Petersen-Braun, 1975). While the primary supression of sexuals can be due to the inhibitory pheromone, the development of sexual larvae may depend upon food.

Monomorium pharaonis, in tropical climates lives in suitable plant cavities outdoors, while in the temperate climates it lives in any crevice or cavity in warm and humid places. It thrives in temperatures between 27-30°C and the nest has a relative humidity of about 80% (Peacock et al, 1955). It feeds on any kind of food which is another reason for its ease of adaptation to unstable environments.

The workers' maximum recorded life span is 9-10 weeks, while the queen's maximum life span is 39 weeks (Peacock & Baxter, 1950). The colonies may consist of 150 to 2000 workers, 2 to 110 queens and 3 to 4 times less males (Petersen-Braun, 1975). Since their odour trails intersect at many points, many colonies infesting a certain area can be considered as one very large supercolony.

1.11 Foraging and recruitment

The study of communication in M. pharaonis has concerned recruitment to a food source (Sudd, 1957a, 1957b, 1960, 1967). Sudd was the first to make detailed observations on the behaviour of M. pharaonis. What we know so far can be summed up as follows:

Scouts leave the nest and begin searching for food independently from each other. They basically follow a well defined route and wander around returning to it at times.

As soon as a scout finds food, it eats and returns to the nest. The return journey is quite different. First, it moves faster, touching the abdomen on the ground. The sting is out and is lifted from time to time, leaving a non-continuous trail of a substance which, although volatile, it remains on the ground for 24 hrs (Blum, 1966). The route the ants follow is almost a straight line from the food to the nest and it seems that in this case it is guided not only by the trail on the ground, but also by distant stimuli such as light for example.

Shortly after the return of the scout to the nest, a group of ants appear and head towards the food, following the trail left by the scout. The workers, after feeding, return too, and another group emerges. Soon, we observe a continuous movement of ants coming in and out of the nest. Initially, the number of workers arriving at the food is greater than the number of those that leave. Soon, an equilibrium is established which lasts as long as the food lasts.

This rapid recruitment is caused by two main factors. The scouts, while entering the nest, make a series of fast movements when they meet another worker. In general, they move in a very agitated manner. This rise in "excitement" stimulates the neighbouring inhabitants of the nest, which in turn stimulate the rest. In other species of the genus Monomorium (M. subopacum, M. venustum), a specific behavioural pattern has been observed which includes head pushing, oscillatory movements and accelerated antennal beatings (Szlep, 1970; Szlep & Jacobi, 1967). This pattern, which has been named by Szlep, Oscillatory Recruitment Display (ORD), has not been observed in *M. pharaonis*.

The general rise of excitement inside the nest, does not give the workers the direction they must take to find the food. This information comes from the odour trail which the scouts leave and which is reinforced by workers returning to the nest.

The odour trail is left by the workers only on their way to the nest. It seems that its components come from both the poison and the Dufour's gland (Ritter et al, 1975). There was an earlier controversy on this matter. Blum(1966) believed that the recruitment pheromone comes from the poison gland, while Hölldobler (1973) believed that it comes from the Dufour's gland and that the poison gland secretes a substance which repels workers of other species.

1.12 Chemical analysis

More work has been done towards elucidating the structure of the substance or substances that cause recruitment or other kinds of behaviour. Ritter et al (1973) homogenised and extracted workers of *M. pharaonis* in methylene chloride. The extract was gas chromatographed and produced three basic biologically active zones. The first of these three, showed the best biological activity.

The bioassay method which was used involved two tubes placed in a V. Their common end was placed in a dish which contained the workers. One of the tubes contained a filter paper with the substance to be tested. The other tube contained a blank filter paper.

Chemical analysis of the first zone with mass spectrometry, IR and MMR, showed that it consisted of one compound, 5-methyl-3-butyl-octahydroindolizine (fig 1.1).

Because there are four possible stereo-isomers of this compound (fig. 1.2), all of them were individually synthesised by Oliver and Sonnet (1974). Behavioural bioassays with all four isomers suggest that the all-cis isomer is the naturally occuring monomorine I, as it was subsequently called (Edwards & Pinninger, 1978).

The second zone had little or no biological activity. GLC-MS analysis gave the possible formula 2-butyl-5-pentyl pyrrolidine, (fig. 1.1). This compound can be related biogenetically with 5-methyl-3-butyl-octahydroindolizine (fig. 1.3).

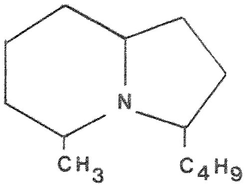
The third compound to be isolated was monomorine III, the trans isomer of 2(5'-hexenyl)-5-pentyl pyrrolidine which was also active in choice tests. Two other homologues of monomorine III were also found in trace amounts: monomorines IV and V, the 5-heptyl and 5-nonyl derivatives of III (Ritter et al, 1975) (fig. 1.1).

An unsaturated bicyclic hydrocarbon with the formula $C_{18} H_{30}$ was also isolated. It was called monomorene.

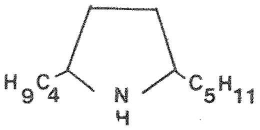
Finally, a very active compound, faranal, was isolated, identified and synthesized (Ritter et al, 1977). This was the most active in both choice tests and trail following tests (fig. 1.1f).

The sources of these compounds vary. The poison gland of workers and queens contained the first three monomorines and probably some monomorene, although the latter was not found in older wingless queens. The Dufour's gland of the workers contained monomorene but not monomorine I, II and III. The Dufour's gland of the queens contained different compounds which could have some sex attractant activity.

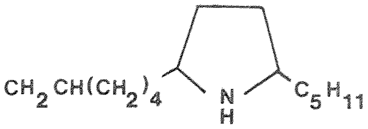
The ratio of the compounds differed in the various castes such as the ratio of monomorine I to monomorine III from 1:3 in workers up to 1:30 in queens. Therefore, the compounds may have many functions according to their relative concentration.



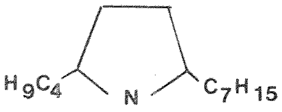
mI



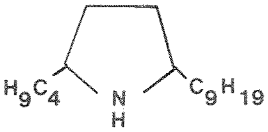
mII



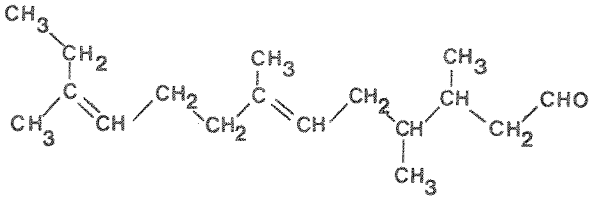
mIII



mIV

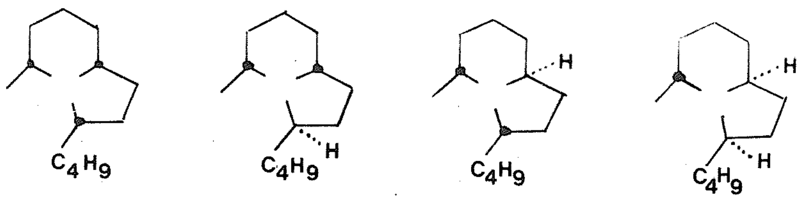


mV



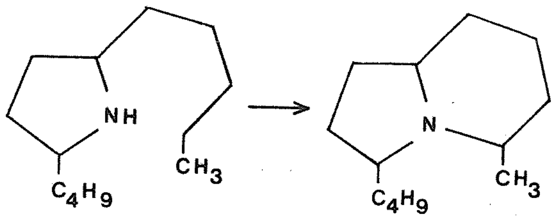
f

Fig 1.1. Structures of M. pharaonis pheromones
(mI: monomorine I, 3-butyl-5-methyl octahydroindolizine;
mII: monomorine II, 2-butyl-5-pentyl pyrrolidine;
mIII: monomorine III, trans-2(5'-hexenyl)-5-pentyl pyrrolidine; mIV: monomorine IV, 2-butyl-5-heptyl pyrrolidine;
mV: monomorine V, 2-butyl-5-nonyl pyrrolidine;
f: faranal, (6E, 10Z)-3,4,7,11-tetramethyl-6,10-tridecadienal-1)(from Ritter et al, 1973, 1975, 1977).



1a 1b 1c 1d

Fig 1.2. Stereo-configuration of the four isomers of monomorine I.
1a: 3(S)5(R)9(R)-3-butyl-5-methyl octahydroindolizine.
1b: 3(R)5(R)9(R)-3-butyl-5-methyl octahydroindolizine.
1c: 3(S)5(R)9(S)-3-butyl-5-methyl octahydroindolizine.
1d: 3(R)5(R)9(S)-3-butyl-5-methyl octahydroindolizine.
(from. Edwards & Pinninger, 1978).



m II m I

Fig 1.3. Possible biogenetic relation between monomorine I(mI) and monomorine II (mII). (From Talman et al, 1974).

CHAPTER 2

MORPHOLOGY OF THE SENSORY AND NERVOUS SYSTEMS

2.1 Introduction

The mechanism of olfaction in animals is largely unknown and although many of the relevant organs of olfaction have been described, the exact means by which a chemical signal is transformed into a particular behaviour pattern is still hypothetical. No attempt is made here to provide a complete picture of olfaction in M. pharaonis, but some basic elements will be discussed that may indicate possibilities of further research.

This chapter will deal with two parts of the animal that play the most important roles in olfaction: the antennae as sensory centres and the brain as an integrating centre.

2.2 Antennae

Antennae and whole heads were covered with a gold-palladium film and observed in the scanning-electron microscope.

The antennae of M. pharaonis workers consist of twelve articles. The first, the scape, is almost as long as the rest, the second is elongated, the third up to the ninth are approximately cuboidal and the last three are larger and form a club (pl. 2.1).

Hair-like processes are apparent throughout the length of the antenna. The most widespread are those that look like sensilla trichodea curvata (pl. 2.2). They are present in all articles. They are curved in shape with a distinct pit at the point where they attach to the cuticle.

The olfactory sensilla are situated on the last three articles

pl. 2.1. Final segments of antenna of M. pharaonis.
(magnification x300)

of the antenna. Three types were observed, although the possible presence of others cannot be excluded. Most abundant were the sensilla trichodea (pl. 2.3) comprising approximately 70% of the total number of sensilla on the last article. They are elongated structures lying almost parallel to the surface of the antenna. They do not articulate with the cuticular surface.

The sensilla basiconica (pl. 2.4) are shorter and thicker than the s. trichodea. They are aligned more vertically with the cuticular surface, and comprise about 5% of all sensilla on the last article.

The sensilla placodea (pl. 2.5) are round plates projecting only a little above the cuticular surface. There are very few of these on the antennae.

Apart from the olfactory sensilla, there are also tactile sensilla on the last three articles comprising about 25% of the total. They can be distinguished from the olfactory sensilla by an articulation at the point of contact with the cuticle and a pit usually visible at the base. They are thicker than the s. trichodea and can be usually seen at various angles to the surface.

2.3 Brain

The term brain is used here to describe both the cerebral or supra-oesophagal and the suboesophagal ganglia which are fused in one mass. The brain is the main association centre of the body receiving sensory input from the sense organs and gives motor and other outputs to the rest of the nervous system and the muscles. It also organises many behaviour patterns.

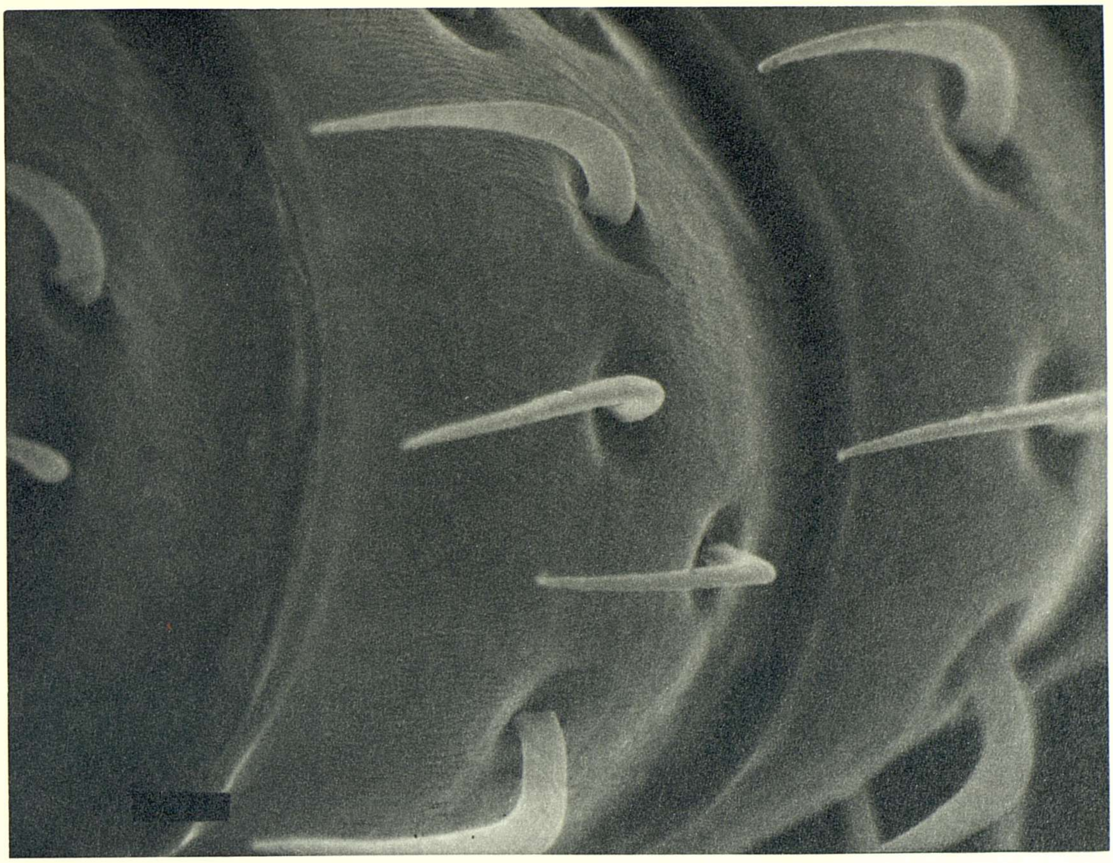
2.3.1. Materials and methods

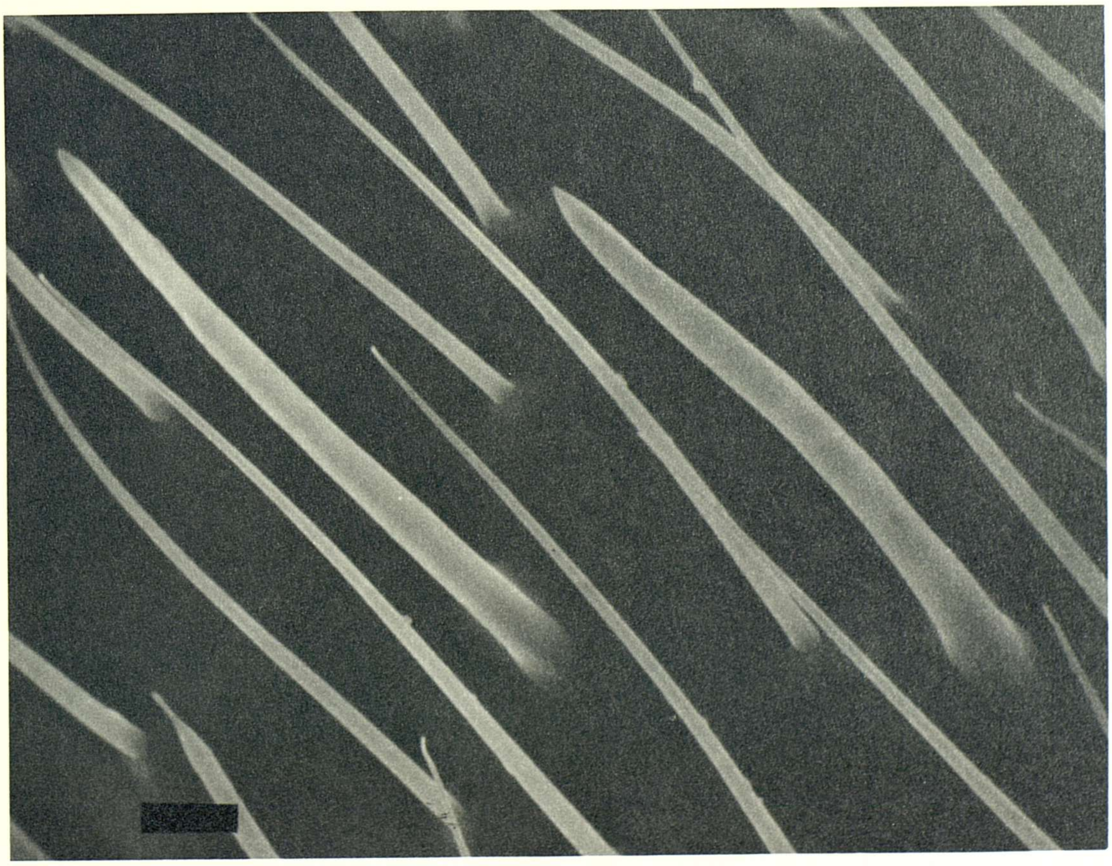
The histological techniques for *M. pharaonis* are described in Chapter 10. The sections of *Atta cephalotes*, *Formica sanguinea* and *Oecophylla longinoda* had been subjected to the following fixing and staining techniques.

a. Brains were fixed in situ by the injection of either Bouins, pl. 2.2. Sensilla trichodea curvata on middle segments of the antenna of _M. pharaonis_ (magnification ×3000).

pl. 2.3. Sensilla trichodea on last segment of the antenna of _M. pharaonis_.

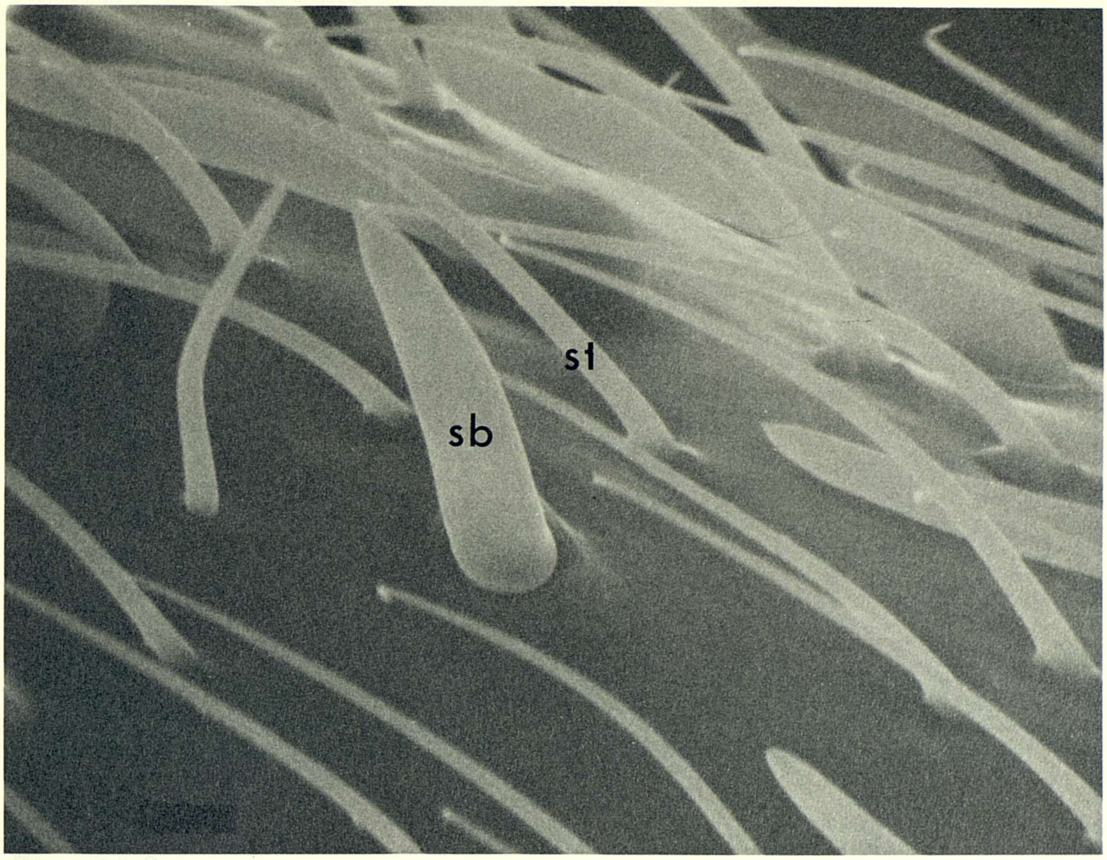
(magnification ×3000)

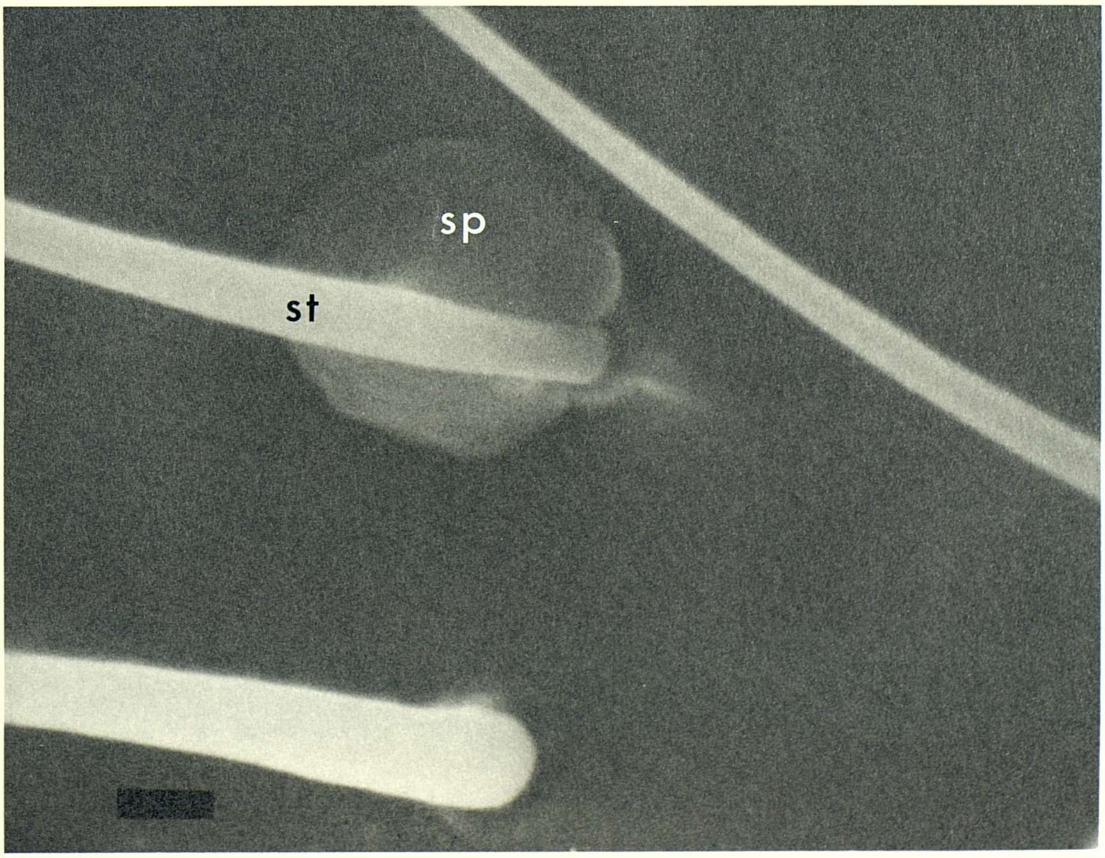




pl. 2.4. Sensillum basiconicum on last segment of the antenna of *M. pharaonis*.
(sb: sens. basiconicum, st: sens. trichodeum)
(magnification ×10000).

pl. 2.5. Sensillum of undetermined nature, possibly sensillum placodeum on last segment of the antenna of *M. pharaonis*.
(magnification ×30000).
(sp: sens. placodeum, st: sens. trichodeum)


st
sb


sp
st

Zenker's fluid or buffered glutaraldebyde into the head capsule. They were then washed, dissected away from the head capsule, dehydrated, cleared and embedded in paraffin wax. The sections were stained in Heidenhain's or Ehrlich's haemotoxylin with counter staining in eosin.

b. Wigglesworth's osmium tetroxide-ethyl gallate method with modifications (Wigglesworth, 1957). First fixing the brain in situ with 6.25% glutaraldehyde (Fisher GL51) phosphate buffered at pH 7.0 to 7.2 according to Colonnier (1964) prior to washing in distilled water and secondarily fixing in 1% osmium tetroxide in distilled water. The dehydrated material was cleared and embedded in paraffin wax and sectioned.

c. Blest's modification of Holmes' pyridine method (Blest, 1961), a reduced silver method, was used on Bouins or phosphate buffered glutaraldehyde fixed brains which were paraffin wax embedded and sectioned.

2.3.2. Results

The brain of *M. pharaonis* can be divided into the following parts: the protocerebrum which contains the corpora pedunculata and the central body, the optic lobes, the deutocerebrum which contains the antennal lobes and the fused tritocerebrum and suboesophagal ganglia.

i) *The protocerebrum* (fig. 2.1p). The protocerebrum consists of two lobes surrounded by cortical cells of one type. The most prominent organs in the protocerebrum are *the corpora pedunculata* (mushroom bodies)(figs. 2.1cp, 2.2, pl 2.8). These are two pairs of mushroom shaped cups lying postero-dorsally to the rest of the brain. Each corpus pedunculatum consists of a calyx and a stem (fig 2.2 c,s). The stems of each pair fuse into a stalk that enters the rest of the brain before dividing into two lobes, the α- and the β- lobe (fig 2.2). The cell bodies of the neuropile that make up the corpora pedunculata, known as Kenyon cells, lie within and over the calyx. The calyx consists of three clearly discernible regions: the lip which is the outermost, the collar which is in the middle, and the basal ring that pl. 2.6. Longitudinal section of head of *M. pharaonis* (al: antennal lobe, cp: corpora pedunculata, phg: pharyngeal gland, p: protocerebrum)

pl. 2.7. Longitudinal section of thorax of *M. pharaonis* (pr: prothoracic ganglion, ms: mesothoracic ganglion, mt: metathoracic ganglion, 1a: 1st abdominal ganglion).


cp
p
al
phg
0 ·1 ·2mm

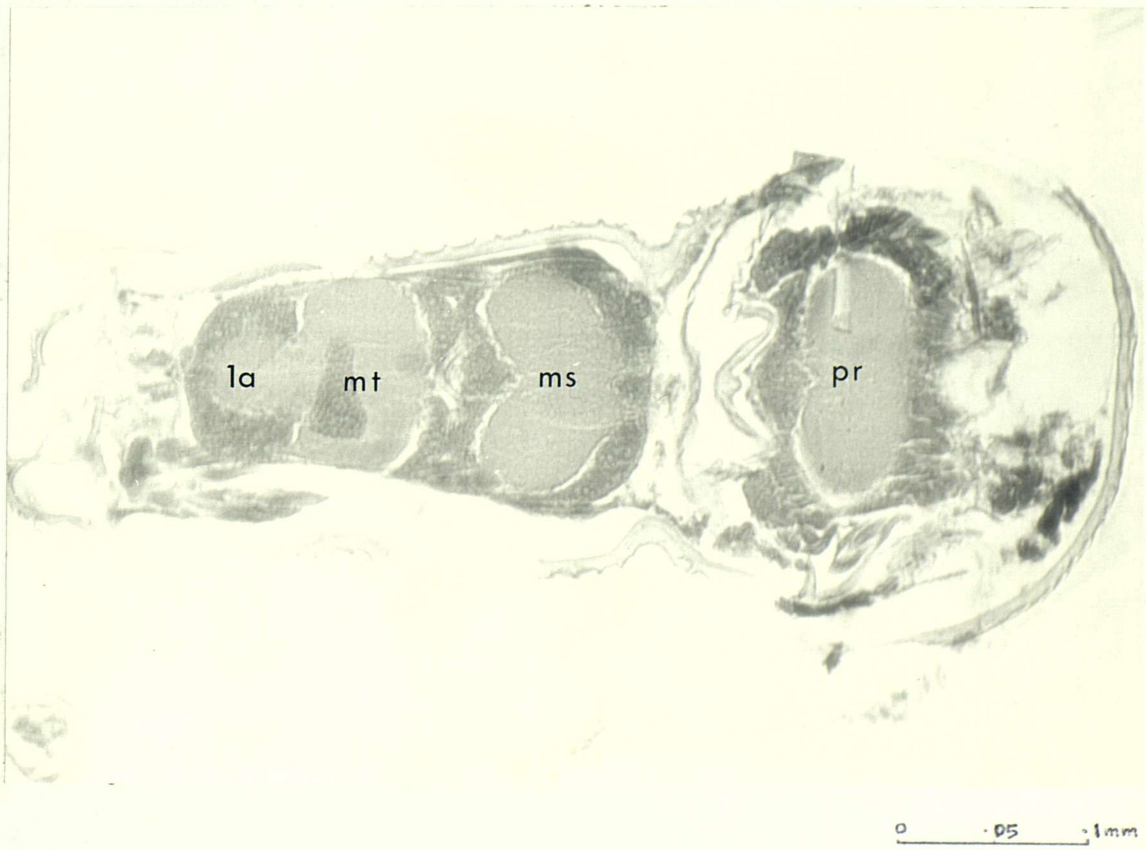
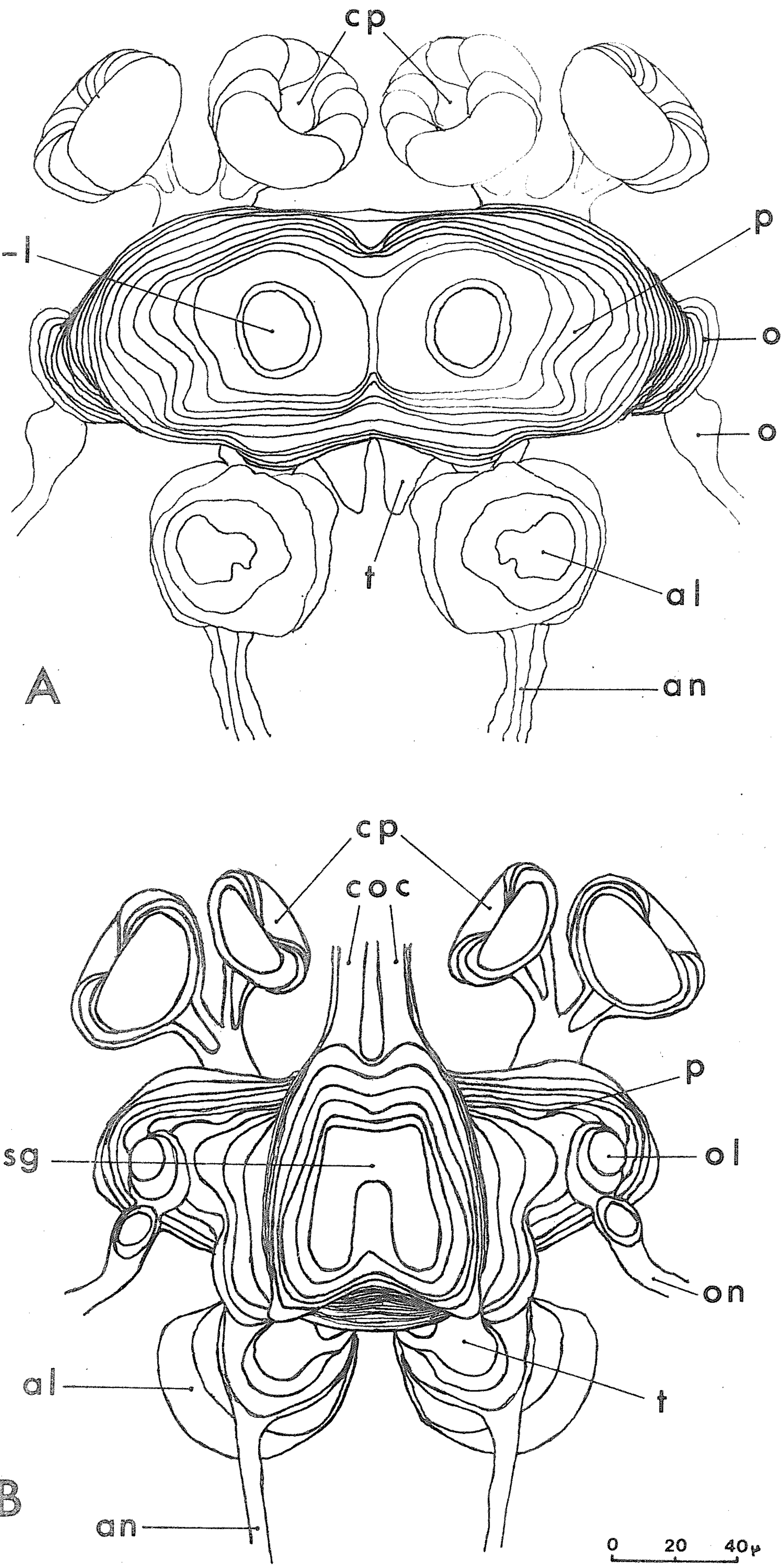

la
mt
ms
pr
0 ·05 ·1mm

Fig.2.1. A) Dorsal and B) Ventral views of the brain of *Monomorium pharaonis*.

al : antennal lobe
a-l : α - lobe
an : antennal nerve
coc : circum-cesophageal connective
cp : corpora pedunculata
ol : optic lobe
on : optic nerve
p : protocerebrum
sg : suboesophagal ganglion
t : tritocerebrum.


cp
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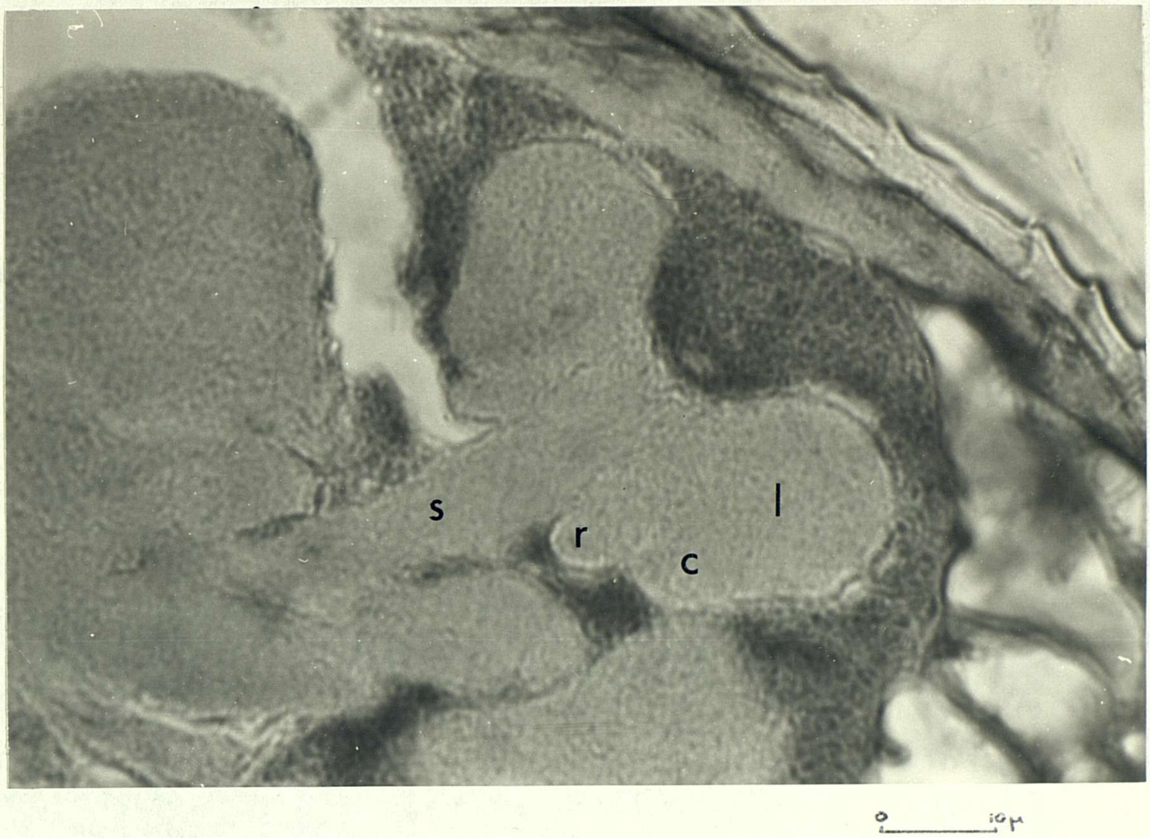
0 20 40μ

pl. 2.8. Corpus pedunculatum of *M. pharaonis*
(l: lip, c: collar, r: basal ring, s: stem)

pl. 2.9. Central body and pons cerebralis of *M. pharaonis*
(cb: central body, pc: pons cerebralis, b-l: β -lobe,
st: stalk of cor.pedunc.)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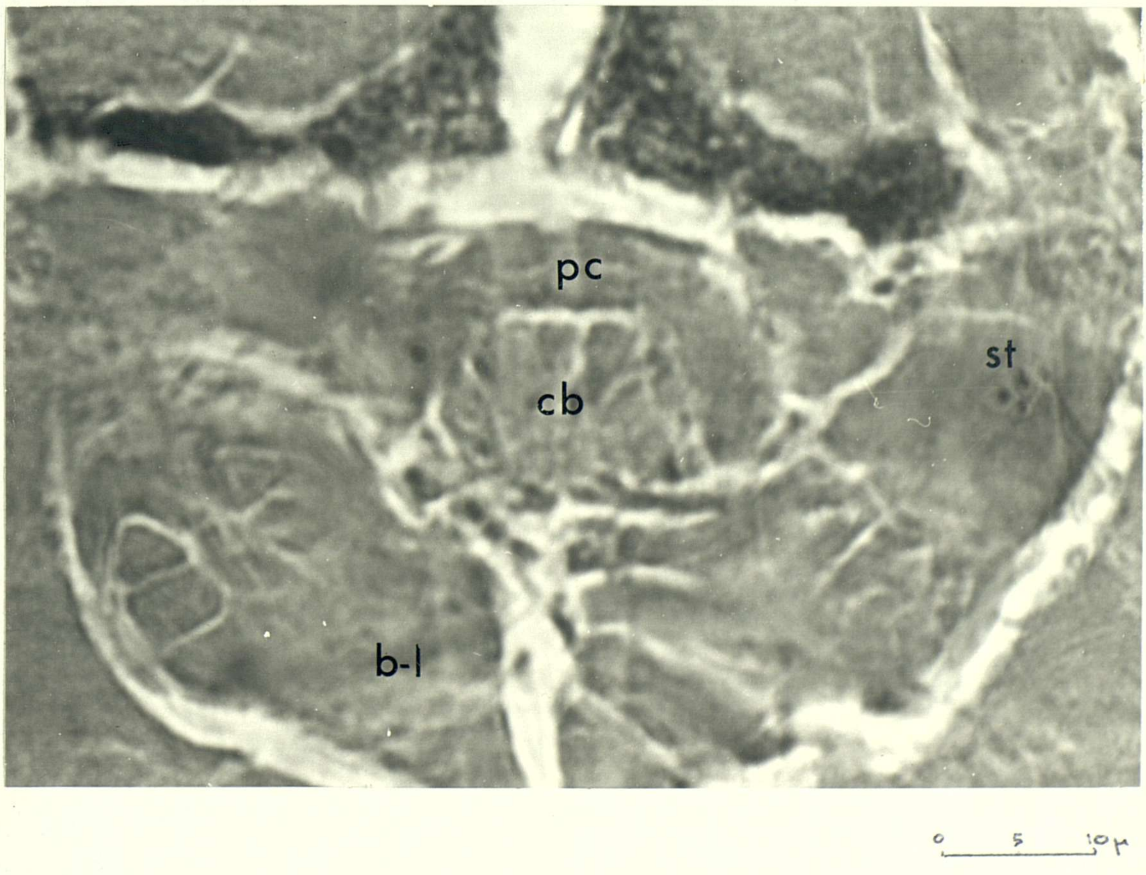
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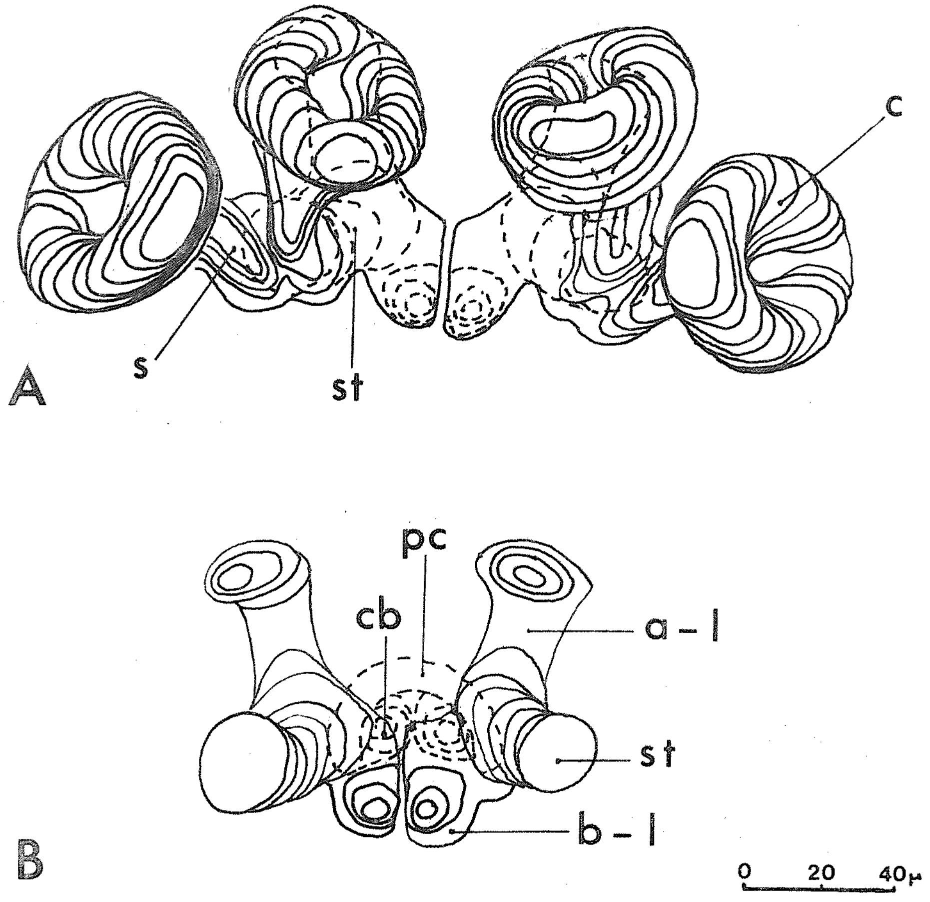


Fig 2.2. Posterior view of corpora pedunculata of M. pharaonis
A. Calyces B. α-and β- lobes. (a-l: α-lobe, b-l: β-lobe, C: calyx, cb: central body, pc: pons cerebralis, s: stem, st: stalk)

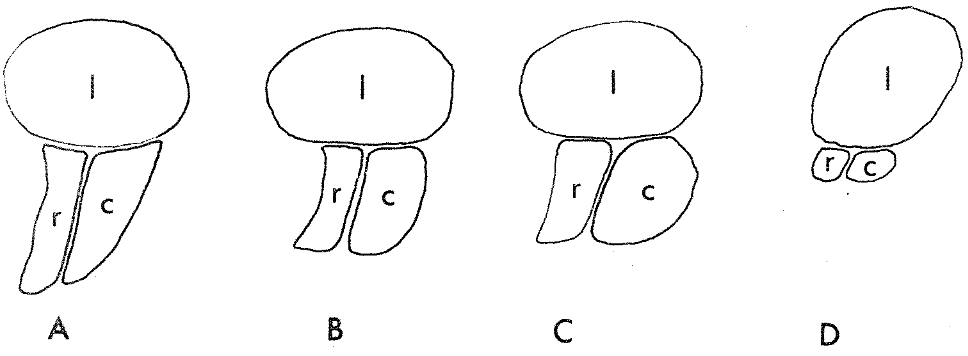


Fig 2.3. Longitudinal sections of corpora pedunculata of four ant species. A: Atta cephalotes, B: Formica sanguinea, C: Oecophylla longinoda, D: Monomorium pharaonis (l: lip, c: collar, r: basal ring) (not drawn to scale).

lies nearest to the stem of the calyx (pl 2.8). Of the two "roots" or lobes of each pair of corpora pedunculata, the α-lobe passes forwards and upwards to the frontal surface of the brain, while the β-lobe turns downwards and inwards under the central body where it meets the β-lobe from the other side.

The function of the corpora pedunculata is not yet completely clear. It is generally believed that they contain at least part of the inhibitory system of the brain and they also act against the central nervous system for the control of movement. In general, they must play an important integrative role in the control of complex muscular patterns (Huber, 1965). Especially, the two lobes may be used to integrate behavioural sequences (Howse, 1975). It has been observed that the ratio of the volumes of the corpora pedunculata to the volume of the central body is proportional to the complexity of behaviour, and is higher in social insects (Howse, 1974).

Another observation is that part of the calyx wall in Hymenoptera is associated with optical memory which is used in foraging, while another part is associated with olfactory memory. On these lines, it has been observed that the collar is much more developed in social bees and wasps which use optical stimuli much more than ants, which on the contrary have a more developed lip. (Howse, 1974) Therefore, lip size must be somehow proportional to the importance that olfaction has in the every day life of the insect. In order to test this, the volumes of the lip, collar and basal ring of <u>M. pharaonis</u> were calculated from serial sections and compared with the respective volumes in three other ant species: <u>Atta cephalotes</u>, <u>Oecophylla longinods</u> and <u>Formica sanguinea</u>. The results are summarized in Table 2.1 and fig. 2.2. From these results, it is obvious that <u>M. pharaonis</u> has a much larger lip relative to the other components of the calyx wall and if the hypothesis about the function of the lip is correct, then olfaction plays a much more important role than in other ants, while vision is much less important.

The other important organ in the protocerebrum, is the <u>central body</u>(fig 2.2cb, pl. 2.9). It lies medially, between the α- and the β-lobes of the corpora pedunculata on the dorsal part of the brain. It is concave with the concave part facing the ventral part of the brain. Its function is largely unknown and many hypotheses have been put forward. It has been suggested that it is the site of regulation of levels of responsiveness in insects. (Howse, 1974).

Finally the pons cerebralis (fig. 2.2pc) is a medially situated structure in the posterior part of the brain, behind the central body. It is more elongated and thinner and the concave side is facing the ventral wall as well.

ii) The optic lobes (fig 2.1 ol). The optic lobes in M. pharaonis are found lateral to the rest of the brain and extend towards the middle of the head where the compound eyes are situated. The lobes are not well developed as in other insects such as Schistocerca gregaria (Williams, 1972) and are even less developed than other ants such as Formica sanguinea.

iii) The deutocerebrum (fig 2.1 al). This consists of the two large antennal lobes that originate below the protocerebrum and end in the antennal nerve that leads to the antenna. The neuropile of the antennal lobes is arranged in grape-like groups which stain more dense. These act as correlation centres for the neurons of the antenna (Boeckh, 1975).

In order to test the importance of olfaction in M. pharaonis further, a comparison was made of the ratio of the volumes of the antennal lobe to the volume of the whole brain, excluding the corpora pedunculata and the optic lobes in three ant species. (Table 2.2)

The antennal lobe in M. pharaonis is relatively larger than in other species which may imply again that olfaction is more used in this species.

iv) The tritocerebrum and the suboesophagal ganglia (fig. 2.1t,sg) It is very difficult to distinguish in this ant whether the tritocerebrum is fused with the deutocerebrum or with the suboesophagal ganglia. Both the two latter ones lie ventral to the stomatogastric canal. The tritocerebrum is anterior and the suboesophagal ganglion is posterior. The tritocerebrum continues into the pair of frontal and labral nerves. The suboesophagal ganglion continues through the

Table 2.1. Average volumes of lip, collar and basal ring in four ant species (in pl.) calculated from serial sections of 4 ants.

	A. cephalotes	%	O. longinoda	%	F. sanguinea	%	M. pharaonis	%
Lip	903.2	50.1	396.0	59.1	477.6	63.3	38.2	94.8
Collar	673.6	37.3	218.4	32.6	226.4	30.0	1.6	4.0
Basal Ring	226.4	12.5	55.2	8.2	50.4	6.7	0.5	1.2

Table 2.2. Average volumes and ratios of antennal lobes to whole brain in three ant species (in nl)

	A.cephalotes	F. sanguinea	M.pharaonis
Antennal lobe	2.14	0.58	0.07
Brain	43.78	12.39	0.95
%	4.9	4.75	7.73

oesophagal nerves to the other ganglia of the body.

2.4 Thoracic and abdominal ganglia

M. pharaonis has three thoracic ganglia, the prothoracic, the mesothoracic and the metathoracic that take up most of the space within the thorax (fig. 2.3, pl 2.7). The metathoracic ganglion is fused with the first abdominal ganglion which lies in the first abdominal segment that has fused with the thorax. The second abdominal ganglion is in the petiole and the third is in the gaster. This last ganglion is the product of fusion of several abdominal ganglia.

2.5 Conclusions

The observations on the sensory and nervous system of M. pharaonis show the presence of a typical formicid structure for these systems. There are however, several deviations from this pattern that reflect the specific way of life of this ant.

The most important adaptation is the increased size and complexity of the organs that are related to olfaction. Both the antennal lobe and the lip of the calyx wall are indications of its importance. Indeed, M. pharaonis uses vision very sparingly and only for general orientation purposes when scouts return to the nest from foraging (Sudd, 1957). This visual stimulation is coupled with olfactory cues but the extent of each is not yet known.

The fact that it has only 3 abdominal ganglia may also be of some significance. M. pharaonis does not use its sting for stinging its enemies and therefore the control of its movement is significantly decreased. This can be related to the presence of only one abdominal ganglion in the gaster. Other Hymenoptera with effective stings have significantly larger number of abdominal ganglia. The bee has 5, Panurgus and Sphecodes have 4 and the Ichneumonidoe with the large ovipositor have 6. (Bullock & Horridge, 1965).

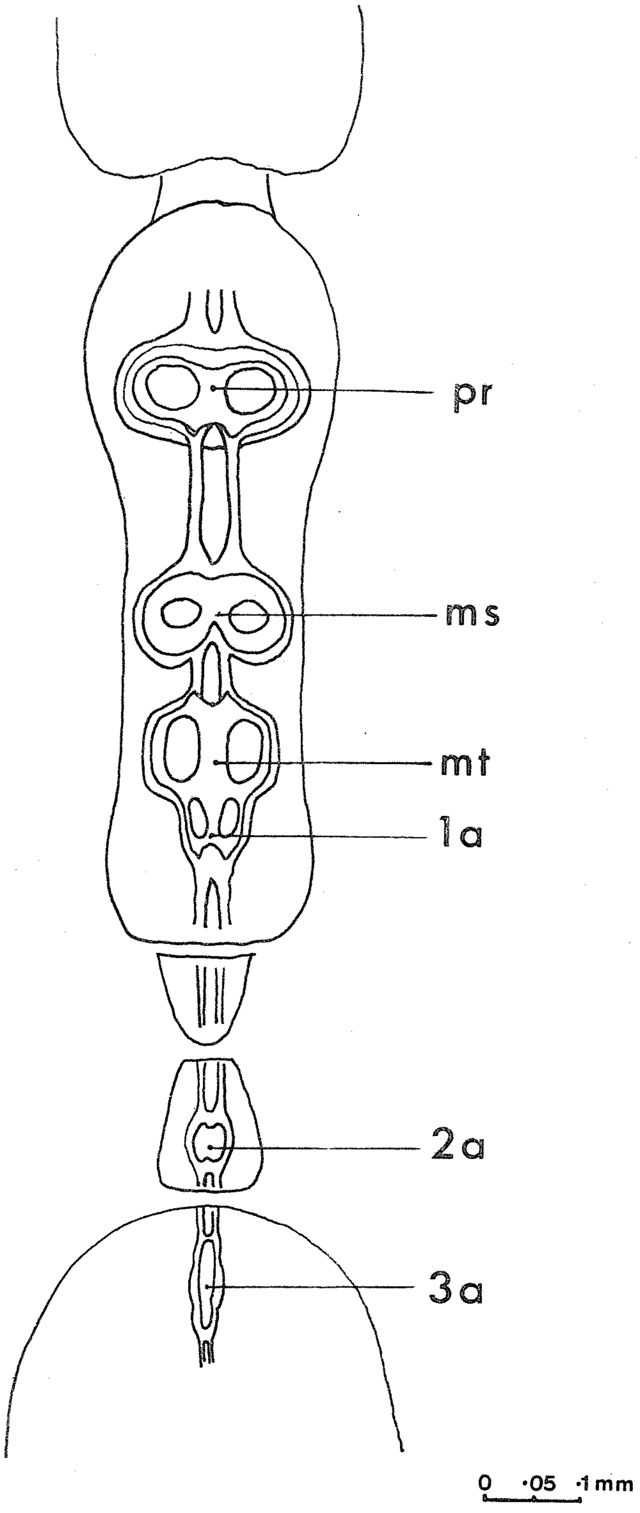


Fig 2.4. Dorsal view of thoracic and abdominal ganglia of M. pharaonis. (pr: prothoracic ganglion, ms: mesothoracic ganglion, mt: metathoracic ganglion, 1a: 1st abdominal ganglion, 2a: 2nd abdominal ganglion, 3a: 3rd abdominal ganglion).

CHAPTER 3

MORPHOLOGY OF THE EXOCRINE GLANDS AND ASSOCIATED STRUCTURES.

3.1 Introduction

Pheromones are usually emitted from specialized exocrine glands present in all parts of the body of an organism. The exocrine glands of the ants have some interest since at least one, the metapleural gland, is present only in this group. The morphology and histology of the glands has been well studied in some species, but in other species such as M. pharaonis, it has been completely overlooked.

The most important exocrine glands in M. pharaonis are the mandibular glands in the head, the metapleural glands in the thorax and the poison and Dufour's glands in the abdomen. Other glands that may play some role in communication are the pharyngeal and maxillary glands in the head, the labial glands of the thorax, the small glands of the 7th abdominal tergite and the glands of the hind gut. Since these glands have other functions apart from the possible function of communication, they will not be studied here.

3.2 Materials and methods

The techniques of dissecting and excising the glands, preparing whole mounts of the sting apparatus and fixing, sectioning and staining serial sections are discussed in Ch.10.

3.3 Mandibular glands

The mandibular glands (fig. 3.1A) consist of a reservoir and a mass of glandular cells (0.06 x 0.02 mm) situated near the base of the mandibles. A duct leads from the reservoir to the anterior mesal border of the preoral cavity. The whole structure of the glands does not differ significantly from the general structure of the glands of other ants (Blum & Hermann, 1978).

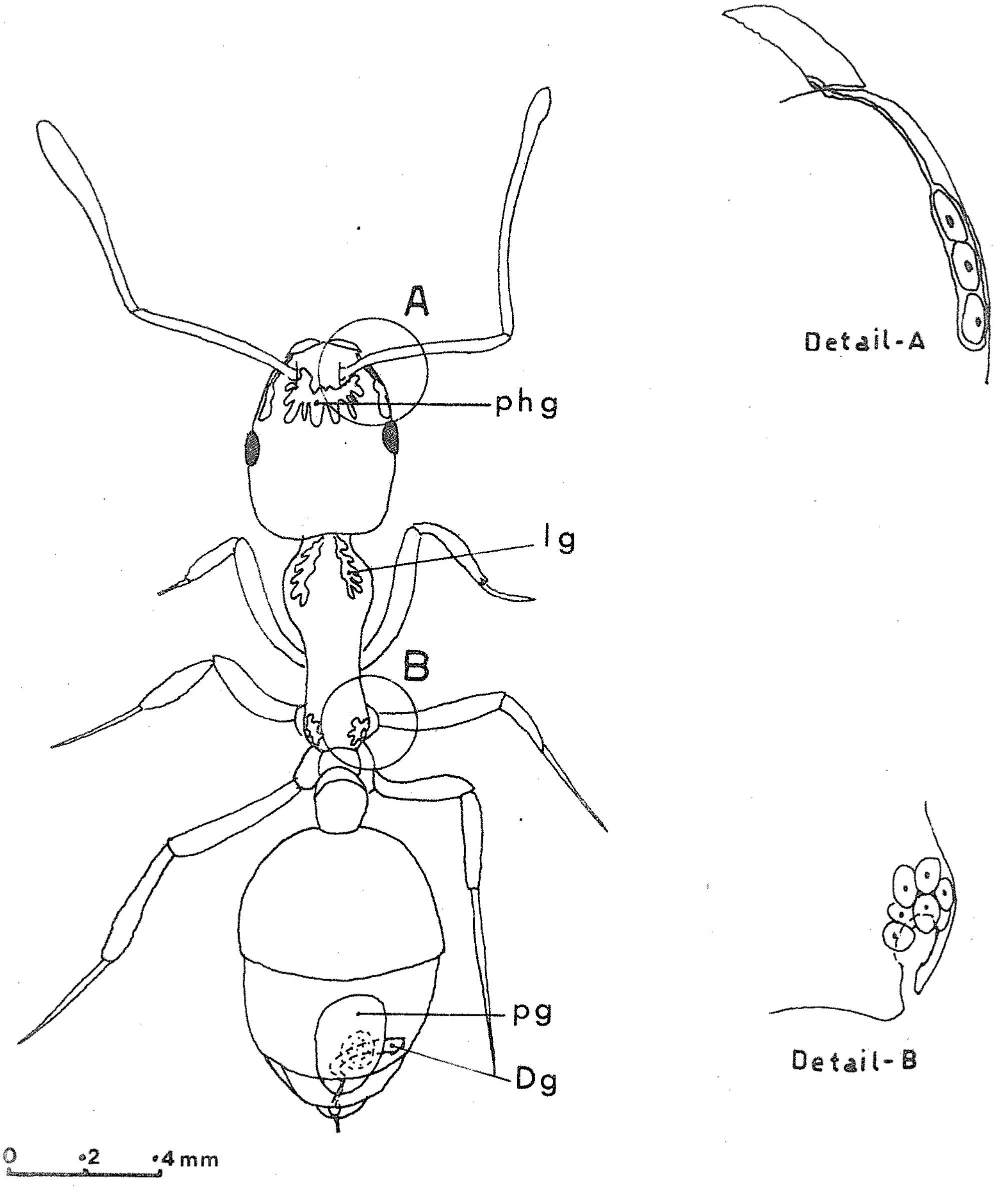


Fig 3.1. Exocrine glands of *M. pharaonis* (A: mandibular gland, B: metapleural gland, phg: pharyngeal gland, lg: labial glands, pg: poison glands, Dg: Dufour's gland).

3.4 Metapleural glands

The metapleural glands (fig. 3.1B) are a pair of multicellular structures (each 0.05 x 0.04 mm) situated at the lateral posterior part of the thorax. Each consists of a number of large secretory cells that open in a sclerotized chamber in the cuticle through a porous plate. There is no significant difference from the common structure observed in all ants. (Blum & Hermann, 1978).

3.5 Poison gland

The poison gland (fig. 3.2.& 3, pl 3.1:j) is situated in the posterior part of the abdomen, dorsal to the Dufour's gland, on the right side of the hind gut. It consists of a convoluted poison gland, a pair of free filaments, a poison reservoir or sac and the main duct of the sac.

a. Poison gland. (fig 3.2 & 3, pl 3.1:j). The convoluted poison gland is oval in shape, and is enclosed in the poison sac. Its distal end is situated near the region where the free filaments start. In the gland, there are many polygonal cells which end in small ducts opening in the poison sac.

b. Free filaments. (fig 3.2 & 3:k). A pair of filaments emerges from the poison sac at a point near the beginning of the main duct. The place where they emerge is slightly thickened. They usually curl towards the anterior part of the abdomen. The filaments arise from the sac individually and are not branched. They have a uniform diameter of approximately 0.005 mm and their length is 0.2 mm. The cells of the filaments are not distinguishable but a small duct running over the whole length can be discerned at 1000x magn.

c. Poison sac (fig. 3.2 & 3:i) The poison sac is subglobular in shape. It resembles the poison sac of Pogonomyrmex badius (Hermann & Blum, 1967) in that the sac is quite distinct from the main duct which is of uniform diameter, while in other species such as Paraponera clavata (Hermann & Blum, 1966), Solenopsis

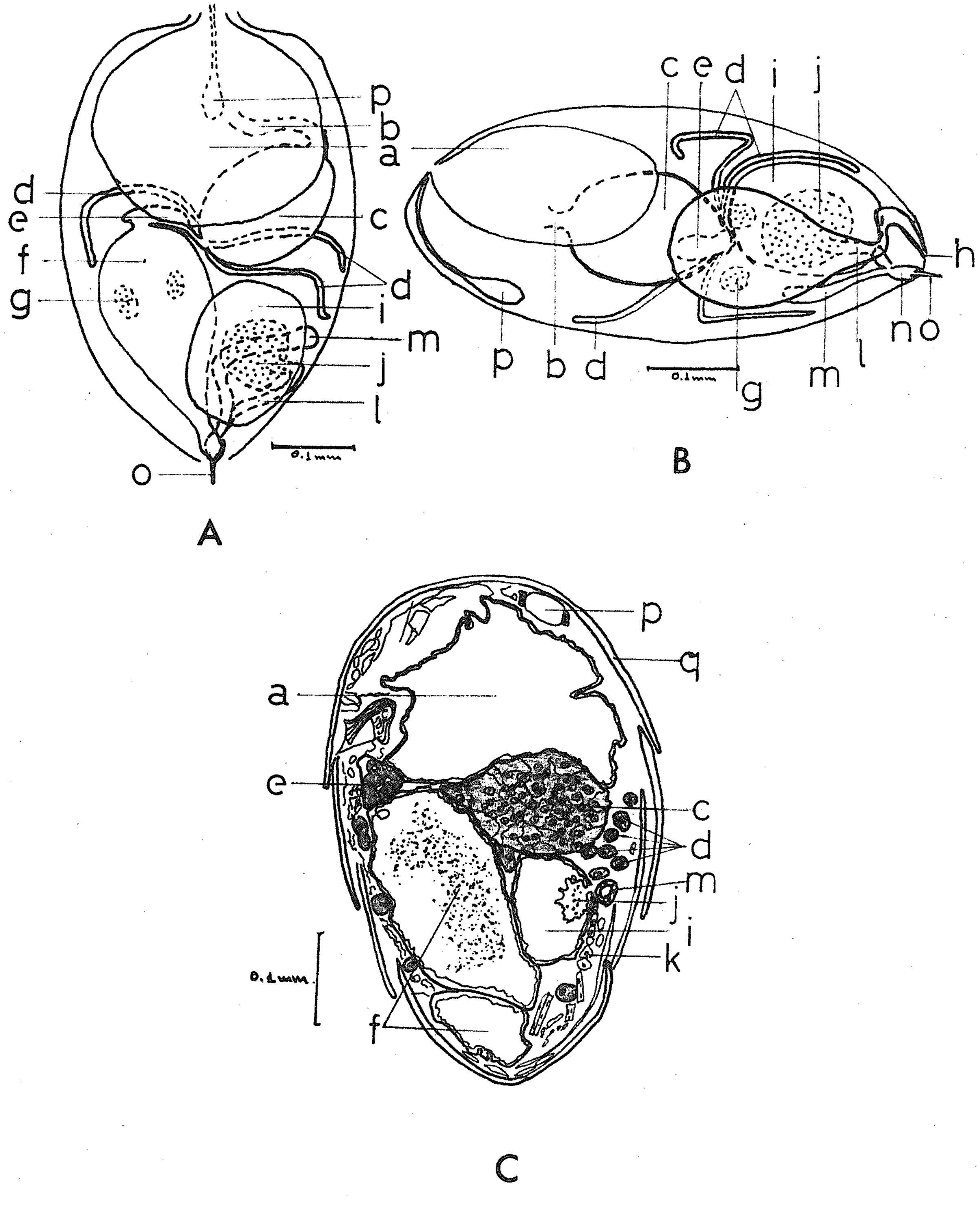


Fig 3.2: Internal morphology of the abdomen of M. pharaonis.
A: dorsal view, B: lateral view, C: horizontal section.
(a: crop, b; proventriculus, c: mid gut, d: malpighian tubules, e: ant. intestine f: hind gut, g: rectal papillae, h: anus, i: poison sac, j: poison gland, k: filaments, l: main duct, m: Dufour's gland, n: sting bulb, o: sting p: abd. ganglion).

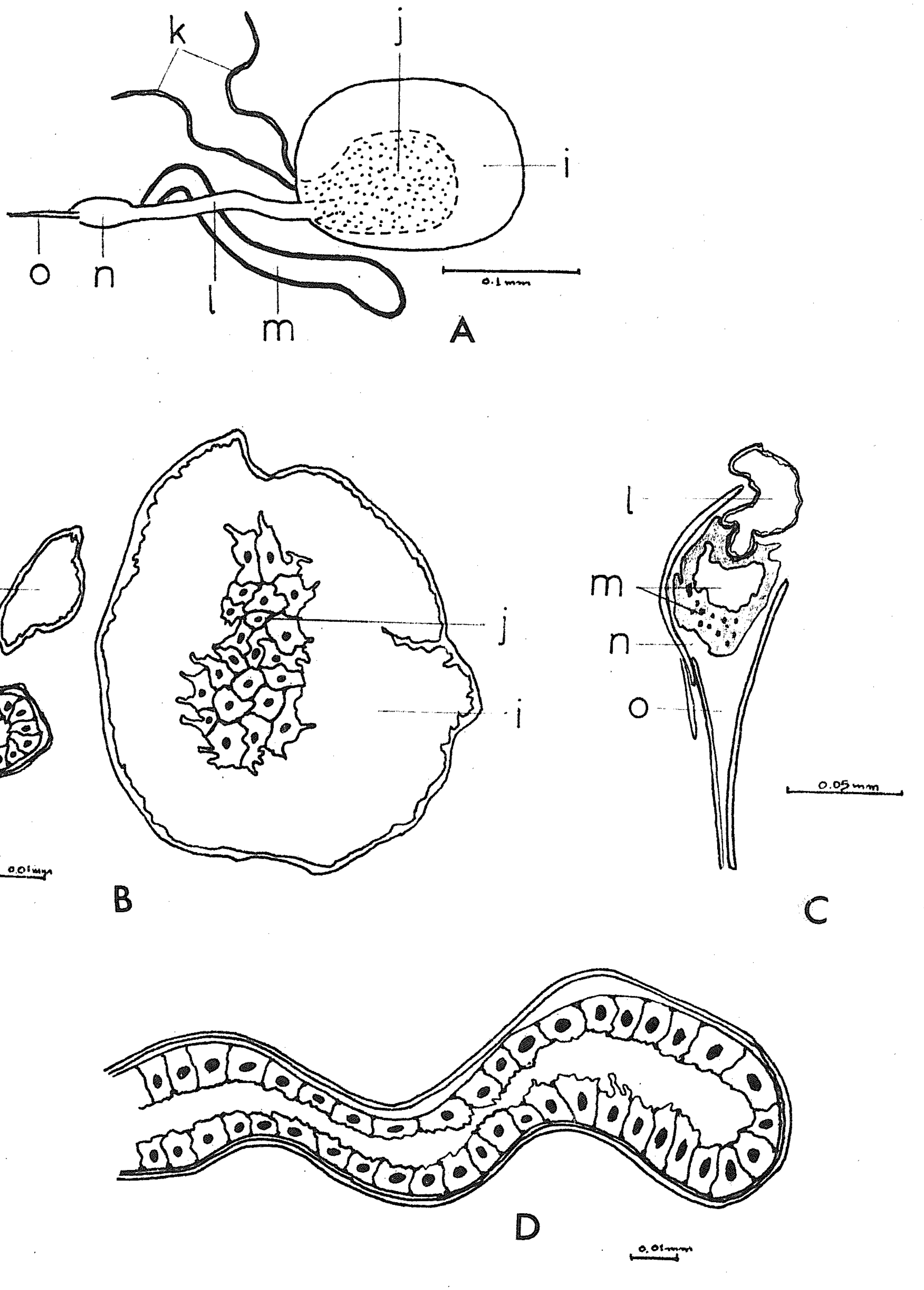
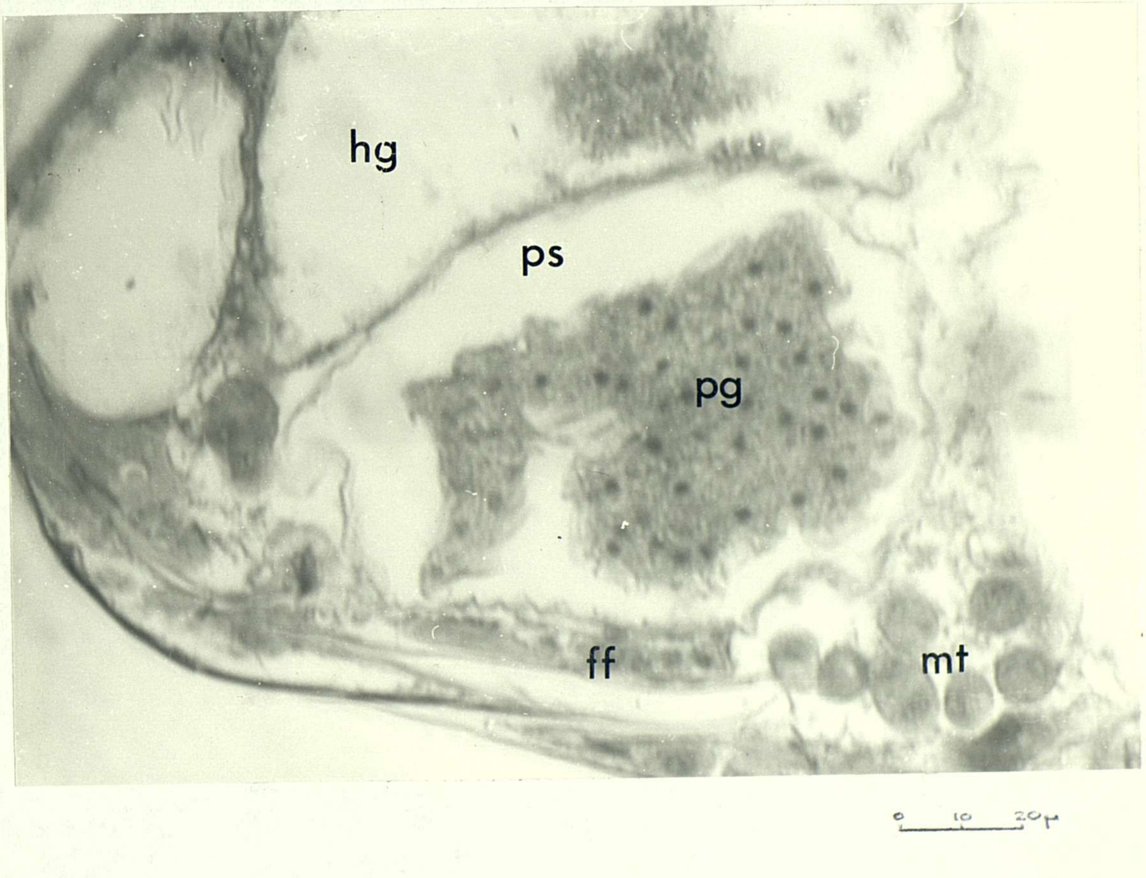


Fig 3.3. Poison apparatus of *M. pharaonis*. A: poison apparatus B. cross section of poison apparatus, C: cross section of sting area, D: cross section of Dufour's gland. (Indices as in fig. 3.2).

pl. 3.1. Longitudinal section of poison sac of M. pharaonis (ff: free filaments, hg: hind gut, mt: malphigian tubules, pg: poison gland, ps: poison sac).


hg
ps
pg
ff
mt
0 10 20µ

(Callahan, Blum & Walker, 1959) and *Pseudomyrmex pallidus* (Blum and Callahan, 1963) the sac is elongate and tapers gradually towards the main duct. The sac has a diameter of approximately 0.15 - 0.2 mm when full, and a volume of 10^{-3} µl. The wall of the sac is composed of achitinous tunica propria and an epithelium whose cells cannot be distinguished. No muscle layer was observed.

d. Main duct (fig 3.2 & 3:1). The main duct of the poison sac is a tube of uniform diameter (0.01 mm) that connects the poison sac and the sting bulb. In the sting bulb,it is found dorsal to the duct from the Dufour's gland. The overall length is approximately 0.2 mm. The walls are similar to the wall of the poison sac with a chitinous outer shell and an epithelium inside.

3.6 Dufour's gland

The Dufour's gland (fig. 3.2 &3:m) is an elongated structure 0.15 mm long, sometimes reaching beyond the poison sac. Its diameter (0.05 mm) decreases gradually towards the junction with the sting bulb. The gland has a thick wall composed of cuboidal cells. It lies ventral to the poison sac and enters the sting bulb at the same point as the main duct of the poison sac.

3.7 Skeletal components of the sting

The abdomen of *M. pharaonis*, as in all ants, consists of 10 segments. One is thoracic, two are petiolar and four are gastral. The last three (8th, 9th and 10th) are concealed within the gastral segments. The skeletal components of the sting consist of the remnants of the 8th and 9th segments and their gonapophyses.

The pair of spiracular plates (8th hemitergites)(fig. 3.4 sp) originate from the 8th tergum. They are the last sclerites. They are closely attached to the exoskeleton of the abdomen. They are triangular in shape and their height from base to top is 0.06 mm.

The pair of quadrate plates (9th hemitergites)(fig. 3.4 qp) originate from the 9th tergum. They are associated with the spiracular plates but they are a more integral part of the sting apparatus. They lie inside the spiracular plates, dorsal and lateral to the rest of the sclerites and are connected by a sclerotized bridge, the anal arc (fig 3.4 aa). The arc bears a structure with numerous setae which may be of sensory nature. The quadrate plates are trapezoidal with approximate dimensions 0.02 x 0.06 mm.

At their proximal end, the quadrate plates are articulated to the pair of triangular plates (1st valvifer)(fig 3.4 tp, fig. 3.5) that originate from the 8th sternite. The triangular plates are triangular in shape measuring 0.02 mm at their base. One of their apodemes extends into the long and thin 1st ramus (fig 3.4 lr) which in turn is connected to the lancets (fig 3.5 l) or stylets (1st valvulae) that are the most protruding parts of the sting. The lancets originate from the genapophyses of the 8th sternite. They are a pair of thin structures approximately 0.15 mm long, forming part of the poison canal through which the secretions of the poison and Dufour's gland flow. Throughout their length, each lancet bears a pair of ridges on their dorsal side. At the proximal end, near the connection with the 1st ramus, each lancet bears a valvular lobe or valve (figs 3.4, 3.5 u). These valves function to pump the secretions out of the glands as the lancets move backwards and forwards.

At the distal end, the lancets bear a number of very indistinct barbs. The barbs were originally used for cutting into wounds but since the sting of *M. pharaonis* is no longer used this way, the barbs have almost disappeared.

The ventral apodeme of the triangular plates, articulates with the pair of oblong plates (2nd valvifer) (figs. 3.4, 3.5 op). They are the lateral remnants of the 9th sternite. They are approximately 0.1 mm in length and their dorsal border is highly sclerotized. At the distal end, they bear a pair of elongate structures, the gonostyli (3rd valvulae) (fig. 3.4 g) that represent the gonapophyses of the 9th sternite. The gonostyli bear a number of setae and are presumably used both as sensory organs in mechanoreception and for protection of the sting. They are hollow and concave posteriorly and form a dorsal and lateral sheath for the sting when it is contracted. Apart from the larger setae, they bear smaller bristles that project from the ventral and mesal borders and may also be used as sensory organs.

At the proximal end, at the point of articulation with the triangular plates, the oblong plates bear 3 sensory pegs that may function as mechanoreceptors, (fig 3.5). The oblong plates continue into the 2nd rami (fig 3.4, 2r) that run next to the 1st rami and are connected to the base of the sting bulb near the point where the fulcral arms arise. The 2nd rami bear 4 sensory pegs on their posterior surface and a number of other sensory pegs on their lower part near the point where they attach to the sting bulb.

The sting bulb is a structure that originates from the 9th sternite and is 0.1 mm long. At its anterior end, it bears a small sclerite, the furcula (figs. 3.4, 3.5 f) which is responsible for the control of the movements of the sting. It is V shaped and large muscles are attached to it.

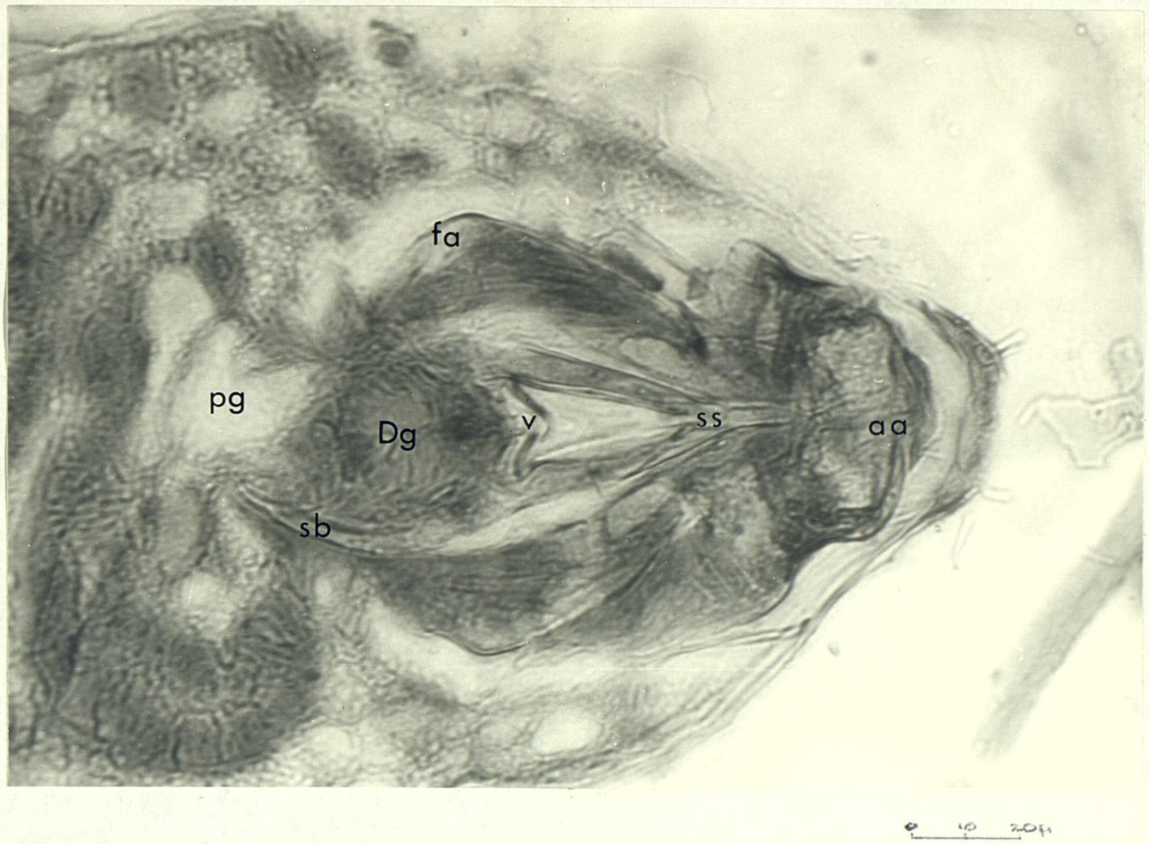
At the base of the sting bulb, two fulcral arms (fig 3.4 fa) arise. They originate from the 8th sternite and are joined to the bulb by membranous connections. They are triangular in shape and only their anterior border is sclerotized.

The sting bulb continues into the sting shaft (2nd valvula)(figs. 3.4, 3.5 ss) which together with the lancets comprises the external part of the sting. It represents the gonapophyses of the 9th tergite. It consists of a fused pair of sclerites forming a concave sheath over the lancets which are at the ventral side. The shaft has a ridge on each of its ventromesal borders that articulates with a groove in the dorso lateral border of the lancet and functions as a guide on which the lancets move back and forth. The sting shaft and the lancets form the cavity through which the secretions of the poison and Dufour's glands flow.

3.8 Muscles associated with the glands

Two muscles are important in the control of the emission of pl. 3.2. Longitudinal section of sting apparatus of *M. pharaonis* (aa: anal arc, Dg: Dufour's gland, fa: fulcral arm, pg: poison gland duct, sb: sting bulb, ss: sting shaft, v: valves).

pl. 3.3. Transverse section of sting bulb of *M. pharaonis* (Dg: Dufour's gland, m: muscles controlling emission, pg: poison gland duct)


fa
pg
Dg
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aa
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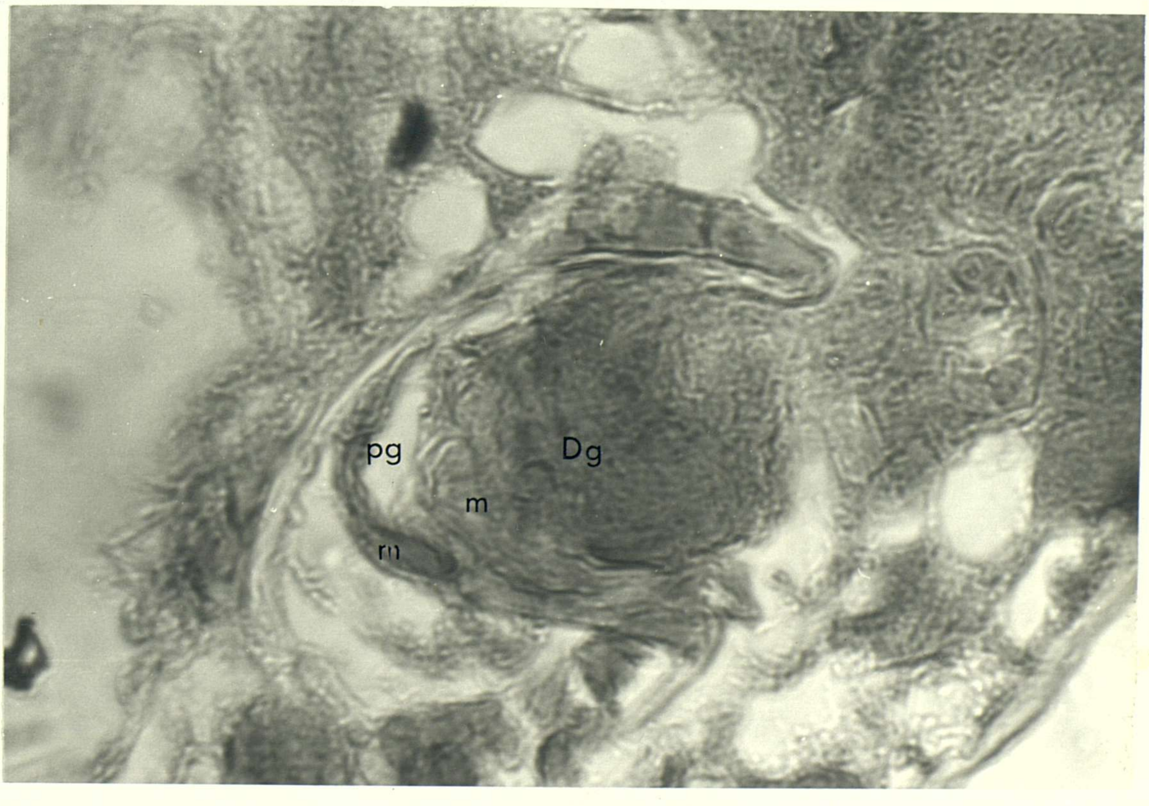
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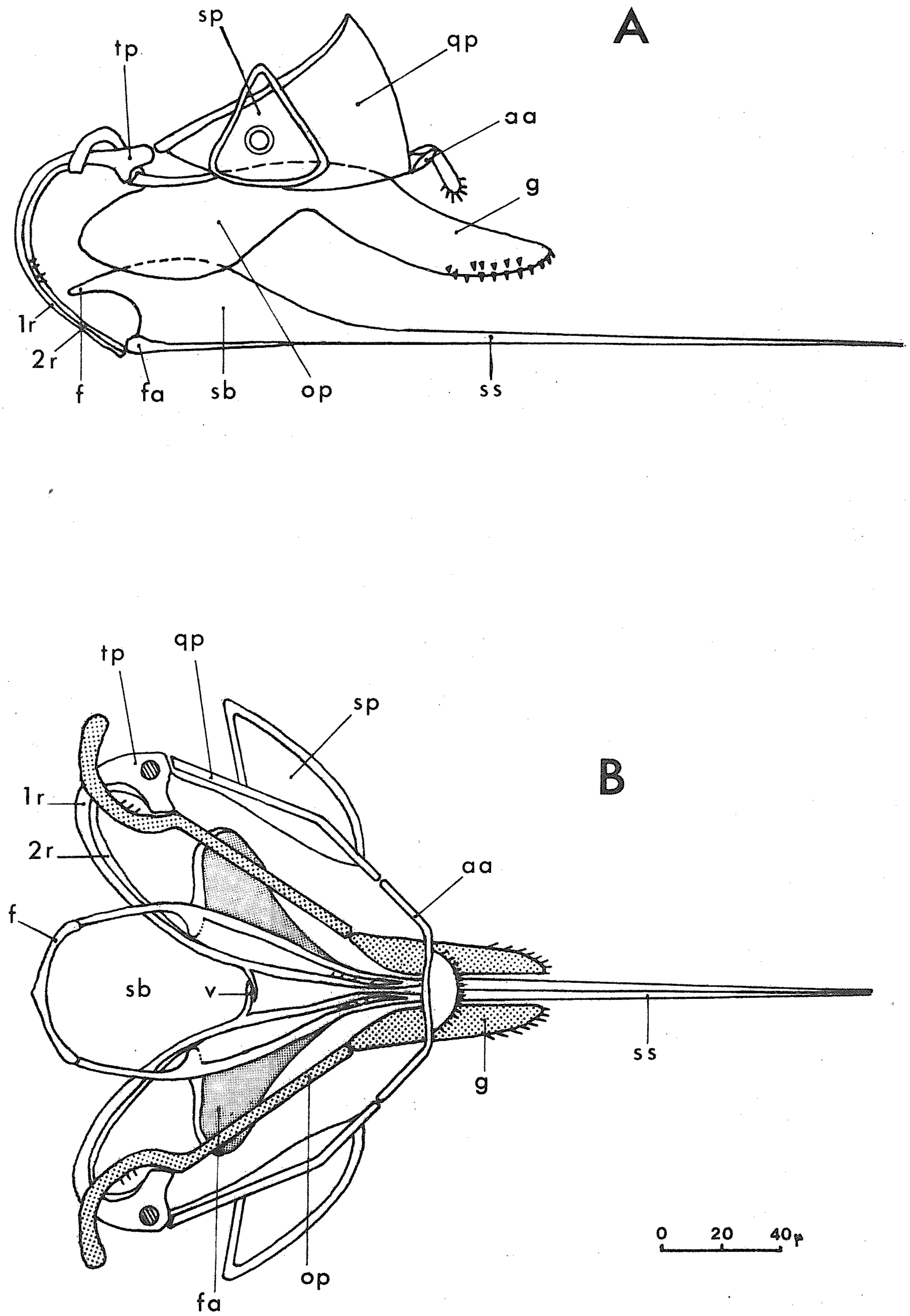


Fig 3.4. Sting apparatus of M. pharaonis. A: lateral view, B: dorsal view (aa: anal arc, f: furcula, fa: fulcral arm, g: gonostyli, op: oblong plate, qp: quadrate plate, 1r: 1st ramus, 2r: 2nd ramus, sb: sting bulb, sp: spiracular plate, ss: sting shaft, tp: triangular plate, v: valve).

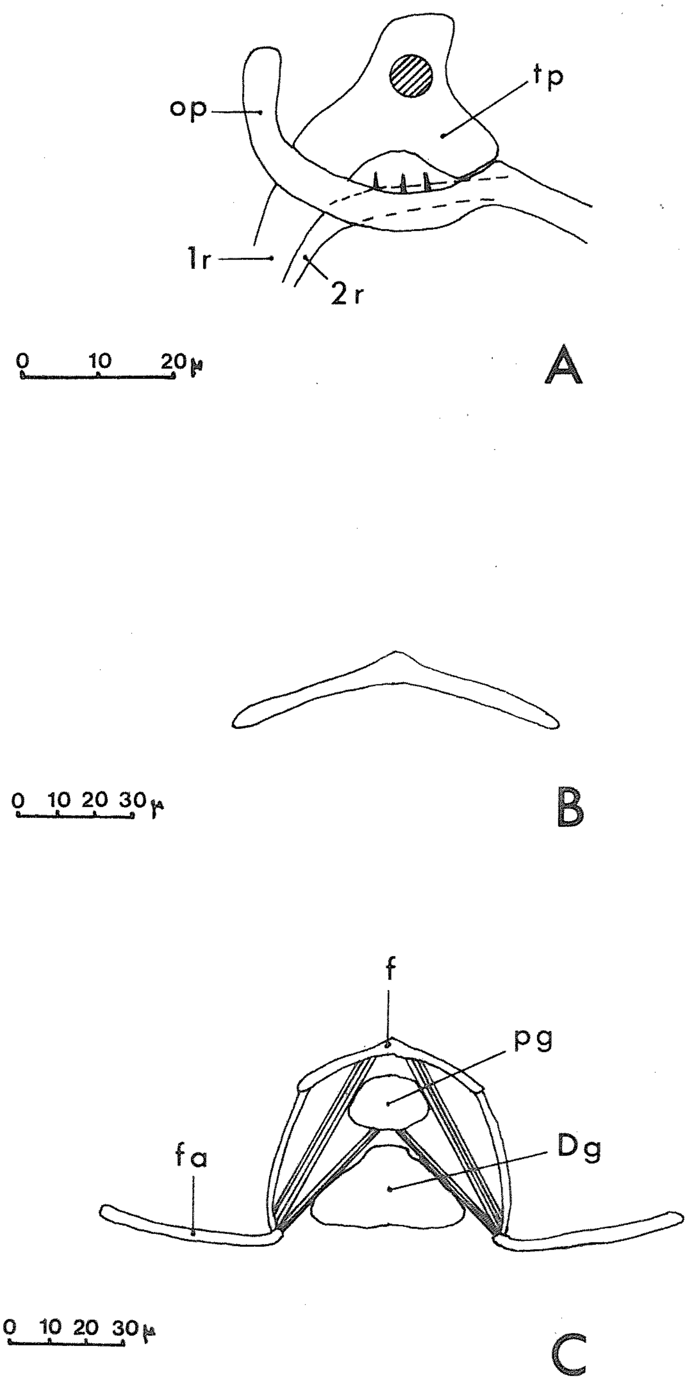


Fig 3.5. Details of the sting apparatus. A:lateral view of area of connection between triangular and oblong plates, B:furcula, C:muscles associated with the control of emission of pheromones. (Dg: Dufour's gland, f:furcula, fa:fulcral arm, op: oblong plate, pg: poison gland, 1r: 1st ramus, 2r: 2nd ramus, tp: triangular plate).

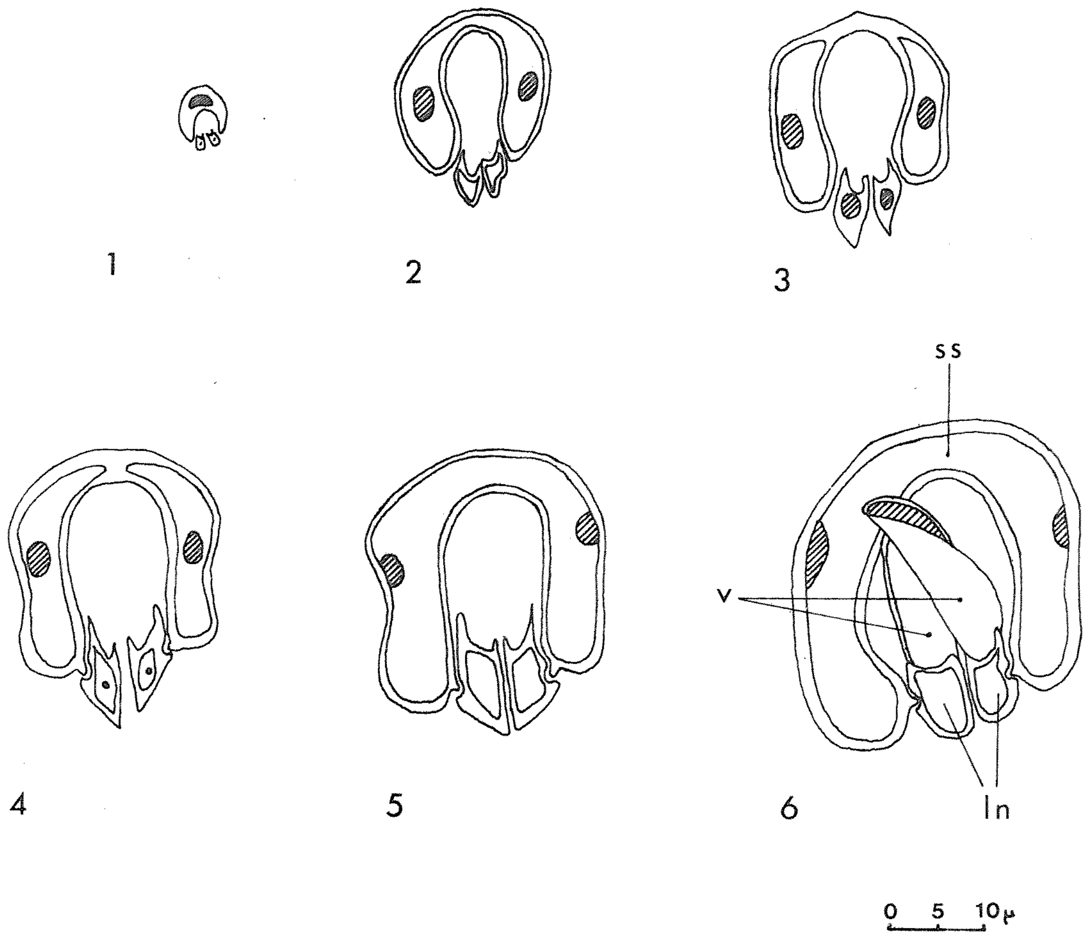


Fig 3.6. Serial transverse sections of sting shaft and lancets from the tip of the sting (1), to the area of the valves (6). (In: lancets, ss: sting shaft, v: valves).

the contents of the poison and Dufour's gland (fig 3.5). One muscle originates from the mesal and ventral border of one fulcral arm, passes dorsal to the duct of the poison sac and connects with the fulcral arm of the other side. The other muscle extends from the ventral mesal border of one fulcral arm, attaches to the ventral wall of the poison duct and again connects with the other fulcral arm. The first muscle, when active, keeps the poison duct hermetically closed. The other opens the duct when the gland is activated. It also controls the opening and closing of the Dufour's gland. The co-ordinated action of these two muscles ensures that the correct proportion of poison and Dufour's gland's contents are emitted.

3.9 Discussion

The structure of the whole sting apparatus of M. pharaonis suggests an ant that does not use its sting for defence, but only for pheromone emission. The interesting points are a) the small size of the furcula. The furcula is essential in the control of movements of the sting and the presence of large furculas in the Ponerinae and Dorylinae is associated with its use as defensive weapon and in stinging. The myrmicine ants that have lost the ability to sting such as Solenopsis and Crematogaster usually have small and weak furculas. b) the almost complete absence of barbs on the lancets. Barbs are often associated with stinging and the infliction of wounds. The absence of barbs and the presence sometimes of spatulate lancets is associated with the use of the sting as an organ for pheromone emission.

CHAPTER 4

CHEMICAL ANALYSIS OF THE ANT AND ITS SECRETIONS

4.1. Introduction

After the description of the glands that are responsible for the emission of pheromones, the chemical analysis of the volatile components of the ant is the next step towards the analysis of communication.

As the previous chapter has shown, the main areas of pheromone production are in the head, the thorax and the abdomen. Chemical analysis of these parts was carried out separately by gas liquid chromatography. The glands that could be excised were also analysed chromatographically.

Three of the various secretions of the ant were also analysed chemically: the trail, a secretion used in defence against intruders in the nest which will be termed the "defensive secretion" and a secretion that causes alarm which will be termed the "alarm secretion". All three were employed very frequently by the ants and had very immediate and sometimes long lasting effects. The behaviour patterns that accompany these secretions will be described in the following chapter.

Finally, the four geometric isomers of one of the major volatile components of the abdomen, monomorine I, were gas chromotographed in order to compare their retention times with the retention time of the naturally occuring monomorine I and to identify which of the four isomers is the naturally occurring one.

4.2 Chemical analysis of the ant

4.2.1. Materials and methods

The heads, thoraces and abdomens of a number of *M. pharaonis* workers were separated and placed inside small glass capillary tubes which were then sealed from both sides. Individual glands were excised from the workers and placed on small pieces of filter paper which were then placed inside the small glass capillary tubes and sealed. The tubes were placed inside a solid sample injection apparatus, secured at the open end of a 5% OV-101/CLQ $\frac{1}{8}$ inch gas chromatographic column and heated up to approximately 170°C by a standard injection port heater. After 5 min. of heating, the tube was crushed and the volatile chemicals were taken up by the carrier gas.(Morgan & Wadhams, 1972).Relative percentages of compounds were determined by calculating the areas under each peak.

4.2.2. Results

i. Abdomens

The gas chromatogram of the abdomens (fig.4.1) showed 17 peaks. Four of these peaks are most prominent (no. 1, 3, 12 and 16). They comprise respectively 12%, 3%, 75% and 5% of the total volatile components of the abdomen. The percentage of peak 10 fluctuates from almost zero up to 1%.

The peaks in the beginning of the chromatogram also fluctuate. It has not been possible to assign a role to them. They may be products of metabolism, components of pheromones or contaminants from other parts of the body.

The retention times of peaks 1, 3 and 12 correspond to the retention times of compounds MP.1, MP 2-1 and MP 3-1 (Talman et al, 1975) which were named monomorine I, II and III respectively. Compound 16 was named (Ritter et al, 1975) monomorene.

ii. Thoraces

The retention times of the peaks of the volatile compounds of the thorax were similar to those of the abdomen (fig. 4.2). However, the relative percentages

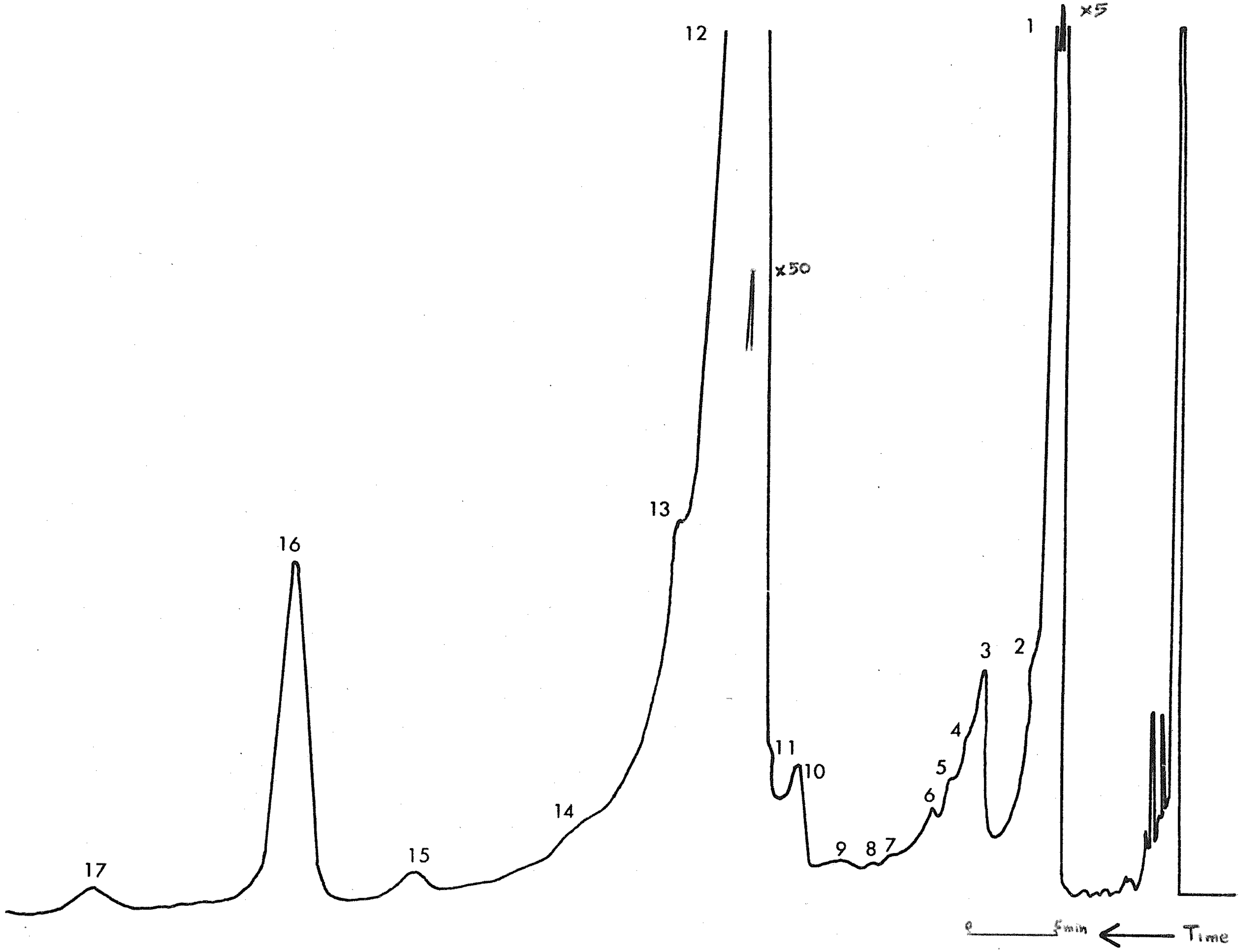


Fig. 4.1. Chromatogram of the abdomen of *M. pharaonis*. 5% OV-101/CLQ at 170°C.

were different. Peak 16 did not appear at all, peaks 1 and 3 were much lower, 2.5% and 3.3% respectively, peak 12 was approximately at the same level, 80% and only peak 10 was considerably higher up to 5%. There was also a number of peaks in the beginning of the chromatogram whose presence again cannot be accounted for. Also, the total amount of volatiles was much less than in the abdomen, approximately 15%.The fact that the proportion of peak 12 to peak 1 is larger than in the abdomen may be due to a metapleural gland secretion that has the same retention time as peak 12 and is therefore masked by it.

iii. Heads

The total amount of volatiles in the head is much less than in both the thorax and the abdomen (fig. 4.3). It is 41% of the total in the thorax and only 6% of the total in the abdomen. There is also one new peak which represents 4.5% of the total in the head and which is not present in the chromatograms of the other parts of the body. It is possible that this compound originates from the mandibular glands of the ant. A very large proportion, 57%, is taken up by the very volatile compounds observed in the beginning of the chromatogram.

In general, 5% of the volatiles can be found on or in the head, 12% can be found on or in the thorax and 83% on or in the abdomen. Because some of the compounds found on the head and the thorax may be contaminants from abdominal secretions, it is calculated that at least 95% of all volatiles originate in the abdomen.

iv. Poison gland

The chromatogram of the poison gland reveals the presence of 12 of the 17 volatile compounds that were observed in the abdomen (fig. 4.4). Compound 1 amounts to approximately 12% and compound 12 approximately 80% of the total volatiles. Their relative ratios are comparable to the ratios observed in the abdomen. Here again, the amount of compound 10 fluctuates up to 3% of the total in the gland. The peaks of the early volatiles were again observed.

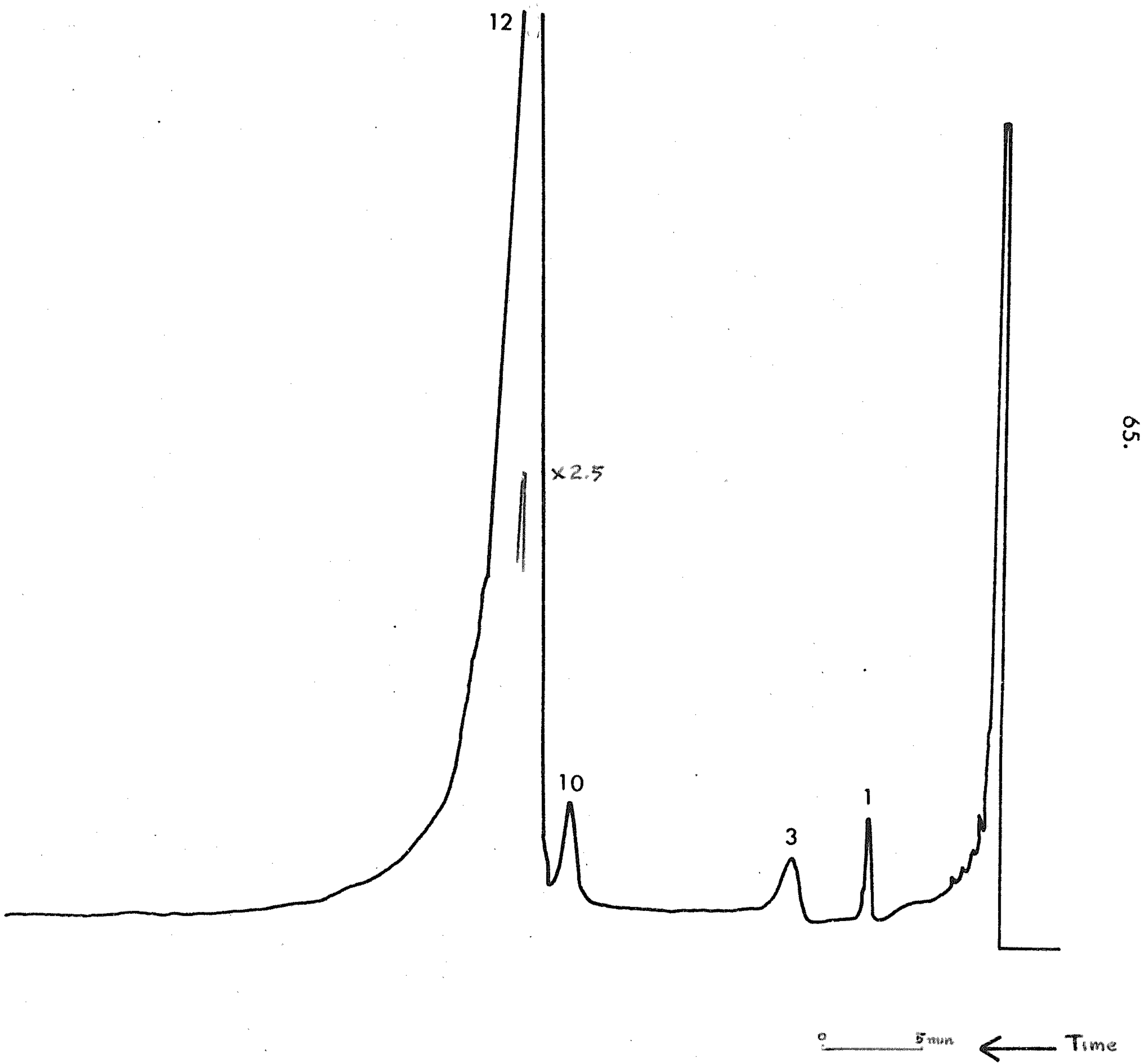


Fig. 4.2. Chromatogram of the thorax of M. pharaonis. 5% OV-101/CLQ at 170°C.

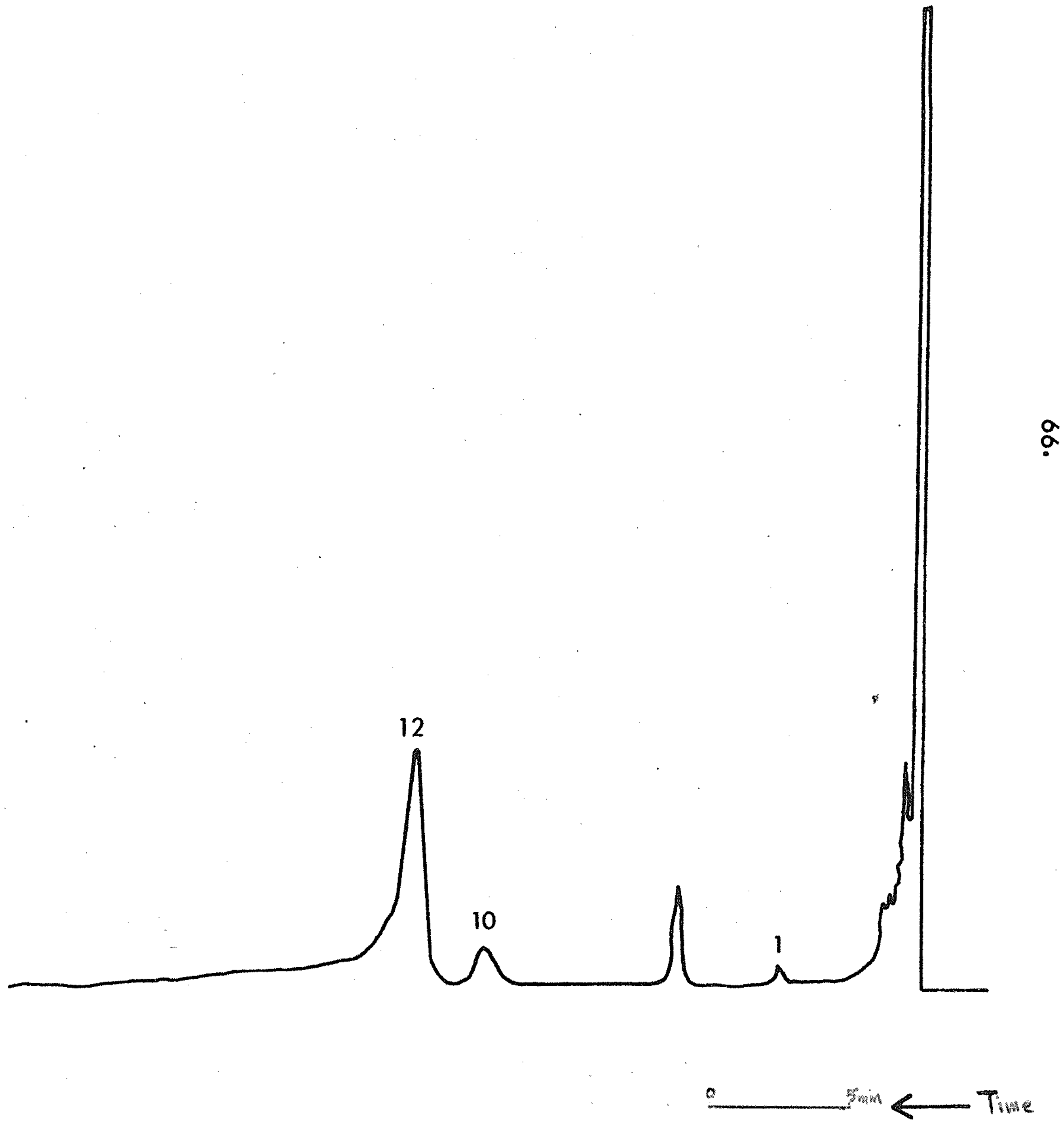


Fig. 4.3. Chromatogram of the head of M. pharaonis. 5% OV-101/CLQ at 170°C.

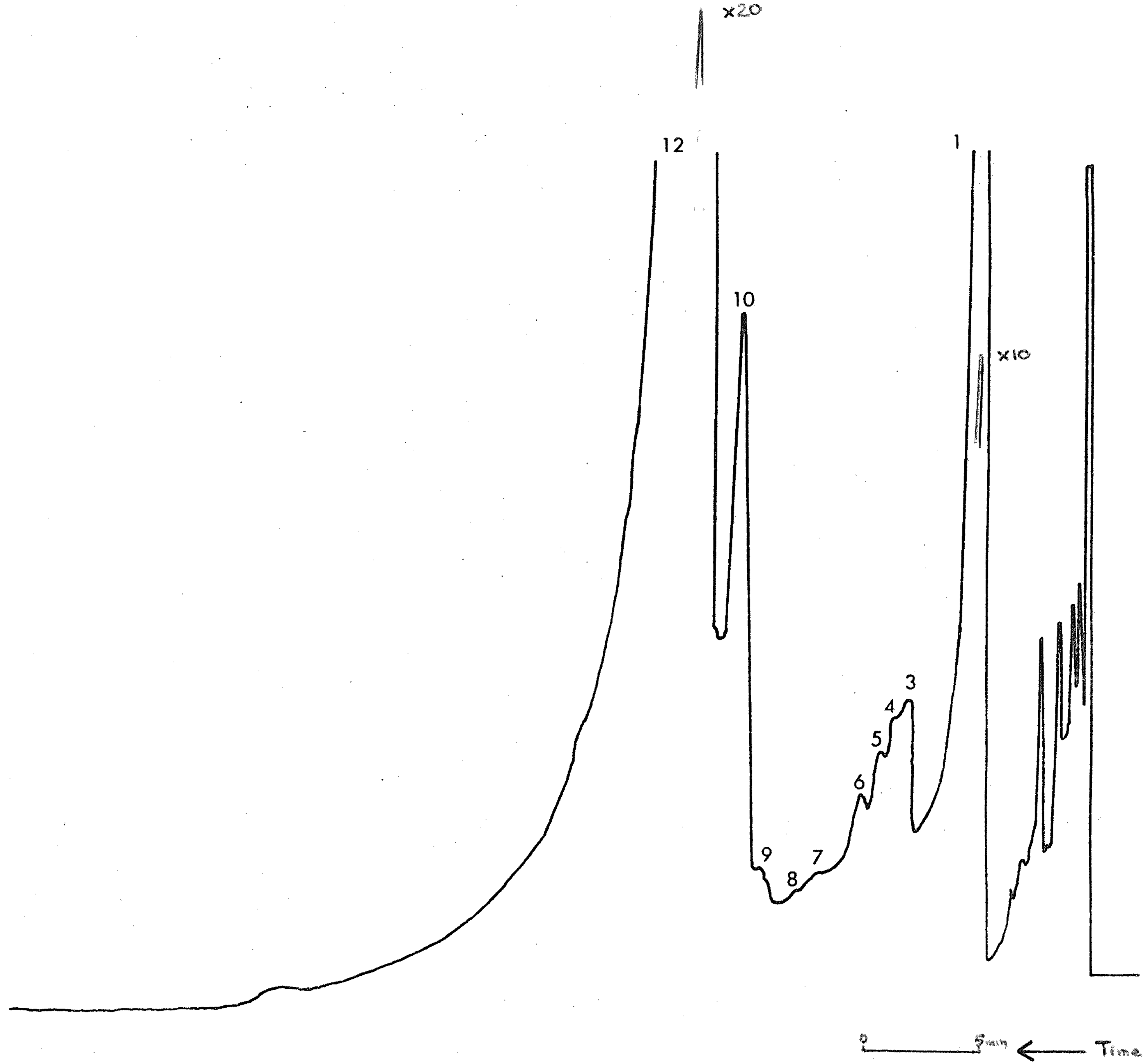


Fig. 4.4. Chromatogram of the poison gland of **M. pharaonis**. 5% OV-101/CLQ at 170°C.

v. Dufour's gland

The Dufour's gland contains the 5 remaining compounds, 13-17 (fig. 4.5). Their retention times in the column are longer than those of the compounds in the poison gland. There is a very large amount (70%) of unidentified very volatile compounds. Of the remainder, compound 16 comprises 80%, compound 13, 7% and compounds 15 and 17, 4% each.

4.2.3. Conclusion

The chemical analysis has shown that the majority of volatile compounds that could be used as pheromones are present in the abdomen of the ant. The mandibular glands may secrete a volatile compound, and there is a possibility for the presence of a metapleural gland volatile secretion. In the abdomen, the poison gland contains the majority of volatiles, including the pheromones so far identified, monomorine I, II and III. Dufour's gland contains a smaller amount of volatiles including the unsaturated hydrocarbon monomorene.

4.3. Chemical analysis of secretions

4.3.1. Materials and methods

i. Trail: A narrow plastic bridge was established between a nest area and a foraging arena of the ants where a food source had been placed. As soon as the trail was established, pieces of filter paper with a diameter of 1 mm, were placed at various points in such a way that the trail would pass over them. The ants were left lying on the trail for approximately 1 h. The pieces of filter paper were then removed and placed inside small glass capillary tubes which were sealed and inserted in a solid sample injection apparatus, connected to a 5% OV-101/CLQ $\frac{1}{8}$ inch GLC column as mentioned above.

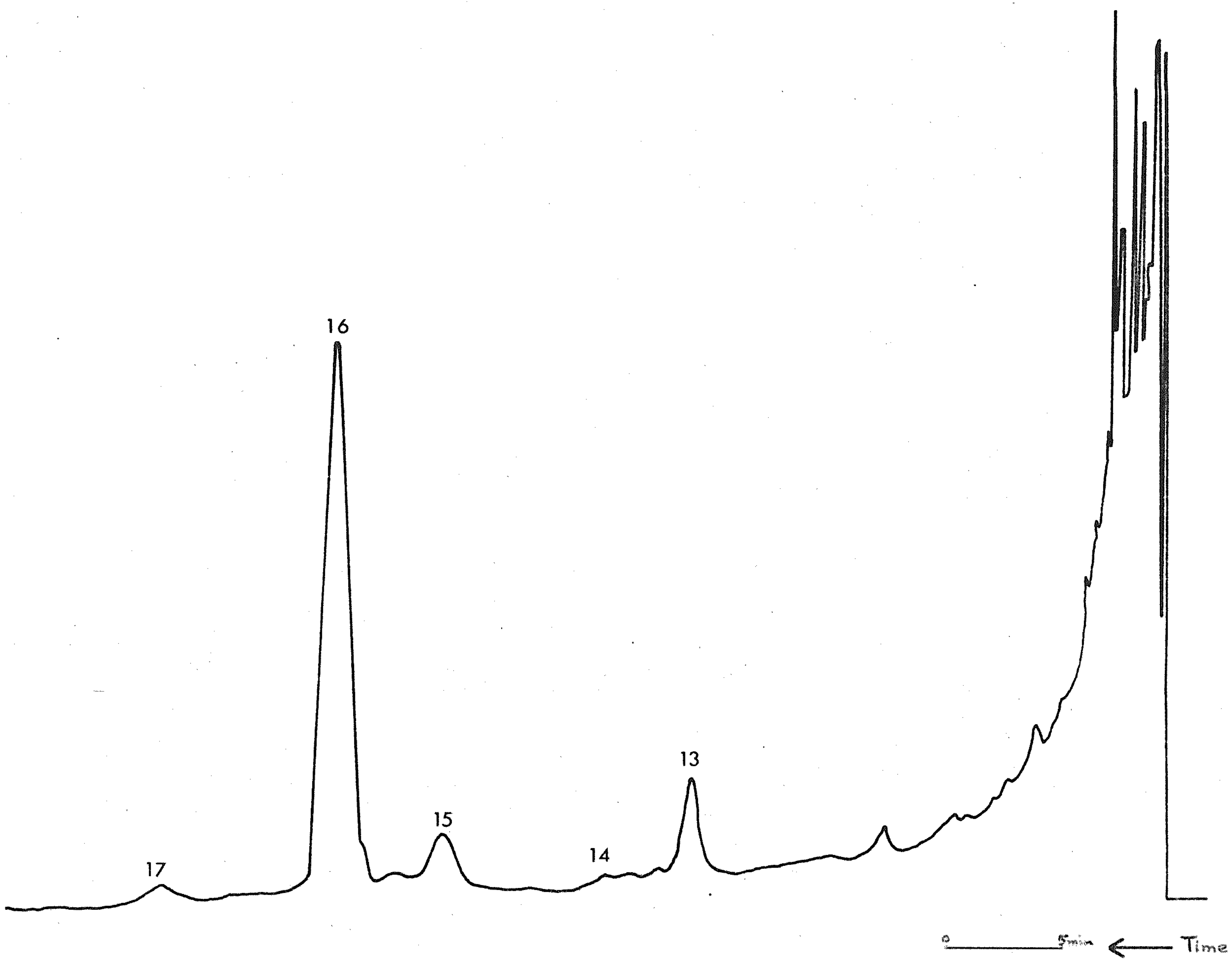


Fig. 4.5. Chromatogram of the Dufour's gland of M. pharaonis. 5% OV-101/CLQ at 170°C.

ii. Defensive secretions: A metal pin was inserted in the entrance of a nest and moved back and forth a few times. When the ants adopted the position described in the following chapter, they secreted a droplet of liquid from their sting. Two hundred and fifty such droplets were collected on a very small piece of filter paper, which was then placed inside a small glass capillary tube and the same procedure for solid sample gas chromatography as described above, was followed.

iii. Alarm secretion: When heavily disturbed near the nest, some ants adopted the characteristic position described in the following chapter and secreted a droplet of liquid. Again 250 such droplets were collected on a piece of filter paper and gas chromatographed with the aid of a solid sample injection apparatus.

4.3.2. Results.

i. Trail

The trail of M. pharaonis contains several compounds from the poison gland and several others from Dufour's gland (fig. 4.6). Compounds 1 (monomorine I) and 12 (monomorine III) from the poison gland were present in the highest quantities comprising about 40% and 20% of the secretions respectively, and compounds 14 and 17 from Dufour's gland comprised approximately 8% of the total amount of volatiles. When these results are compared with the results from the poison and Dufour's glands, it is noted that monomorine I and compound 17 are present in relatively higherquantity in the trail. Also, compound 16 of Dufour's gland, monomorene, which represents 80% of the volatiles in the gland, is relatively lower in the trail.

Taking into account the volatility of these substances it is calculated that the ratio of Dufour's gland secretion over poison gland secretion in the trail is in the

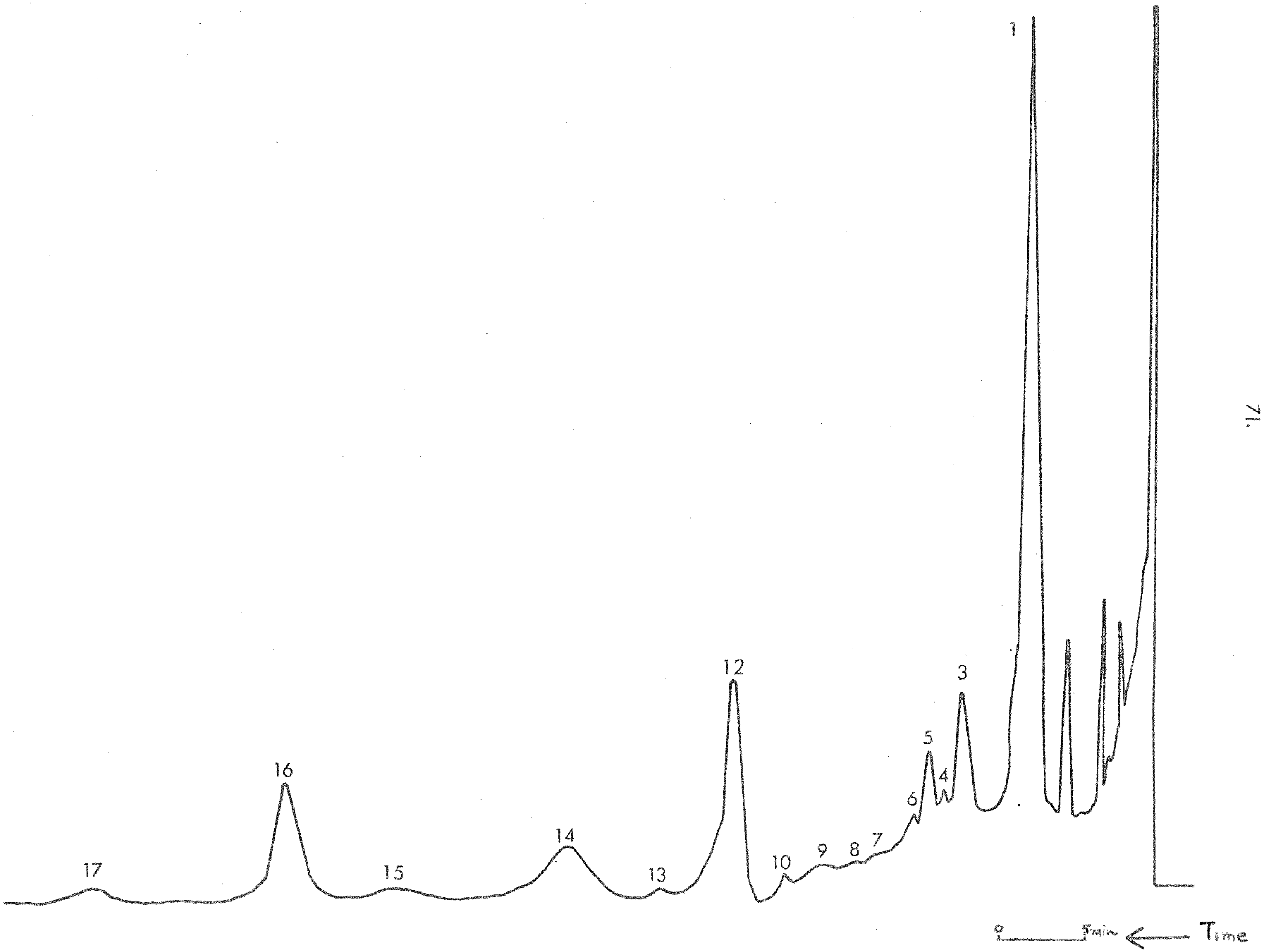


Fig. 4.6. Chromatogram of the trail of M. pharaonis. 5% OV-101/CLQ at 170°C.

range of 15:1. The least volatile compounds seem to be monomorine I and compound 17. They possibly act as long-term trail pheromones, while the other compounds such as monomorine III and monomorene function as short-term trail pheromones.

ii. Defensive secretion

The components of the defensive secretion of *M. pharaonis* are very similar to the components of the poison gland (fig. 4.7). Present are 12 of the 17 compounds of the abdomen, the same as those in the poison gland. Their relative quantities are also similar. Monomorine III comprises approximately 80% of the volatiles while monomorine I is 10%. Compound 10 is approximately 5% of the total volatiles.

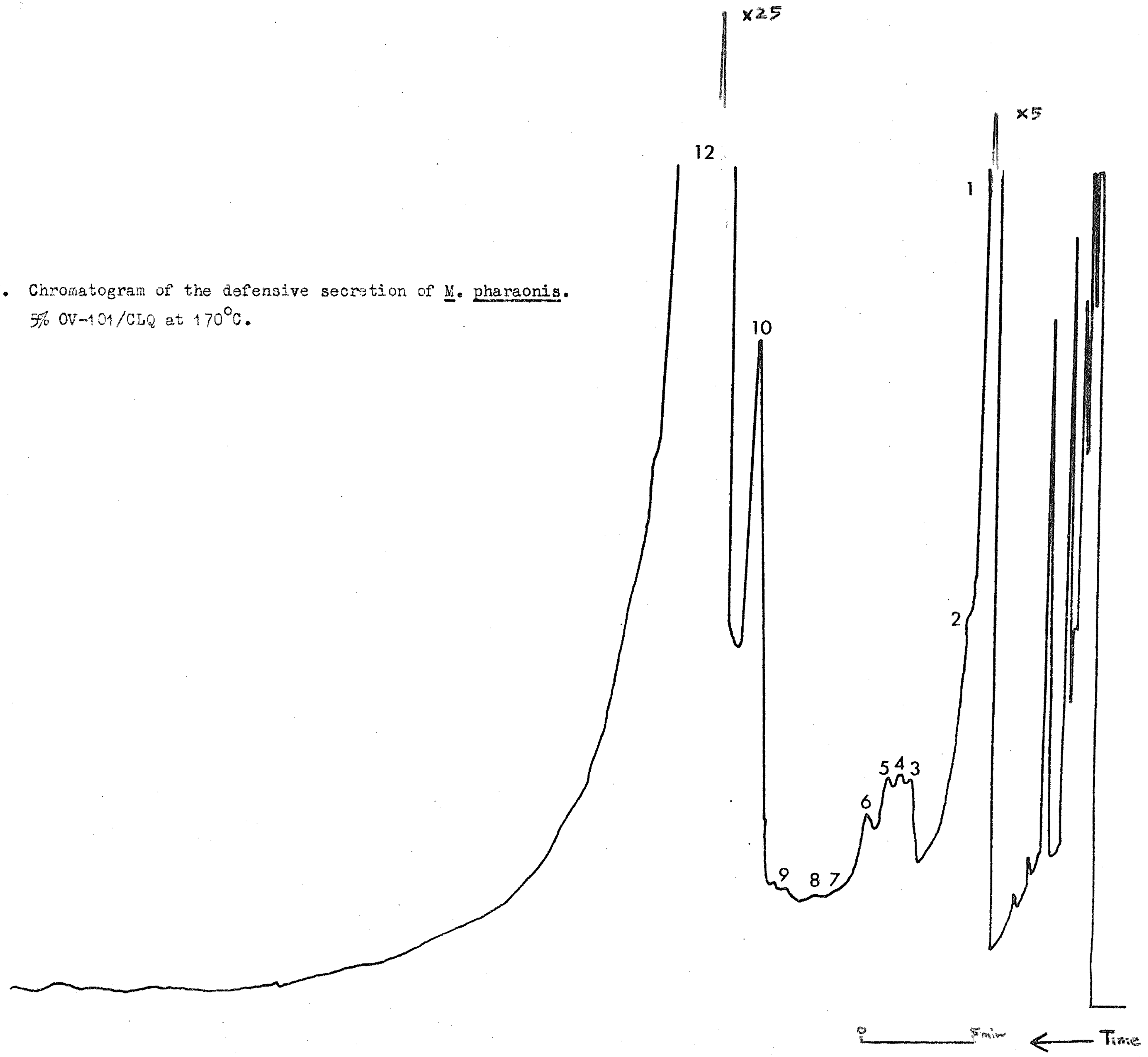
iii. Alarm secretion

The components of the alarm secretion are exactly the same as those of the defensive secretion and the poison gland (fig. 4.8). There are again 12 peaks in the chromatogram and the relative quantities are similar. The difference between the alarm and the defensive secretion is the total amount of volatiles. Two hundred and fifty droplets of the alarm secretion contain five times the amount of volatile compounds that the defensive secretion contains.

4.3.3. Conclusions

The results suggest that the defensive and the alarm secretions originate from the poison gland but larger amounts of the poison glands are emitted in the latter case. It seems that the same mixture of compounds in lower quantities will elicit only a slight recruitment and a low level of excitement, while at higher quantities it will elicit alarm.

Fig. 4.7. Chromatogram of the defensive secretion of M. pharaonis.
5% OV-101/CLQ at 170°C.



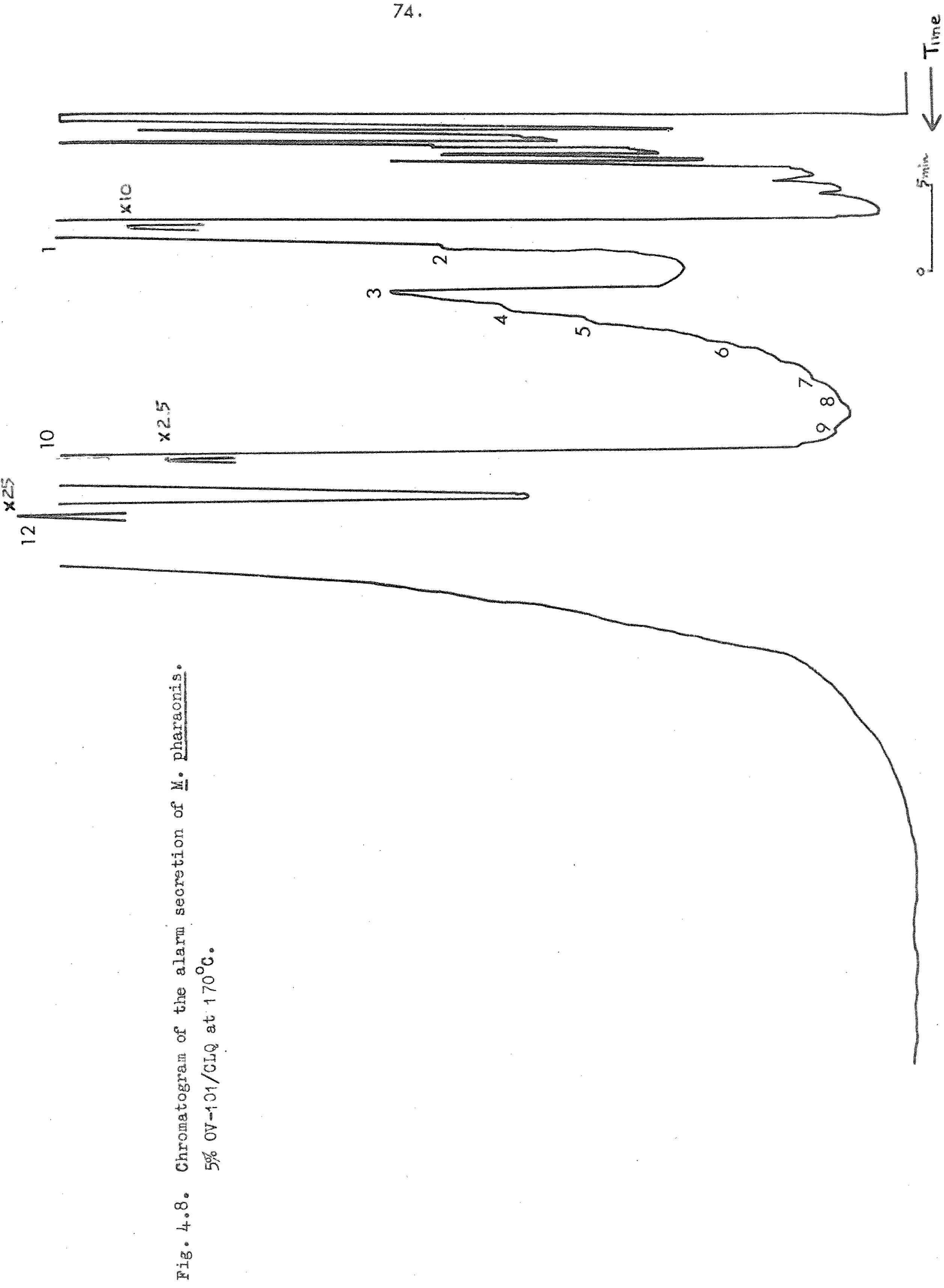


Fig. 4.8. Chromatogram of the alarm secretion of *M. pharaonis*.
5% OV-101/CLQ at 170°C.

The trail appears to consist mainly of the Dufour's gland secretion with some synergistic effect from the poison gland secretion. Some of the compounds in these two glands, especially those with higher volatilities, may act as attractants to the trail in the short term, while another group of compounds may act as trail substances in cases where the trail is used for longer periods of time. When the food source or whatever else elicits trail laying is quickly depleted, then the trail must be destroyed very soon.

In this case the most volatile compounds will act as attractants but the less volatile compounds will not have enough time to accumulate, and the trail will soon disappear. However, when the source is permanent or at least long lasting, less volatile compounds will accumulate and make a trail which will be much more stable.

4.4. Chemical analysis of the monomorine I isomers.

The four geometric isomers of monomorine I are 1a: 3(S) 5(R)9(R) -3-butyl-5-methyl octahydroindolizine, 1b: 3(R)-5(R)-9(R)-3 butyl-5-methyl octahydroindolizine, 1c: 3(S)-5(R)-9(S)-3-butyl-5-methyl octahydroindolizine and 1d: 3(R)-5(R)-9(S)-3-butyl-5-methyl octahydroindolizine. The isomers were injected separately in an $\frac{1}{8}$" 5% OV-101/CLQ column at 120°C and their retention times were calculated. The results are summarized in table 4.1.

Isomers	Abdomen	1a	1b	1c	1d
Ret. time(min)	13.0	13.0	14.2	15.6	14.3

The results suggest that isomer 1a, the all-cis isomer, is the naturally occuring isomer. This confirms other results obtained by Edwards and Pinninger (1978) who reported similar behavioural responses elicited by the naturally occuring monomorine I and the synthetic 1a isomer.

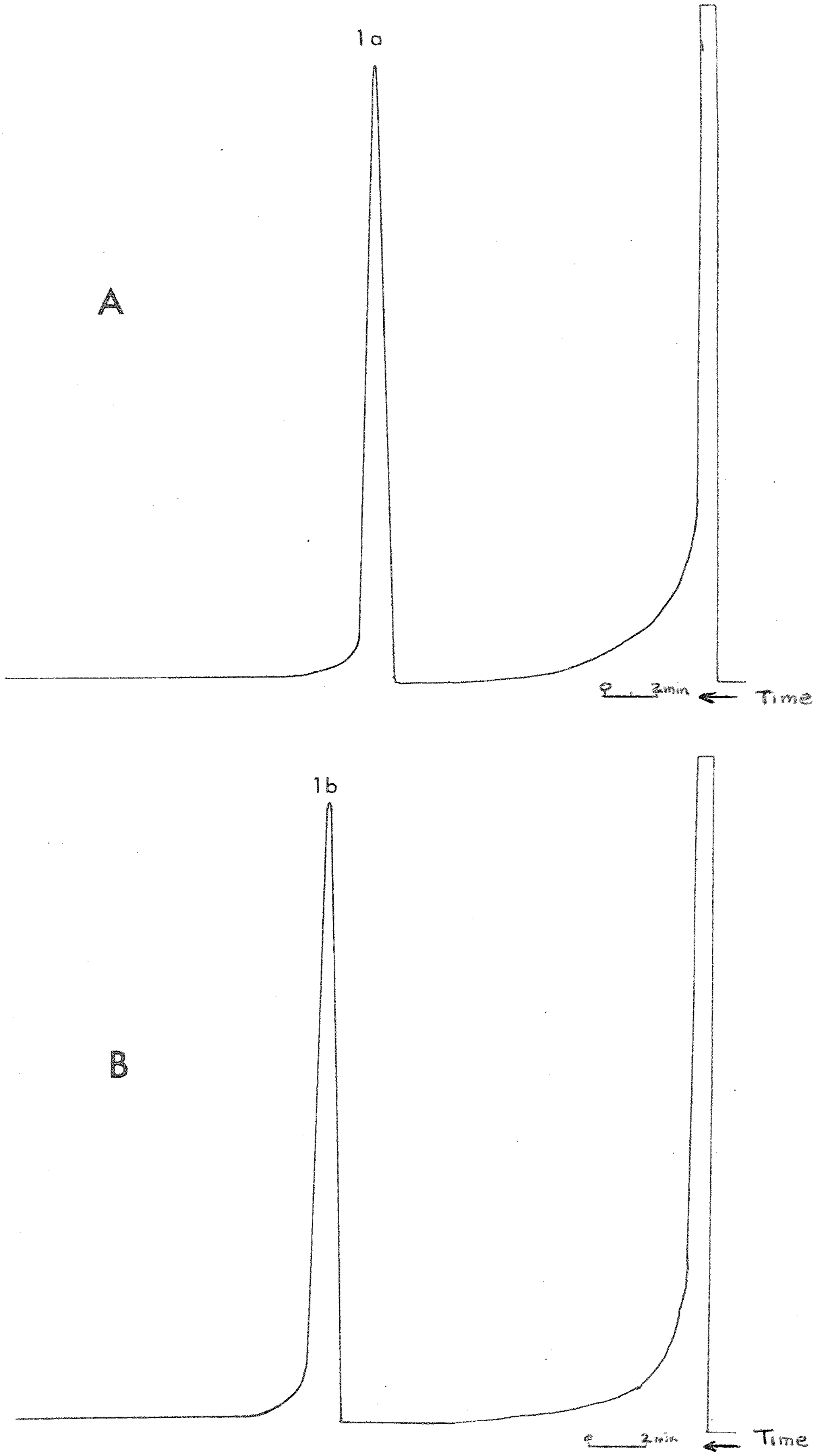


Fig 4.9. Chromatograms of A: Isomer 1a of monomorine I, 3(S), 5(R), 9(R)-3-butyl-5-methyl-octahydroindolizine and B: Isomer 1b of monomorine I, 3(R), 5(R), 9(R) -3-butyl-5-methyl-octahydroindolizine. 5% OV-101/CIQ at 120°C.

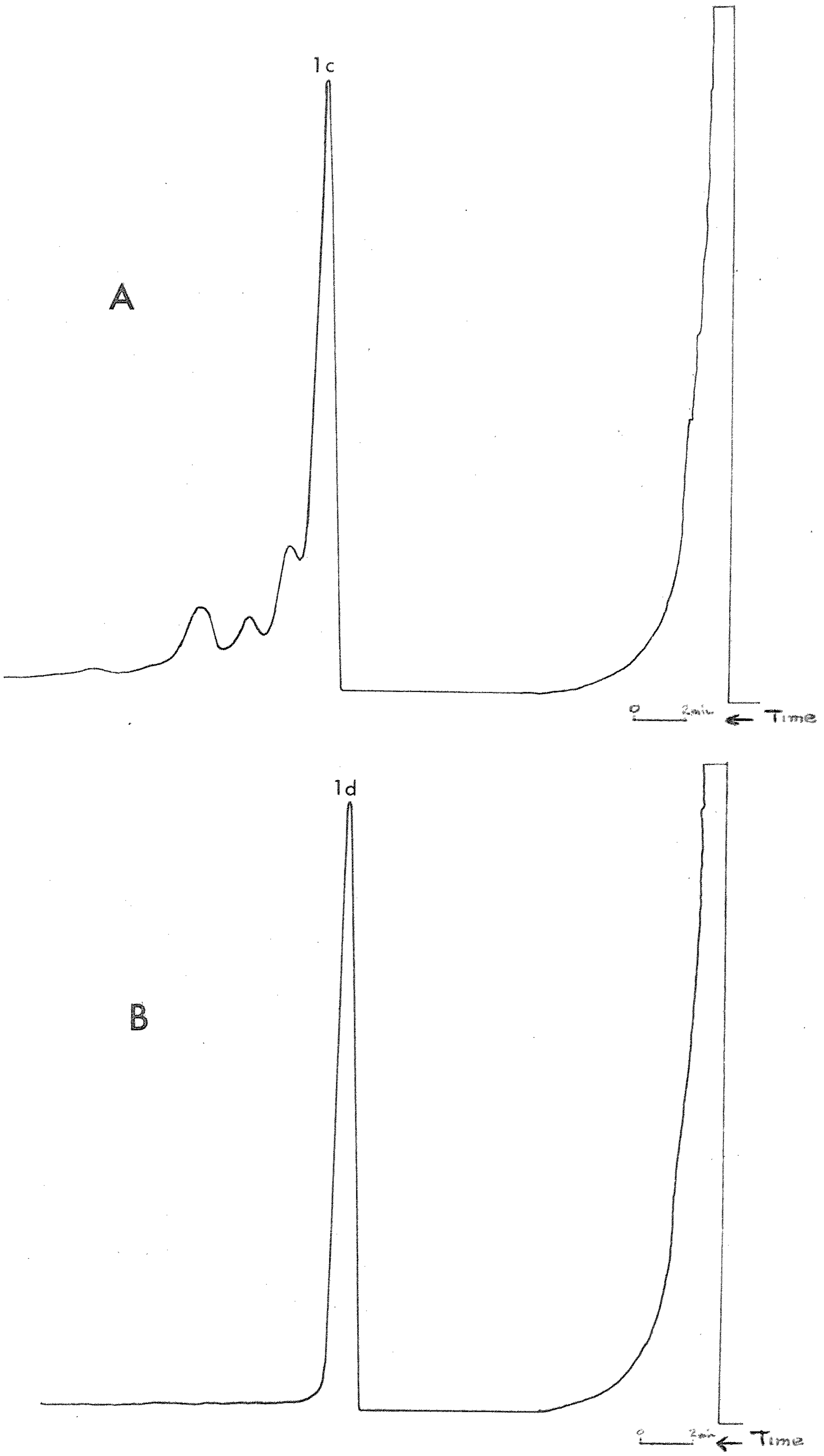


Fig 4.10. Chromatograms of A: isomer 1c of monomorine I, 3(S), 5(R), 9(S)-3-butyl-5-methyl-octahydroindolizine and B: isomer 1d of monomorine I, 3(R), 5(R), 9(S)-3-butyl-5-methyl-octahydroindolizine. 5% OV-101/CLQ at 120°C.

The chromatograms also show that the 1c isomer is unstable since it produces more than one peak (fig. 4.10). Finally, as was previously reported (Oliver and Sonnet, 1974) it is not possible to distinguish isomers 1b and 1d by gas chromatography.

CHAPTER 5

BEHAVIOUR PATTERNS OF PHARAOH'S ANT

5.1. Introduction

Social insects such as ants, have evolved a complex variety of behavioural patterns that enable them to communicate and to carry out a number of activities inside and outside their nest. The description of these behavioural units is essential in the study of the more subtle quantitative and causative factors that were not immediately apparent to the descriptive naturalist of the last century. Chemical studies of one ant, pharaoh's ant, *Monomorium pharaonis* (L), have recently appeared (Ritter et al, 1975, 1977) but the underlying behaviour associated with the functionality of the chemicals so far identified has not been clarified. The description of these behavioural patterns is the first step towards an integrated understanding of both the behaviour and the factors causing it.

5.2. Materials and methods

One large colony of *M. pharaonis* was used in the observations. The materials and the methods of observation of the behaviour patterns are elaborated in Ch.10.

5.3. Results

5.3.1. Individual behaviour

a) *Inactive position*. Most of the ants spend some of the time standing motionless either inside or outside the nest. The body is near the ground, the legs outstretched. The antennae are pulled backwards, the scape and funiculus forming a V, the angle of which varies up to a maximum of 90° (fig. 5.1A).

In another more alert position, the body is held higher and the legs are less outstretched. The antennae are held forward forming more or less a right angle between scape and

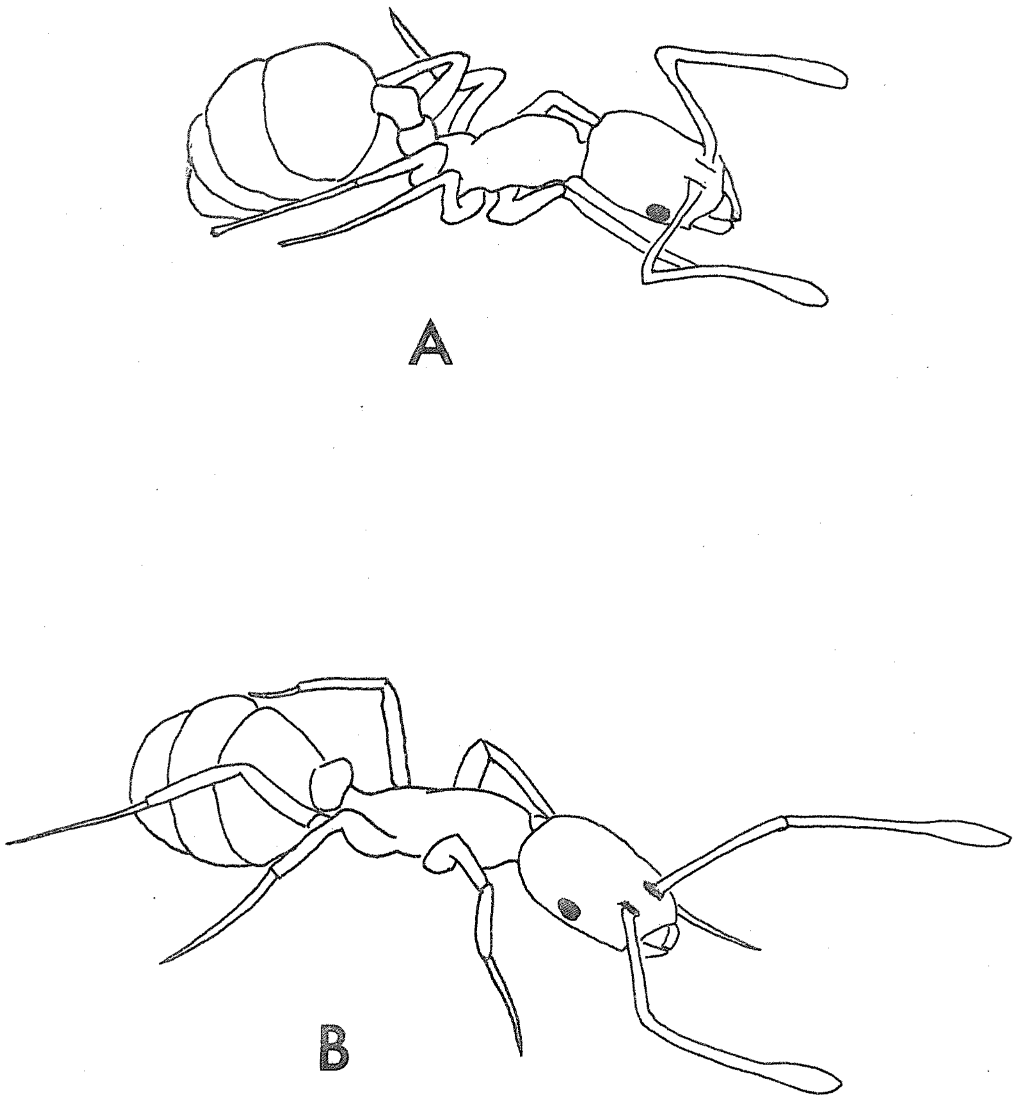


Fig. 5.1. Inactive (A) and active (B) postures of *M. pharaonis*

funiculus (fig. 5.1B). Intermediate postures can also be observed.

b) Cleaning. Self-cleaning or self-grooming is a behaviour often observed in ants. The cleaning movements are very stereotyped and can be easily divided into the following eight categories.

i) The ant brings one front leg over the antennae of the same side and the whole of the antenna is pulled through the groove between the tarsus and tarsal spur. This is repeated two or three times. (fig. 5.2A).

ii) Following or preceding the previous pattern, the ant brings the leg to the mouth from the spur distally and passes its labial mouthparts over it two or three times. The whole operation of (i) and (ii) is repeated again. These two patterns were frequently observed when the lamp used for lighting was placed over the nest with only the transparent cover on. They could serve to cover the antennae with saliva in order to replace the moisture lost due to the high temperature.

iii) The two front tarsi are brought together and each one is brushed on the other from the spur distally. Pattern (ii) then follows.

iv) The ant leans slightly on the side opposite to that which is cleaned and rests on the abdomen and the middle and hind legs. The front tarsus of this side is passed over the three legs of the other side which have been raised from the surface and brought together. The three legs are brushed against each other at the same time as the front tarsus of the other side passes over them. Then the front leg is licked with the mouthparts. This sequence is then repeated two or three times. Then the ant changes position and cleans the three legs of the opposite side in the same manner. (fig. 5.2B).

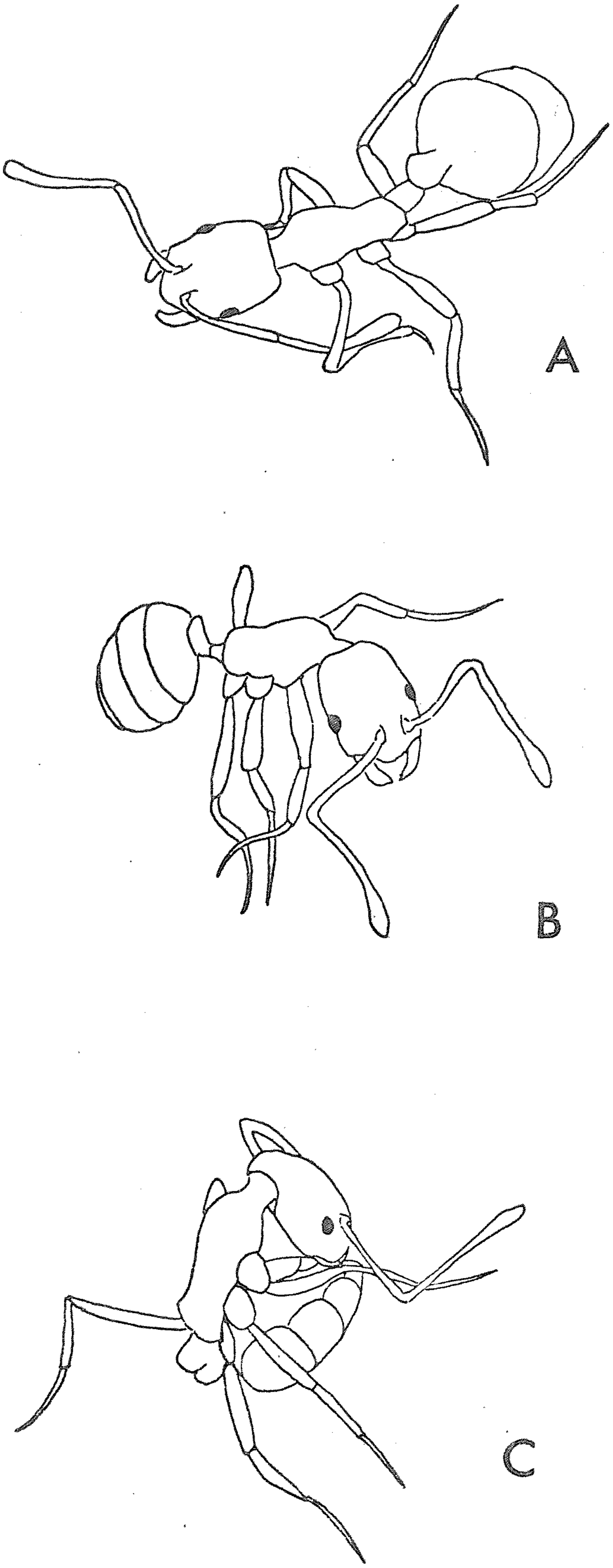


Fig 5.2. Cleaning postures of M. pharaonis A: foreleg and antenna B: Hind and middle leg, C: abdomen.

v) The front and the middle legs are brushed against each other in the same fashion as when cleaning occurs between the fore legs.

vi) The ant rests on the four hindmost legs. The two front legs are brought backwards on the side of the thorax and over the other legs rubbing over the whole length. Then they are rubbed together and brought to the mouth.

vii) The abdomen is slightly lowered and the hind legs sweep its sides.

viii) The abdomen is drawn under the thorax between the legs which are straightened. The head is bent downwards and the mouthparts touch and move over the posterior of the abdomen and the sting. The abdomen is also cleaned by the fore tarsi sweeping its sides at the same time (fig. 5.2C).

c) Feeding. The mouthparts are extended over the food. The antennae are drawn back touching the food and move very slowly over its surface. The mandibles are half open.

d) Exploring substrate. The ant moves over the surface with outstretched antennae palpating the substrate. Both the head and the body are turned alternately left and right. The abdomen is raised slightly above the ground.

e) Trail following. The ants move relatively much faster, rarely deviating from their course. The antennae palpate more rapidly and are held closer to the substrate. The head is turned alternately to the left and right but the body remains more or less on the same line.

f) Examining objects. When encountering an object, the ant keeps the head and thorax very low, the legs are bent and the antennae are extended forward, without touching the object, keeping the head as far as possible away from it. In a positive reaction, the ant draws nearer very slowly, moving the antennae over the object. In withdrawal, it draws the antennae back rapidly forming a V between scape and funiculus.

g) Carrying objects and brood. The objects or the brood are grasped between the mandibles. The head is kept level with the ground, unless the object carried, is large.

h) Carrying corpses. The ant grasps the head, thorax or legs of the corpse between the mandibles and carries it above its head. The ant moves in a straight line towards the entrance of the nest if the corpse is in the nest, or towards the periphery of the foraging arena if it is outside. Its linear speed is 1,5-2 times greater than when transporting other materials. Some ants stop the porter on its way and try to examine or grasp the corpse. The porter resists and pulls back the corpse while at the same time it moves its antennae over the antennae of the other workers. Outside the nest, it heads towards the periphery and appears to search for a suitable place to drop its load. It is not left at the nearest spot but is usually taken to places where other corpses have been deposited.

5.3.2. Aggression and defence

a) Threat. The ant moves twice as fast as normal. It opens its mandibles wide, stops and moves the head forward towards the object. The antennae are stretched wide (fig. 5.3A).

b) Seizing. The ant is seized between the mandibles and held tightly. When the ant seized belongs to the same colony, it remains passive holding back its antennae. An inactive posture is also adopted by the seizing ant and they both remain in this position for a considerable time, f.e. 5 min. When an ant of a different species is seized, seizing is followed by rapid side to side movements of the head. Areas which are commonly seized in this manner are the neck, the petioles, the antennae and the legs.

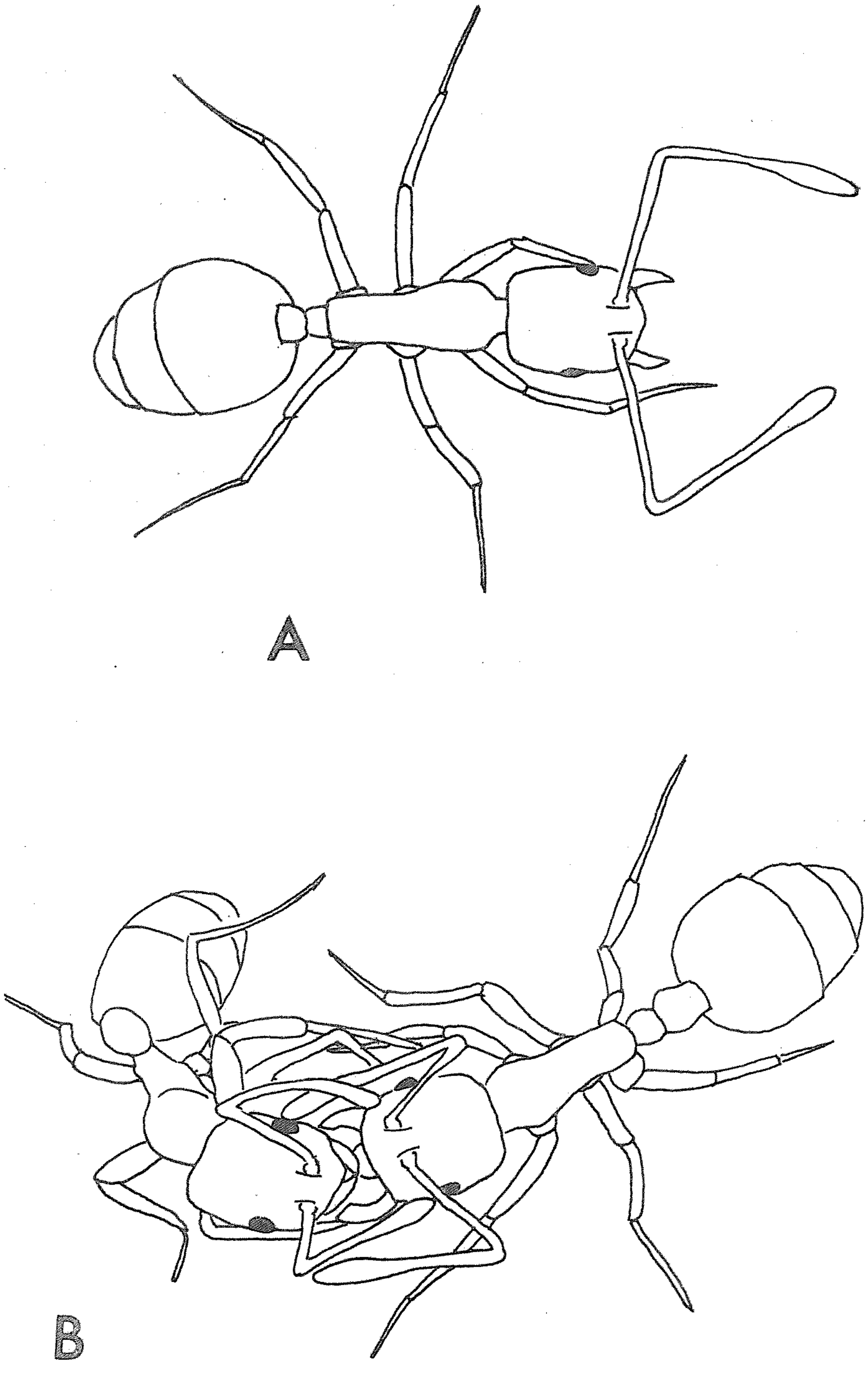


Fig 5.3. Threatening (A) and food sharing (B) in *M. pharaonis*

c) Dragging. Dragging is sometimes observed after seizing. The ant stretches the legs and moves backwards. The antennae are also pulled backwards.

d) Restraint of the queen. When the artificial nest is shaken or seriously disturbed, the queens are the first to leave it. If the disturbance does not continue, the workers run out of the nest and stroke the antennae of the queens rapidly standing in front of them or running besides them. Sometimes they grasp the legs and pull them backwards. The queens then usually stop and turn back to the nest.

e) Defence. Two or three ants stand in the entrance of the nest. One of them puts the abdomen under the legs, lifting its body high and a droplet is seen coming out of the sting. (fig. 5.4A). The ant remains motionless. After sometime, it retracts the abdomen and another ant takes its place. Sometimes there are two ants in this position. This behaviour is observed when an intruder attempts to invade the nest, for example when a pin is inserted two or three times in the entrance hole. If the disturbance is continuous then alarm behaviour ensues.

f) Flight. This consists of a sudden cessation of movement, a rapid turn through 135° - 180° and swift running. Usually, the antennae are also pulled back upon encounter.

5.3.3. Group activities

a) Alarm. The ant palpates its antennae rapidly above its head. The head and thorax are lifted higher than the abdomen and the ant runs very fast, often in circles. It does not stop to examine anything on its way. Sometimes it falls over as a result of the rapid running.

When this behaviour occurs, some ants are seen standing and lifting the abdomen at a 45°angle and a droplet of secretion is seen coming out of the sting. The antennae are outstretched

45^{o} apart (fig. 5.4B).

b) Examining individuals. An ant meeting another ant, raises and stretches its antennae towards it and palpates them over the antennae and the head of the other ant. The other ant usually responds in the same way. The antennae may or may not touch the funiculus of the other's antennae. Examination is brief, usually lasting about one second and is not often followed by other behaviour patterns. Examination may sometimes occur before other patterns such as licking or food exchange.

c) Trail laying. The abdomen is placed close to the ground and the sting touches it periodically, for relatively long periods. The head moves alternately left and right. The antennae do not palpate very rapidly.

d) Recruitment. Ants entering the nest usually examine those ants that they meet and also exchange food. Ants returning from a food source exhibit different behaviour. They move very fast and do not stop to examine others but palpate their antennae very rapidly while running. They also do not exchange food. Soon after, they leave the nest again returning to the food source.

e) Licking. Licking of one ant by another often occurs inside the nest. The mouthparts are applied to the body surface. The antennae are held close to the head and they move slowly over the surface in a manner similar to that observed when feeding. Areas more frequently licked are the mouth, the legs, the antennae and the abdomen, especially the posterior part. Apart from live ants, freshly killed corpses present inside the nest are also vigorously licked.

Licking seems to serve more than the function of cleaning, since most of the areas licked are areas that can be reached by the ant itself. One possible function is the removal of odours, foreign to the colony odour of the ants. The licking of ants

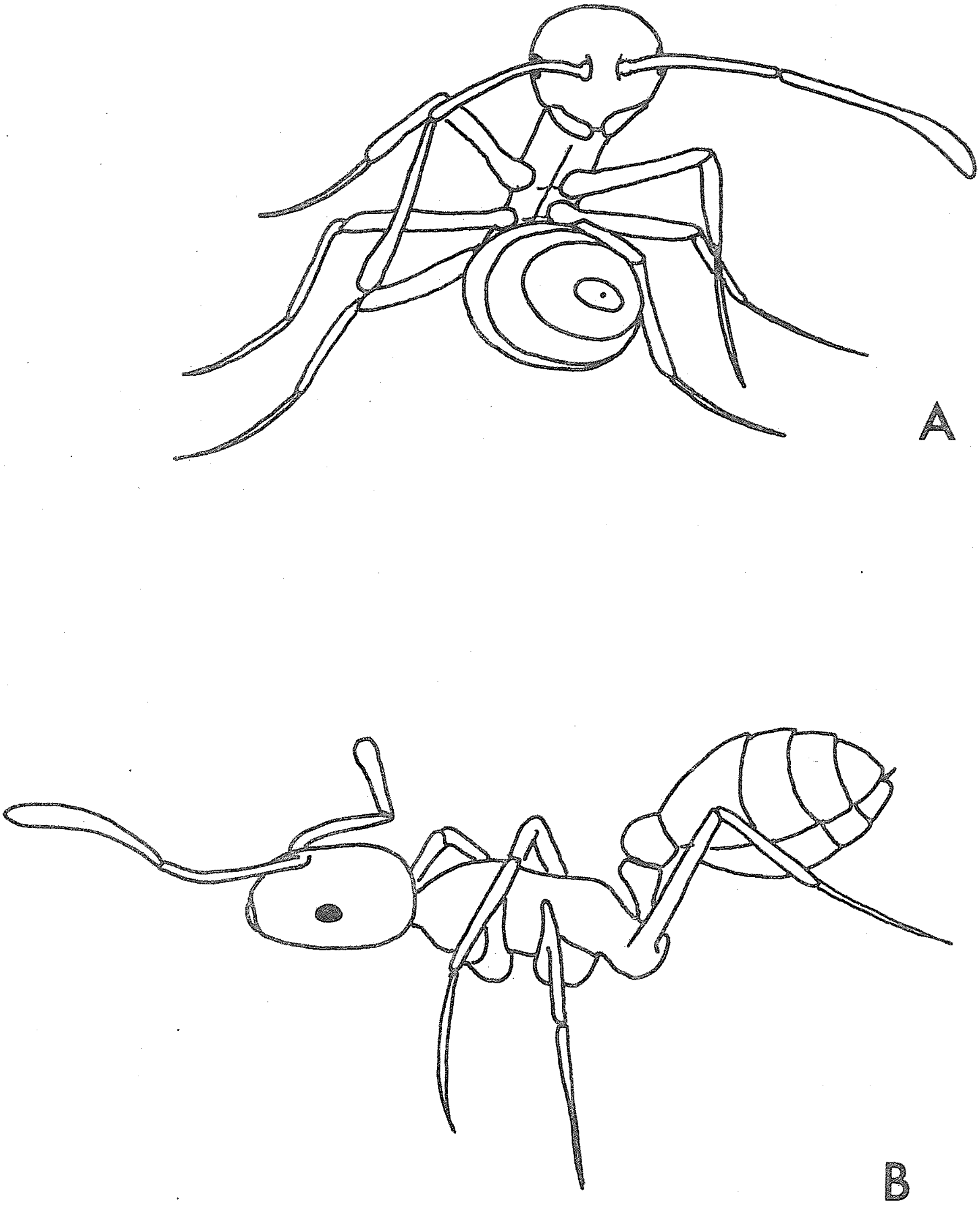


Fig 5.4. Defence (A) and alarm (B) postures of *M. pharaonis*

that have been away from the nest for some time and therefore may have acquired new odours and the licking of fresh corpses which may start releasing large amounts of pheromones or decomposition products, tend to support the argument. Similarly, licking can be seen as coating the ant with saliva or other secretions for possibly the same reasons.

Apart from the adults, all stages of the brood are also licked, possibly in order to keep them moist.

f) Food exchange. Food exchange or trophallaxis is a frequently observed sequence of behaviour inside the nest. Various patterns can be distinguished (fig 5.3B):

i) Antennal palpation. A soliciting ant meeting a possible donor, palpates its antennae rapidly over the front part of the head and on the mouthparts and at the same time turns the head slightly on its horizontal axis. The donor may respond by the behaviour described below or by palpating its antennae over the antennae of the soliciting ant in the same manner as in examining, whereupon no food exchange follows and the soliciting ant proceeds to solicit from another ant.

ii) Donor behaviour. The donor opens its mandibles wide and extends the lateral mouthparts. The antennae are held wide, forming a W shape over but not directly towards the head of the acceptor. Sometimes they are palpated slightly. The head is parallel with the surface and the rest of the body, but the whole anterior part of the body is slightly raised so that the head of the donor is slightly higher than the head of the acceptor.

iii) Acceptor behaviour. The body in general is held low and the legs are outstretched. The head is pointing upwards at an angle to the body and is also turned at an angle from the horizontal plane. The mandibles are closed and the antennae are directed towards the mouthparts or the head of the donor always inside its antennae. The antennae are palpated slightly but palpation increases when the donor begins to move away. The forelegs are off the ground, usually touching the genae or the mandibles of the donor. Sometimes, the abdomen of the acceptor is seen moving up and down.

iv) Offering. Upon encountering another ant, an ant may sometimes open its mandibles and extrude its mouthparts without any soliciting behaviour occurring. This behaviour may or may not be followed by food exchange.

v) Gaping. After being fed, an ant is sometimes seen standing motionless with its mandibles wide open and the mouthparts slightly extruded. The head is bent downwards and the antennae are held at the resting position.

vi) Brood feeding. When feeding liquids to the larvae, the ant extrudes its mouthparts and sets them on the mouth of the larva. The mandibles are kept closed and the antennae remain motionless over the head of the larva. When feeding solid food, a small piece is deposited in the mouth of the larva.

Food exchange is not only observed among ants returning from a liquid food source but is equally distributed among all ants, castes and stages. Workers with inflated abdomens are often seen soliciting food and larvae also regurgitate droplets of liquid. The significance of trophallaxis in communication, physiology and caste differentiation is yet to be fully explained.

5.4. Discussion

This list of behaviour patterns is certainly a selective list. It only includes those patterns that were observed under the specific circumstances. Also, no effort was made to quantify all of them because this chapter has served as a checklist of behaviour patterns and some that are closely related to chemical communication will be discussed in more detail in later chapters.

CHAPTER 6

NECROPHORIC BEHAVIOUR

6.1. Introduction

One of the most important activities of ants is maintaining hygiene in the nest and foraging area. Included in this, is necrophoric behaviour in which dead members of the colony are removed, often to localized cemeteries.

Although there have been many descriptions of necrophoric behaviour in social insects (Wilson, 1971), there have been few objective experimental studies dealing with the underlying causes. Apart from the harvester ant, Pogonomyrmex badius (Wilson, Durlach and Roth, 1958) and the fire ant, Solenopsis invicta (Howard & Tschinkel, 1976), very few species have been studied in detail. In Pogonomyrmex, pieces of paper treated with acetone extracts of corpses were treated like intact corpses. Separation and behavioural assays of the principal components of the extract implicated long-chain fatty acids and their esters as releasers of necrophoric behaviour. Oleic acid, a common decomposition product of insect corpses, was effective for both Pogonomyrmex badius and Solenopsis saevissima (Wilson, Durlach and Roth, 1958). In Solenopsis invicta, contact chemical cues were found to release necrophoric behaviour, but they were not identified (Howard & Tschinkel, 1976). In pharaoh's ant, Monomorium pharaonis, there has been only one study of the reaction of workers towards corpses (Sudd, 1957). Here, it was mentioned that workers meeting fresh corpses of their own species on food trails were repelled by them.

In the present study, an attempt has been made to establish the components of necrophoric behaviour and their sequential organization in Monomorium pharaonis and to elucidate the stimuli involved.

6.2. Materials and methods

One large colony of Monomorium pharaonis was used in the experiments. The materials and the methods of observation are elaborated in Ch.10.

Two bioassays for necrophoric behaviour were investigated,
1) Corpses were placed on the arena. Initially, they were placed at a maximum distance of 5cm from the nest entrance. This proved ineffective because of the small number of ants moving on the arena. Subsequently, corpses were placed on a trail leading to a food source where more attention was given to them by the ants. 2) Corpses were placed inside the nest through a gap between the glass cover and the walls of the nest without disturbing the ants inside. This was more effective as there were always workers near the spot where the corpses fell and observation under the microscope was easier in this limited space. Other objects such as pieces of food, wood and corpses of other species could also be easily introduced into the nest. Observations were carried out until the objects were removed from the nest by the ants (fig. 6.1).

6.3. Results

6.3.1. General description of necrophoric behaviour

Observation of ants carrying their dead nestmates led to the following categorisation of components of necrophoric behaviour in M. pharaonis which could be used in subsequent bioassays. An ant grasps the head, thorax or legs of the corpse between its mandibles and carries it above its head. The ant moves in a straight line rarely deviating from its course, towards the entrance of the nest if the corpse is in the nest, or towards the periphery of the foraging arena if it is outside. Its linear speed is 1.5 -2 times greater than when transporting other materials such as pieces of rubbish. Some ants stop the porter on its way and try to examine or grasp the corpse. The porter resists and pulls back the corpse while at the same time it moves its antennae rapidly over the antennae of the other workers. Outside the nest it heads towards the periphery and appears to search for a suitable place to drop its load. It is not left at the nearest spot but is usually taken to places where other corpses have been deposited.

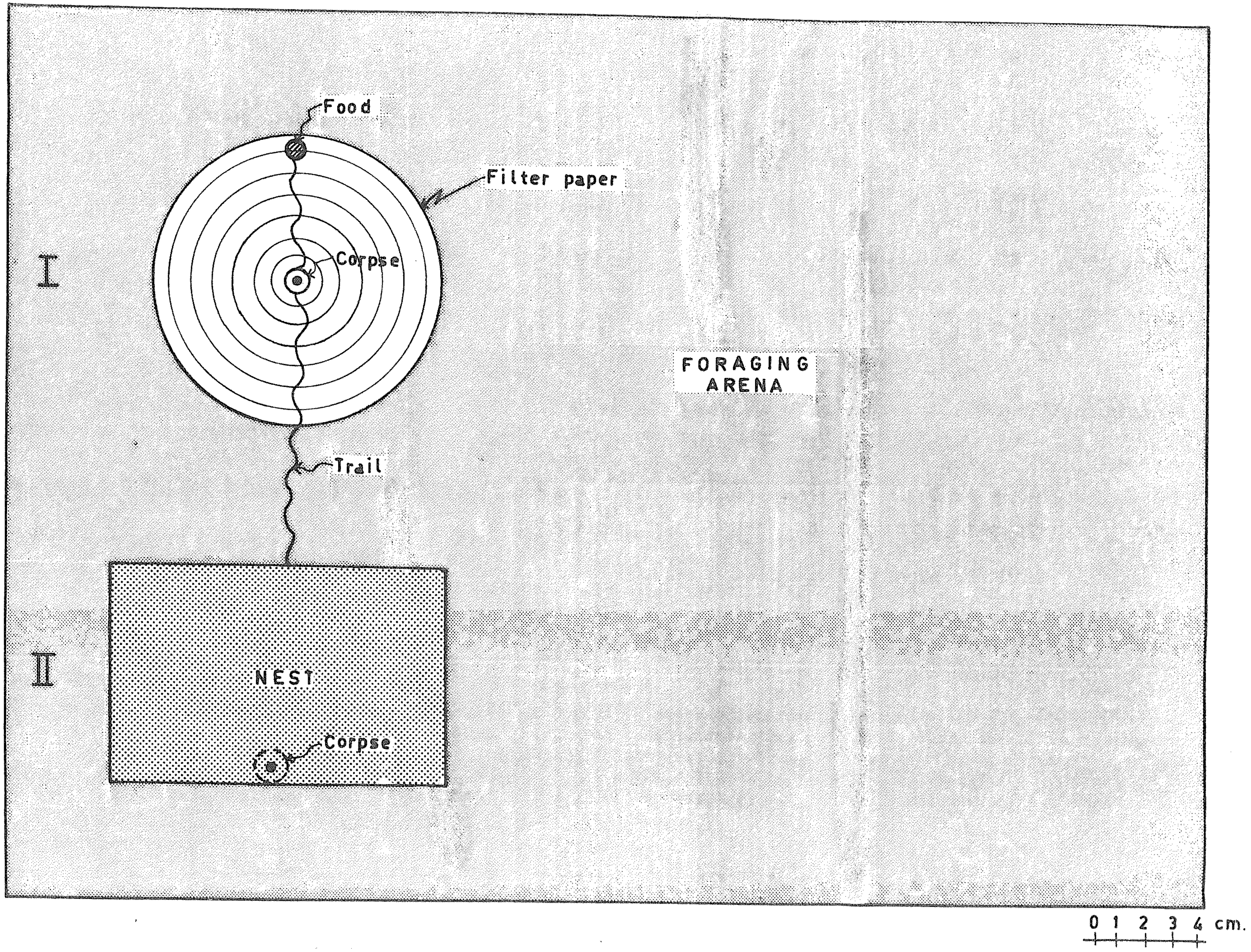


Fig. 6.1. Layout for the bioassays of necrophoric behaviour.

Observations in the nest revealed the presence of refuse piles in the four corners. These piles contained pieces of wood, uneaten food, queens' wings and faecal material, but no dead ants or brood. These were observed in the periphery of the foraging arena, scattered below it, or on the paraffin wax layer. There were no distinct refuse piles. A count of the number of corpses in the periphery was carried out which showed two areas of high concentration, in the upper left corner and the lower right corner (fig 6.2). These areas are at the maximum distance from the two other corners, at one of which the nest is located and at the other the nest had been positioned two months previously. Another observation tends to support the hypothesis that there are specific areas for the deposition of corpses. A very large number of corpses was observed under a food tray on the arena. The tray was at a distance from the nest equal to the distance between the nest and the upper left corner of the arena.

It is not clear whether the workers and sexuals are carried or wander to the periphery by themselves before death. It is more likely that both events occur since workers carrying dead brood and corpses placed in the nest were seen depositing their load at the periphery, and old queens were seen wandering on the paraffin wax layer and remaining there. This supposition is also reinforced by the finding that when the nest is disturbed and queens move out immediately, the workers force them to return by surrounding them, beating their antennae intensely and even pulling them, while queens about to die are left completely unattended. It seems, in laboratory colonies at least, that most injured and dying ants leave the nest, but when death or injury occurs inside the nest, the ant is carried out by its sister workers. A worker whose abdomen had been crushed but was still alive was carried outside the nest by another worker.

6.3.2. Responses to objects on a trail

a) Whole corpses

Preliminary observations showed that corpses of

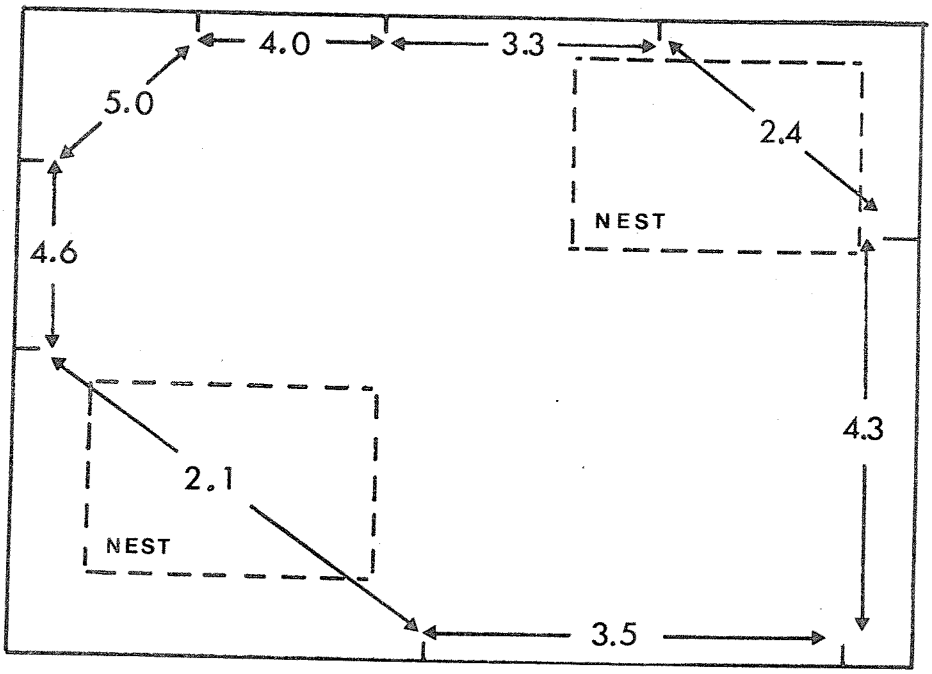


Fig 6.2. Concentration of corpses in a zone 2cm wide along the periphery of the foraging arena. Numbers in corpses/cm.

M. pharaonis were carried away only if they were placed in the nest or on a foraging trail.

A piece of filter paper, 11 cm diameter, with concentric circles at 0.5cm intervals, was placed near the nest on the foraging arena. Two Tenebrio larvae were placed 2cm from the centre on the far side of the filter paper and soon afterwards a trail of M. pharaonis workers was established between the nest and the food. The trail passed almost through the centre of the filter paper. When the trail following reached its maximum, a M. pharaonis corpse was placed on the trail as near as possible to the centre of the paper. The corpse was placed at a moment when the nearest in - or outgoing ant was at least 2cm from the centre to prevent alarming the ants. The reaction of the ants towards corpses of different ages was observed and noted.

Corpses which had been left in the open for various periods elicited different types of behaviour. We can distinguish two categories of behaviour according to corpse age.

i) Corpses up to 24h old. The main type of behaviour is withdrawal. The ants that came near the corpse stopped immediately when approximately 1 cm away, turned through 180°, and ran back to the nest without necessarily following the trail. After approximately 10 min., and after about 30 ants had encountered the corpse, a deviation from the trail was established about 1 cm from the corpse in either direction. The repellency was very persistent, lasting up to 5h. Sometimes the workers that first encountered the corpse were slightly alarmed and ran around very rapidly in circles lifting the front part of the body and moving the antennae rapidly. As the age of the corpse increased, the repellency decreased (Fig. 6.3). The ants came closer to 12-24 h old corpses and did not run away immediately upon encounter, but stayed away for a few seconds. The number of ants encountering the corpse before it was picked up decreased (Fig. 6.4). The picking up and transport of the corpse always followed the typical necrophoric pattern of behaviour described above.

Table 6.1. Average minimum distance between object on a trail and ant encountering it. (in mm).
* = significant difference from whole corpse at 5% level.

Corpse age(hrs)	Whole corpse		Head and thorax		Abdomen		*Plagiolepis* sp	
	mean	s.d ±	mean	s.d ±	mean	s.d ±	mean	s.d ±
0	10.0	2.4	10.0	2.0	9.0	1.8	0.0 *	0.0
2	6.5	1.8	7.1	1.7	6.5	1.5	0.0 *	0.0
4	4.5	1.2	6.0	1.4	4.2	1.2	0.0 *	0.0
6	3.2	1.0	4.0	1.3	3.1	0.9	0.0 *	0.0
9	1.7	0.8	2.0	0.9	1.5	0.8	0.0 *	0.0
12	1.1	0.8	1.1	0.6	1.1	0.4	0.0 *	0.0
18	0.7	0.6	0.8	0.4	0.9	0.5	0.0 *	0.0
24	0.5	0.4	0.6	0.4	0.4	0.3	0.0 *	0.0
36	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
72	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
96	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
120	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acetone extracted	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

(mean= mean of 5 replicates each consisting of the mean of observed distances.
s.d.= standard deviation of the sample of 5 replicates)

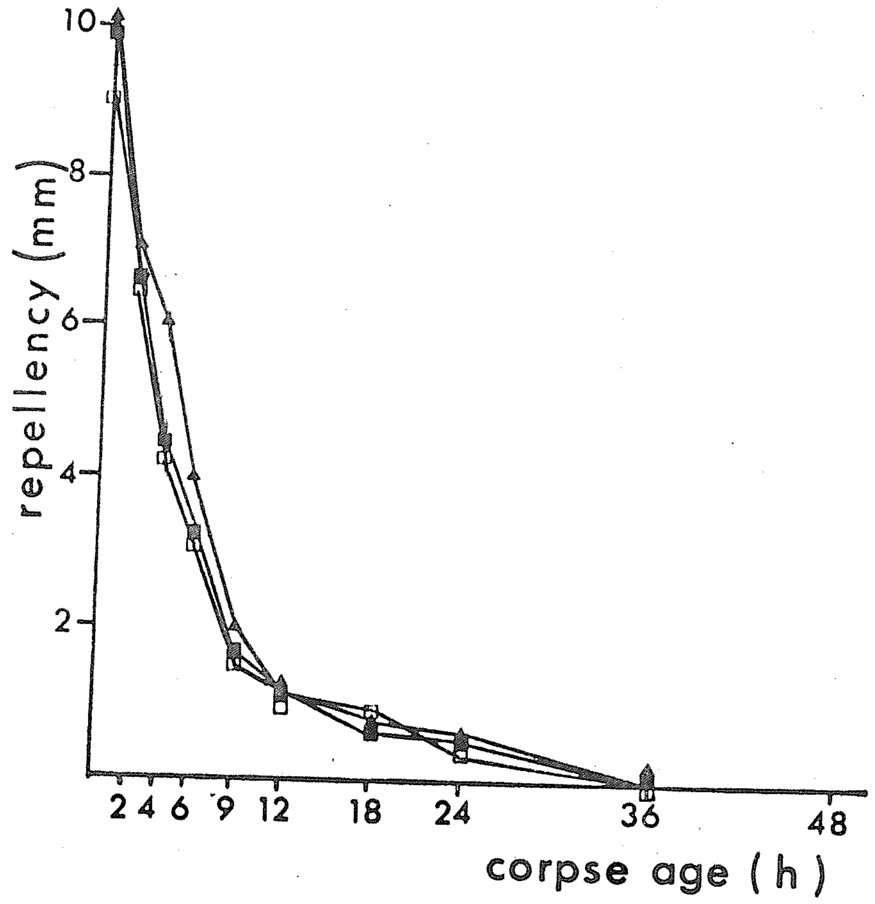


Fig 6.3. Repellency of corpses. Mean distance (in mm) from corpse on a trail, at which ants stop and turn back. (■ = whole corpse, ▲ = head and thorax, □ = abdomen). (Full data from table 6.1)

Table 6.2. Average duration of examination of object on trail by each ant (in sec)

Corpse age(hrs)	Whole corpse		Head and thorax		Abdomen		*Plagiolepis* sp	
	mean	s.d ±	mean	s.d ±	mean	s.d ±	mean	s.d ±
0	0.8	0.4	0.8	0.5	0.4	0.2	0.2	0.1
2	0.9	0.5	0.8	0.4	0.5	0.3	0.3	0.2
4	1.0	0.4	0.9	0.4	0.6	0.3	0.2	0.1
6	1.0	0.6	1.0	0.6	0.6	0.2	0.1	0.1
9	1.0	0.5	1.2	0.6	0.8	0.3	0.2	0.2
12	1.2	0.7	1.4	0.5	0.9	0.4	0.2	0.1
18	1.4	0.6	1.6	0.7	1.0	0.3	0.3	0.2
24	1.6	0.9	2.0	0.8	1.2	0.7	0.3	0.1
36	1.7	0.6	2.0	1.3	1.6	0.9	0.5	0.2
48	2.0	0.9	2.0	1.2	2.0	1.2	0.3	0.2
72	3.0	1.7	3.0	1.9	2.5	0.9	0.1	0.1
96	5.0	2.7	4.0	2.3	3.0	1.9	0.2	0.1
120	8.0	3.4	7.0	3.2	3.0	1.5	0.1	0.1
Acet. extracted	1.0	0.6	2.0	1.1	0.2	0.2	0.3	0.1

(mean= mean of 5 replicates each consisting of the mean of observed time periods.

s.d.= standard deviation of the sample of 5 replicates).

Table 6.3. Number of ants encountering object on trail before pick-up.

* = significant difference from whole corpse at 5% level.

Corpse age(hrs)	Whole corpse		Head and thorax		Abdomen		*Plagiolepis* sp	
	mean	s.d ±	mean	s.d ±	mean	s.d ±	mean	s.d ±
0	36	11	40	13	56 *	15	2 *	2
2	26	8	30	12	44 *	13	2 *	1
4	18	4	20	8	36 *	12	1 *	0.8
6	14	4	16	6	28 *	12	2 *	1.0
9	10	3	13	3	20 *	9	1 *	0.7
12	9	3	8	4	13	8	1 *	0.8
18	6	3	6	3	9	6	1 *	1.0
24	4	2	5	4	6	4	1 *	0.7
36	4	3	4	3	6	4	2 *	1.2
48	5	3	4	2	5	3	1 *	0.8
72	5	2	4	3	4	3	2 *	1.0
96	4	3	6	3	4	3	2 *	1.3
120	6	2	6	4	4	2	1 *	0.8
Acetone extracted	18	5	12	7	8	5	1 *	0.6

(mean= mean of 5 replicates each consisting of observed number.
s.d.= standard deviation of the sample of 5 replicates).

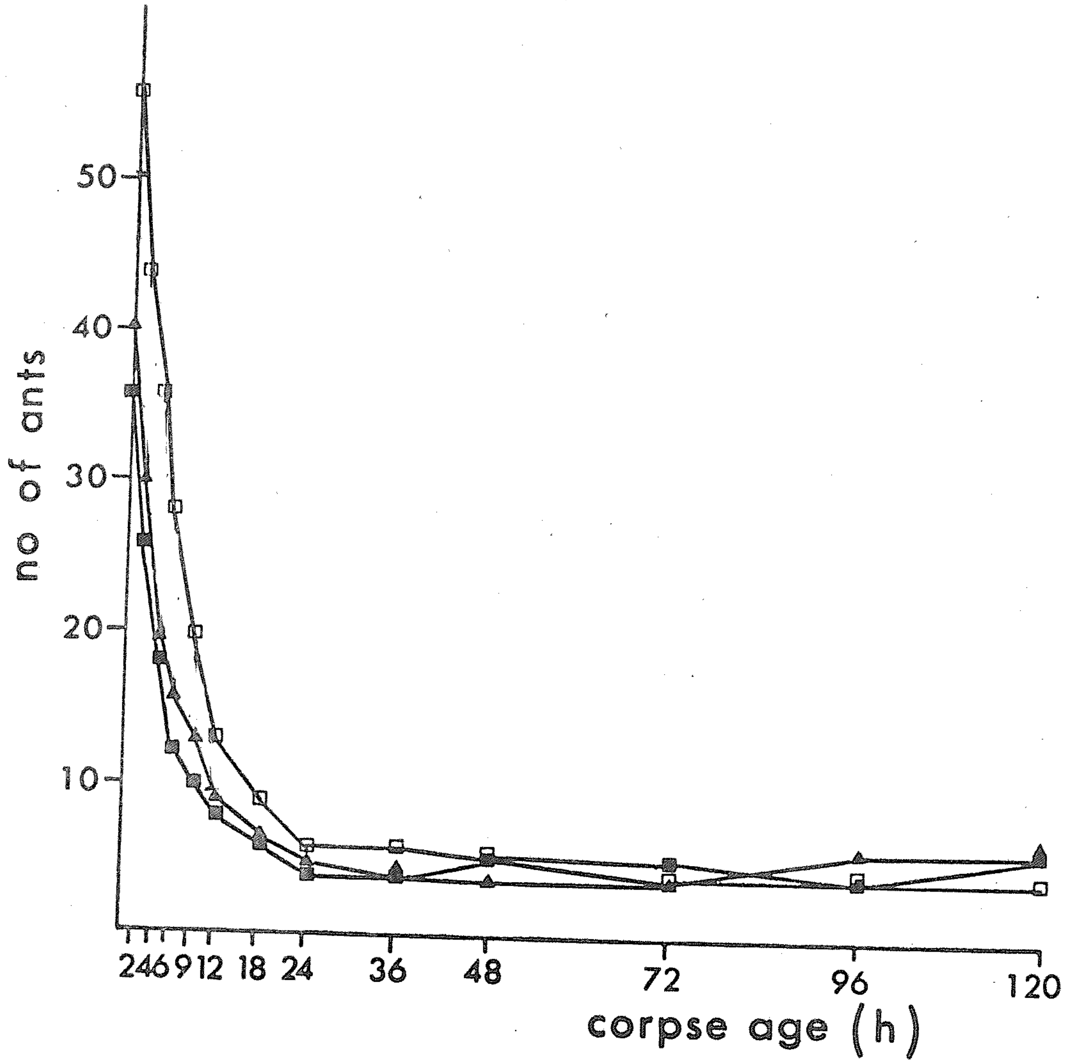


Fig 6.4. Mean number of ants examining corpses on a trail before pick-up. (■ = whole corpse, ▲ = head and thorax, □ = abdomen.)
(Full data from table 6.3.)

Table 6.4. Presence of alarm, aggression, high speed and orientation towards the periphery and depositing of load on the periphery towards objects on a trail (WC=Whole corpse, HT=Head and thorax, A=abdomen, P=*Plagiolepis* sp) (+= presence, -= absence).

Corpse age(hrs)	Alarm				Aggression				High speed and orient.				Depositing			
	WC	HT	A	P	WC	HT	A	P	WC	HT	A	P	WC	HT	A	P
0	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-
2	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-
4	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-
6	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	-
9	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	-
12	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	-
18	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	-
24	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	-
36	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	-
48	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	-
72	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	-
96	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	-
120	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	-
Acetone extracted	-	-	-	-	-	+	-	+	-	-	-	-	+	+	-	-

The high level of repellency and its subsequent decline suggests the presence of volatile chemicals in the corpse which can last up to 24h.

ii) Corpses older than 24h. At approximately 24h after death, the corpses elicited two kinds of behaviour. The ants returning from the food to the nest were repelled by them at an average distance of 1 ± 0.4mm. They turned back or remained there for a mean time of 1.6 ± 0.4 sec waving their antennae. The ants going from the nest to the food were not repelled. They examined the corpse and after a number had done so (mean=5±1) one picked it up and proceeded to carry it to the periphery. We observe here a difference in motivation that might be due to hunger. Corpses older than 24h did not elicit this ambivalent behaviour. The ants examined the corpses for 3 - 8 sec and after only a small number had examined them were the bodies transported in the typical necrophoric pattern. The chemicals that may have caused the repellency seem to be absent here and other stimuli may have taken their place.

b) Heads and thoraces. To investigate the possibility of chemicals eliciting necrophoric behaviour and at the same time identify a possible source, the same observations were carried out using the head and thorax only of *M. pharaonis* under the same experimental conditions.

Again, we can distinguish two categories of behaviour, one towards corpses up to 24h old and the other towards older corpses. In both categories, the behaviour was similar to that towards the whole corpses: repellency with traces of alarm at first, and then gradual examination and removal of the head and thorax towards the periphery (figs. 6.3, 6.4). Again, the ants left a new trail around the position where the heads and thoraces had lain. There was slightly more aggression towards corpses up to 4h old (higher number of ants threatening with open mandibles). These results again suggest the involvement of chemical stimuli, but the increased aggression may be due to the different shape of the corpse as well as the presence of different quantities of the same chemicals or different chemicals.

c) Abdomens. The same observations were carried out using abdomens of dead M. pharaonis workers. Two categories of behaviour can be distinguished again. In the first category with abdomens of up to 24h old, there was an appreciable difference from the previous two bioassays. A larger number of ants was repelled by the abdomen (table 6.3, Fig. 6.4). However, in the second category, with abdomens older than 24h, this difference was eradicated and when the abdomens were 4 - 5 days old, they were more readily picked up than the other two (table 6.3, fig. 6.4). Deviation of the trail from centre was again observed. These results suggest that the source of the chemicals that may cause repellency are in the abdomen but the stimuli that elicit necrophoric behaviour towards corpses 4 - 5 days old are present in smaller amounts than in the rest of the body.

d) Removal of the chemical signal. To test the effect that absence of chemicals has on necrophoric behaviour, a number of fresh nestmates' corpses was left in acetone for one month and then left to dry in open air for another month. The acetone extract was kept for further bioassays. The reaction towards acetone-treated corpses was not markedly different from that towards 5 day old corpses. However, there was an increase in the number of workers examining the corpses before one of them finally picked them up (table 6.3). Also there was a decrease in the time for which each ant examined the corpse (table 6.2). The corpses were not transported in the typical necrophoric pattern. The walking speed of the porters was not higher than their usual speed and they did not head directly towards the periphery. The ants did not make deviations in their trail.

Acetone-treated heads and thoraces elicited slight aggression initially, which was manifested by open mandibles and a fast forward movement of the head towards the objects. They were examined for a slightly longer period by each ant (average 2 ± 0.4 sec) and were carried out after a smaller number of ants had examined them (table 6.3). Again, they were not transported as in normal necrophoric behaviour.

Acetone-treated abdomens elicited a distinctly different behaviour pattern. They were not examined at all apart from a simple tapping of the antennae which lasted on average 0.2 ± 0.1 sec. Very soon they were lifted by an ant and deposited 2 - 5cm from the trail and never taken to the periphery. This suggests complete absence of both necrophoric and recognition stimuli.

Another attempt was made to remove the chemical signal. Freshly killed corpses were covered with a thin gold-palladium film as in preparation for scanning electron microscopy. It was thus hoped that the film would not permit any chemicals to come out of the corpse.

The corpses were again placed on the filter paper and reaction of workers towards them was noted. Ants encountering these covered corpses stopped at an approximate distance of 2mm, extended their antennae towards the corpse and turned back. This lasted for an average 1.2 ± 0.4 sec. After about 10 ants had responded in this way, one lifted the corpse and proceeded to carry it towards the periphery in the typical necrophoric pattern.

The reaction towards covered heads and thoraces and covered abdomens was not different from that towards covered whole corpses. The results of these bioassays with covered corpses show similarity with results from corpses 9 - 12h old. The covering of the corpses cannot inhibit completely the volatilization of all chemicals, but the results presumably rule out any effects of contact pheromones on the cuticle in freshly killed corpses.

These results show that all acetone-treated corpses do not elicit the typical necrophoric pattern of behaviour.

e) Objects treated with acetone corpse extract. The acetone extract of the previous bioassay was concentrated up to 100 μl , and 1 μl of this,was placed on a small piece of filter paper which was then placed on a trail. The filter paper elicited significant repellency in the ants that were following the trail in either direction. The workers stopped at an average distance of 6 mm from the filter paper.

A control filter paper of the same size which was treated only with acetone did not elicit any repellency and was ignored by the workers following the trail.

1 µl of the acetone extract was also put on acetone-treated corpses that had been left for one month to desiccate. These corpses also elicited repellency in the workers following the trail in either direction at an average distance of 7 mm from the corpse. The workers remained at this distance for an average of 1 sec and then turned around and left.

These results show that the acetone extract of the corpses is active in repelling workers. This repellency is a characteristic component of necrophoric behaviour of workers encountering corpses on a trail. (Table 6.10).

f) Objects treated with oleic acid. Oleic acid has been reported to elicit necrophoric behaviour in various species of ants including Pogonomyrmex badius (Wilson et al, 1958), Solenopsis invicta (Blum, 1970) and Myrmecia vindex (Haskins, 1970). One µl of various concentrations of oleic acid in diethyl ether was bioassayed for necrophoric behaviour, both on filter paper and on corpses. The concentrations used were 1µg/ml, 10µg/ml, 100µg/ml, 1mg/ml, 10mg/ml, 100mg/ml and 1g/ml. The controls were pieces of filter paper and corpses treated with diethyl ether only.

Only the filter papers that contained concentrations 10 mg/ml, 100 mg/ml and 1g/ml elicited repellency in workers that encountered them on a trail. The other concentrations and the controls did not elicit any recognisable changes in behaviour. Also, no filter paper treated with oleic acid was picked up and transported to the periphery of the arena. The same was observed with the controls.

The corpses that were treated with concentrations 100 µg/ml, 1mg/ml, 10mg/ml, 100mg/ml and 1g/ml of oleic acid, did repel the workers that encountered them. Neither concentrations of 1µg/ml and 10µg/ml nor the controls elicited any change in behaviour. Again, no corpses were transported to the periphery of the arena.

A drop of oleic acid was deposited on a number of live ants moving on the trail. These ants repelled all the other workers that encountered them.

These results suggest that oleic acid does have one of the characteristics that corpses placed on a trail have, that is, repellency, but they are not transported in the typical necrophoric pattern of behaviour and therefore the acid cannot be classified strictly as a necrophoric behaviour releaser. (Table 6.11).

g) Behaviour towards other objects. Various objects were placed on the trail in order to observe the behaviour of workers towards them and compare it with behaviour towards corpses.

i) Plagiolepis. The corpses of a Plagiolepis species of the brunni group, a small ant 1.5 - 2 mm long, were placed on the trail. They were immediately seized by the first ants that met them and soon they were cut in pieces and transported towards the nest. The behaviour was similar towards corpses of various ages, even towards acetone-treated corpses of Plagiolepis.

ii) Pieces of wood, leaves, 2 - 3mm long. The behaviour was similar to that towards acetone-treated abdomens. They were not examined and very soon they were moved to the side of the trail.

6.3.3. Responses to objects inside the nest.

a) Whole corpses

Corpses which had been left in the open for various periods were placed inside the nest one at a time. The behaviour of the workers towards them was observed and noted.

Corpses which had been left in the open for different periods elicited different kinds of behaviour. We can distinguish three categories of behaviour according to corpse age.

i) Corpses up to 24h old. Examination of the corpse started immediately. During the first minute the workers did not go near the body but stayed away palpating their antennae 1 - 2mm from the corpse, in a similar fashion shown towards fresh-killed corpses on a trail. From the second minute onwards, examination was more thorough. The workers surrounded the corpse and examined it with their antennae, giving more attention to the mouth, antennae and sting of the corpse. Antennal examination continued at various intensities until the corpse was removed from the nest. Five to ten minutes after introduction of the corpse, a new activity, licking, began. It was directed to all parts of the body, but again especially towards the mouth, antennae and sting. Licking was so intense that at times there were five to six workers licking the corpse at the same time. It lasted for a long time, sometimes until the corpse was removed. Eventually, one hour or more after the initial introduction one of the workers which had been licking the corpse, picked it up and carried it outside in the typical necrophoric pattern.

ii) Corpses 1 - 2 days old. The major differences in the behaviour towards these corpses were the absence or low intensity of licking (fig. 6.5) and the decrease in the time that elapsed between the introduction of the corpse in the nest and its removal (fig. 6.7). With fresh corpses it was always over one hour. With 1 - 2 day corpses it exceeded 15 min only once. There were other differences which will be described below.

As in the first group, the first minute after the introduction passed with the workers surrounding the corpse. The antennal examination started immediately afterwards. It was not directed at the head and abdomen only, but was spread over the whole body. Antennal examination continued until the corpse was carried outside. While examination of the corpse was going on, one worker would open its mandibles, grasp the corpse and carry it outside or to a corner of the nest. If the corpse was not carried outside within the first five or ten minutes, some sporadic licking of the mouthparts, the body and the sting was observed. Another kind of behaviour was observed which was more

Table 6.5. Number of ants examining object inside the nest at 30s intervals. Measurements were taken until the end of the examination. (For time period of examination, see relative columns in table 6.8.)

Corpse age(hrs)	Whole corpse		Head and thorax		Abdomen		*Plagiolepis sp*	
	mean	s.d ±	mean	s.d ±	mean	s.d ±	mean	s.d ±
0	4.9	0.5	4.6	0.9	4.0	0.6	6.6	0.9
2	5.0	0.6	6.4	1.0	4.0	0.5	5.8	0.7
4	4.7	0.5	4.4	0.5	3.0	1.0	4.2	0.6
6	5.4	0.8	4.4	0.6	3.5	0.9	5.0	0.5
9	4.6	0.6	4.8	0.8	4.5	0.7	5.2	0.7
12	5.2	0.9	5.6	0.5	5.2	0.7	4.0	0.8
18	4.0	0.6	4.8	0.6	5.7	0.8	5.6	0.9
24	4.8	0.8	3.6	1.0	5.0	0.6	4.6	0.5
30	4.3	0.6	5.0	0.7	4.2	1.0	3.8	0.8
36	5.1	1.0	4.6	0.8	5.2	0.8	4.8	0.7
48	4.7	1.0	4.0	0.9	5.1	0.7	5.4	0.6
72	5.2	0.8	5.6	1.0	4.5	0.5	4.6	0.7
96	4.2	0.7	4.0	0.7	4.0	0.6	5.2	0.8
120	4.9	0.8	4.6	0.5	4.5	0.7	6.0	0.6
Acetone Extracted	4.5	0.9	7.0	1.0	6.2	0.9	6.6	0.8

(mean= mean of 5 replicates each consisting of the mean of observed numbers.
s.d.= standard deviation of the sample of 5 replicates.)

Table 6.6. Number of ants observed licking object inside the nest during each time interval of observation (30s)

(For total duration of observation, see relevant columns in table 6.8.)

* = significant difference from whole corpse at 5% level.

Corpse age(hrs)	Whole corpse		Head and thorax		Abdomen		*Plagiolepis* sp	
	mean	s.d ±	mean	s.d ±	mean	s.d ±	mean	s,d ±
0	0.86	0.09	0.30 *	0.15	0.55 *	0.09	0.0 *	0.0
2	0.70	0.09	0.50 *	0.18	1.20 *	0.21	0.0 *	0.0
4	0.53	0.10	0.40	0.21	0.70 *	0.08	0.0 *	0.0
6	0.22	0.04	0.50 *	0.17	0.15	0.04	0.0 *	0.0
9	0.09	0.01	0.65 *	0.29	0.10	0.03	0.0 *	0.0
12	0.04	0.01	0.65	0.24	0.10	0.02	0.0 *	0.0
18	0.03	0.01	0.30 *	0.05	0.05	0.02	0.0 *	0.0
24	0.02	0.01	0.0 *	0.0	0.0 *	0.0	0.0 *	0.0
30	0.03	0.01	0.0 *	0.0	0.0 *	0.0	0.0 *	0.0
36	0.01	0.01	0.0 *	0.0	0.0 *	0.0	0.0 *	0.0
48	0.02	0.01	0.0 *	0.0	0.0 *	0.0	0.0 *	0.0
72	0.03	0.01	0.0 *	0.0	0.0 *	0.0	0.0 *	0.0
96	0.02	0.01	0.0 *	0.0	0.0 *	0.0	0.0 *	0.0
120	0.02	0.01	0.0 *	0.0	0.0 *	0.0	0.0 *	0.0
Extracted	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

(mean= mean of 5 replicates each consisting of the mean of observed number.
s.d.= standard deviation of the sample of 5 replicates.)

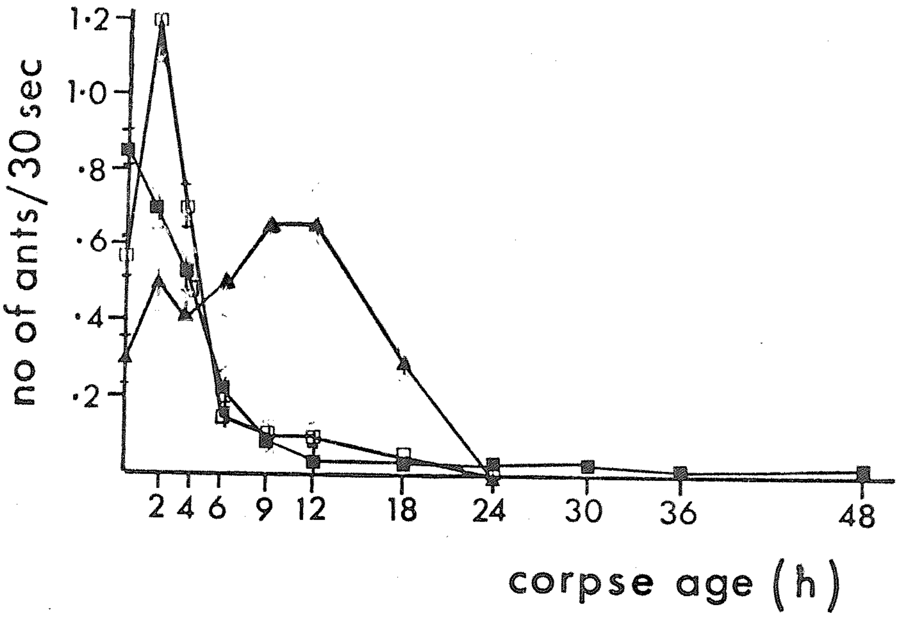


Fig 6.5. Mean number of ants observed licking during each time interval of observation (30s). (■ = whole corpse, ▲ = head and thorax, □ = abdomen).
(Full data from table 6.6.)

apparent towards older corpses. An ant approached the corpse with open mandibles, sometimes remaining in this posture for a few seconds and then leaving, or at other times grasping the corpse by parts of the body and then leaving it. This last type of behaviour was rarely observed.

iii) Corpses older than two days. As before, one minute elapsed before the ants started examining the corpse. The examination was carried out over the whole body. Licking of the body was rarely observed, and the aggressive type of behaviour observed towards group (ii) was also seen. In this third group, this behaviour was much more evident and frequent (fig. 6.6). The workers grasped parts of the body with their mandibles or ran towards the corpse with open mandibles. Eventually, one of the workers grasping the corpse lifted it and attempted to carry it out. It did not go out directly because other ants tried to take the corpse from it. Pulling of the corpse by two opposing workers was frequently observed. The time between introduction and removal never exceeded 15 min.

It must be noted that the limits of these categories were not strict and behaviour changed gradually from one kind to another.

b) Heads and thoraces. The head and attached thorax of corpses of various ages were placed inside the nest and behaviour of workers towards them was noted.

Again, three different phases were observed. The first, as before, was characterized by licking, the majority of which however, was not towards fresh corpses but to corpses 9 - 12h old (fig. 6.5). This indicates that the stimulus for licking appears later and since no other change occurs in the corpse, it must be of olfactory nature, The duration of examination and licking decreased as older corpses were used. However, the duration increased when heads and thoraces 12 - 18h old were placed inside the nest. This duration reached a maximum for 18h corpses and then decreased again (fig. 6.7). In the second phase in which behaviour towards heads and thoraces of one to

Table 6.7. Number of ants observed threatening object inside the nest and/or grasping it during each time interval of observation (30s).

(For total duration of observation, see relevant columns in table 6.8.)

* = significant difference from whole corpse at 5% level.

Corpse age(hrs)	Whole corpse		Head and thorax		Abdomen		*Plagiolepis* sp	
	mean	s.d ±	mean	s.d ±	mean	s.d ±	mean	s.d ±
0	0.07	0.02	0.20	0.05	0.14	0.08	0.50 *	0.22
2	0.08	0.02	0.24	0.08	0.21	0.07	0.80 *	0.27
4	0.08	0.02	0.15	0.07	0.14	0.06	0.80 *	0.20
6	0.08	0.02	0.16	0.09	0.15	0.06	0.89 *	0.17
9	0.07	0.01	0.15	0.07	0.12	0.07	0.85 *	0.24
12	0.07	0.01	0.15	0.09	0.05	0.03	0.90 *	0.27
18	0.08	0.01	0.12	0.06	0.05	0.02	0.95 *	0.25
24	0.07	0.02	0.10	0.07	0.05	0.02	1.00 *	0.28
30	0.08	0.01	0.12	0.05	0.0 *	0.0	1.05 *	0.27
36	0.09	0.02	0.15	0.06	0.0 *	0.0	1.10 *	0.26
48	0.10	0.02	0.20 *	0.08	0.0 *	0.0	1.20 *	0.31
72	0.20	0.03	0.20	0.07	0.07 *	0.04	1.20 *	0.29
96	0.26	0.04	0.50 *	0.15	0.20	0.09	1.30 *	0.33
120	0.25	0.04	0.90 *	0.25	0.20	0.11	1.30 *	0.30
Extracted	0.45	0.21	0.80 *	0.22	0.20 *	0.07	1.20 *	0.27

(mean= mean of 5 replicates each consisting of the mean of observed numbers.
s.d.= standard deviation of the sample of 5 replicates.)

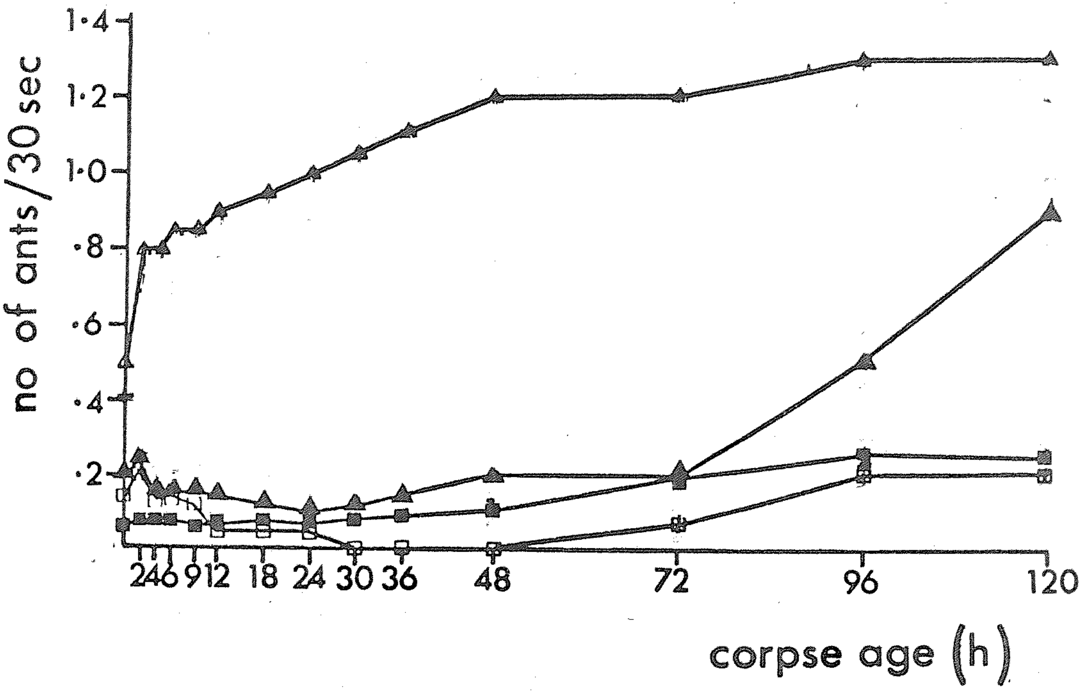


Fig 6.6. Mean number of ants observed threatening the corpse with open mandibles and/or grasping it during each time interval of observation (30s). (■ = whole corpse, ▲ = head and thorax, □ = abdomen, △ = Plagiolepis sp.) (Full data from table 6.7.)

Table 6.8. Time period between the introduction of the object inside the nest and the final pick-up (in min.)
* = significant difference from whole corpse at 5% level.

Corpse age(hrs)	Whole corpse		Head and thorax		Abdomen		*Plagiolepis* sp	
	mean	s.d ±	mean	s.d ±	mean	s.d ±	mean	s.d ±
0	120	29	120	33	120	26	8.0 *	3.0
2	67	15	45 *	15	60 *	13	4.0 *	2.0
4	51	12	32 *	14	50	15	2.5 *	2.0
6	38	15	30	12	45	17	2.0 *	0.8
9	29	7	8 *	6	20	12	1.5 *	0.7
12	25	5	45 *	12	60 *	19	1.5 *	0.6
18	18	4	60 *	15	45 *	12	1.5 *	0.5
24	16	3	13	10	18	7	1.0 *	0.4
30	12	4	16	9	2	1	1.0 *	0.7
36	10	3	14	7	3	1	1.0 *	0.8
48	7.5	2	7	4	6	3	1.5 *	0.8
72	9	3	13	2	18	1	1.0 *	0.9
96	12	4	1	1	1	1	0.5 *	0.4
120	7.5	2	8	3	3	2	1.0 *	0.7
Extracted	5	2	3	1	2	1	1.5 *	0.9

(mean= mean of 5 replicates each consisting of the observed time periods.
s.d.= standard deviation of the sample of 5 replicates.)

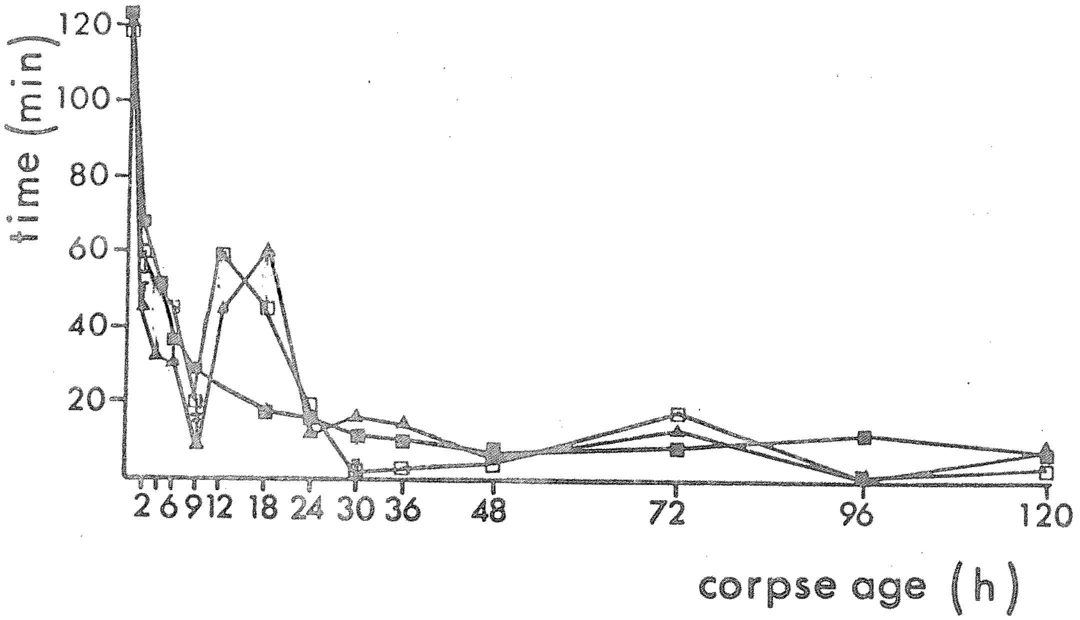


Fig 6.7. Mean time period between the introduction of the corpse and the final pick-up. (■ = whole corpse, ▲ = head and thorax, □ = abdomen).
(Full data from table 6.8.)

three days old was observed, licking was non-existent, again, but aggression towards them was higher than towards whole corpses and the ants took less time to pick them up and carry them outside.

The third phase was characterized by a much higher level of aggression. There was frequent opening of mandibles and fast forward movements of the head and also grasping the body without lifting it or attempting to bite it. The duration of examination before pick-up remained at short levels (fig 6.7). In all three phases, the increase of the duration of examination and the higher levels of aggression may be due to differences in tactile stimulation.

c) Abdomens. Abdomens of corpses of various ages were placed inside the nest for observation. As before, three phases of behaviour could be observed.

The first was very similar to the behaviour towards heads and thoraces with a slight longer period of examination, but the same sudden increase occurred with corpses 12 - 18h old. This again can be explained by the difference in shape. Licking was again very frequent with a peak at 2h, earlier than with the head and thorax and at almost double the intensity (Fig. 6.5). This suggests that the stimulus for licking must originate from the abdomen and if it is olfactory in nature it must reach its highest concentration 2h after death. After 24h there was no licking at all.

The second phase showed lower levels of aggression on the one hand and faster pick-up on the other. The ants seemed to be less interested in abdomens of this age.

This pattern of behaviour remained in the third phase, with abdomens older than three days. The aggression did not increase substantially and the abdomens were picked up and transported within one minute after they were placed inside the nest.

d) Removal of the chemical signal. As in the first bioassay, corpses that had been left in acetone for one month and dried for a further month, were placed inside the nest.

Ants responded immediately to a corpse by running towards it and grasping it by the head. One worker remained in this position for 22 min. while others remained approximately 2-3 min. Grasping of the parts of the body was violent and aggressive. In general, aggression towards whole corpses increased but not to the point where corpses were dismembered.

Aggression remained at the same levels towards heads and thoraces treated with acetone but was still higher than that towards whole corpses, while it remained low towards abdomens which were completely ignored (table 6.7). All three objects were carried outside the nest quite soon after being placed inside, the most attention being given to whole corpses (table 6.8). The objects were frequently deposited not outside but inside the nest in one of the corners where other debris had accumulated. Also, the manner in which the corpses were carried did not exactly fit the description of necrophoric behaviour. The porters did not move very fast and did not go directly towards the entrance. When stopped by other workers, they usually remained in their position for several minutes, allowing the others to examine or even grasp the corpses or parts of them.

These results suggest that, apart from the loss of the typical necrophoric behaviour, the absence of chemical signals is also followed by increased levels of aggression if the object towards which it is directed has a shape like that of another ant.

Corpses were used which were covered with a gold-palladium film used in electron scanning microscopy, as in section 6.3.2d. Covered corpses which were introduced in the nest were met with slight aggression at first, which consisted of open mandibles and temporary grasping of the corpses. Soon afterwards, that is after two minutes, licking was observed. It was relatively high and lasted until 30 min. after the introduction. At that point, a worker lifted the corpse and carried it outside the nest and towards the periphery in the typical necrophoric pattern. Covered heads and thoraces were treated in a similar way. Licking increased slightly but the duration of examination was almost the same. Abdomens were also treated in approximately the same way. There was a longer period of examination but they were carried outside similarly.

The results of both bioassays with covered corpses showed a marked similarity with results from corpses of 9-12h old. It seems that covering a corpse with a metal film cannot completely inhibit the volatilization of all the chemicals. A small proportion does escape and that amount is equal to the amount of chemicals coming from a corpse 9-12h old. It also suggests that these chemicals are not contact pheromones of the cuticle.

e) Objects treated with acetone corpse extract. The concentrated acetone extract of the previous bioassay was put on pieces of filter paper which were then introduced into the nest and the behaviour of workers towards them was noted. The pieces, after being examined by a small number of workers, were lifted and taken out of the nest towards the periphery. No licking or aggression was observed. The same behaviour was observed with control pieces of filter paper that were treated with acetone only. The only difference was the duration of examination. The filter papers that contained the corpse extract were examined for an average of 2 min. while the controls were examined for an average of 7 min.

1 µl of the acetone extract was also put on acetone-treated corpses that had been left to desiccate for one month. The corpses were examined, licked and transported outside after an average of 33 min. Controls that had been treated with acetone only were not licked at all, elicited aggression and were transported outside after only 5 min. of examination. A comparison of the results with corpses treated with the corpse extract with the results obtained from 6h old corpses showed very significant similarities (Table 6.10). This leads to the conclusion that the acetone extract contains those factors that are present in 6h old corpses and cause necrophoric behaviour when inside the nest.

f) Objects treated with oleic acid. One µl of various concentrations of oleic acid in diethyl ether was bioassayed on filter paper and on corpses, for necrophoric behaviour inside the nest. Neither concentrations of 1µg/ml, 10µg/ml, 100µg/ml and 1mg/ml on filter paper nor the controls elicited any licking or aggression and were transported to a corner of the nest after an average of 2.5 min. Concentrations of 10mg/ml, 100mg/ml and 1g/ml on filter paper elicited a significant amount of licking and also some aggression from the workers that examined them. They were examined for an average of 7.5 min. before being taken to a corner of the nest. No filter paper was seen to be taken outside the nest or transported in the typical necrophoric pattern of behaviour.

Corpses treated with concentrations of 1μg/ml and 10μg/ml and the control corpses treated with diethyl ether elicited no licking or aggression and were transported an average 5 min. after introduction. Corpses treated with concentrations of 100μg/ml, 1mg/ml, 10mg/ml, 100mg/ml and 1g/ml were heavily licked but elicited no aggressive behaviour and were transported to a corner of the nest after an average 28 min.

A droplet of oleic acid was deposited on a number of live ants that were standing in the entrance of the nest. Most of the workers that encountered them responded in the same way as towards any other worker. A few licked the ant at the point where the droplet had been deposited.

It seems from these results that oleic acid elicits only one of the characteristic components of necrophoric behaviour, licking, when inside the nest. All the other components, including the typical necrophoric pattern are absent (Table 6.11).

g) Behaviour towards other objects. Various objects were introduced into the nest and the behaviour of workers observed.

Pieces of wood and leaves evoked no aggressive responses. Some workers which were near them examined them with their antennae and left in less than ten minutes. The pieces were eventually lifted and deposited in a corner.

A piece of Musca puparium attracted more attention. The first two workers that met it started licking it after a brief examination

Table 6.9. Responses to various objects on the trail and inside the nest.(- = absence)

	Response	Pieces of wood		Pieces of leaves		fly puparium		Drosophila s.p	
		mean	s.d ±	mean	s.d ±	mean	s.d ±	mean	s.d ±
Outside nest									
	Repellency on trail (in mm)	0.0	0.0	0.0	0.0	Not measured		Not measured	
	Duration of examination on trail(sec)	0.1	0.1	0.1	0.1				
	No encountering on trail before pick-up	6	4	7	4				
	Alarm on trail	-		-					
	Aggression on trail	-		-					
	High speed and orientation	-		-					
	Depositing on periphery	-		-					
Inside nest	No examining inside nest	1.2	0.8	1.2	0.8	2.5	1.2	4.2	2.3
	No licking	0.0	0.0	0.0	0.0	0.20	0.10	0.0	0.0
	No showing aggression	0.0	0.0	0.0	0.0	0.0	0.0	1.40	0.32
	Duration of examination in nest(min)	8	3	8	3	7	4	1,5	1.1

Table 6.10 Responses to corpse acetone extract. (+= presence, - = absence) (For explanations see: Licking- table 6.6., Aggression - table 6.7., Duration - table 6.8.)

	On trail	Inside the nest		
	Repellency	Licking (no.of ants) mean (n=5)	Aggression (no. of ants) mean (n=5)	Duration of examination (min) mean (n=5)
On filter paper	+	0.0	0.0	2
Control	-	0.0	0.0	7
On corpse	+	0.25 *	0.0 *	33 *
Control	-	0.0	0.45	5
6h old corpse	+	0.23 *	0.0 *	38 *

* = significance at the 5% level.

Table 6.11. Responses to objects treated with various quantities of oleic acid. (FP=filter paper, C=acetone-extracted corpse) (+ = presence, – = absence)
(For explanations see : Licking – table 6.6., Aggression – table 6.7., Duration – table 6.8.)

Quantity	Repellency		Licking (no. of ants)		Aggression (no. of ants)		Duration of examination (min)	
	FP	C	FP	C	FP	C	FP	C
1 ng	–	–	0.0	0.10*	0.0	0.0*	6	4
10 ng	–	–	0.0	0.12*	0.0	0.0*	1	6
100 ng	–	+	0.0	0.62*	0.0	0.0*	1	22*
1 µg	–	+	0.0	0.68*	0.0	0.0*	2	24*
10 µg	+	+	0.42*	0.75*	0.0	0.0*	6*	28*
100 µg	+	+	0.70*	0.83*	0.0	0.0*	7*	31*
1 mg	+	+	1.35*	0.95*	0.0	0.0*	9*	35*
Control	–	–	0.0	0.0	0.0	0.36	2	6

* = significance at the 5% level.

After this, they carried it to a corner and left it there.

Much more attention was given to a dying Drosophila which was attacked from all sides after a brief examination. The behaviour was more aggressive than that shown towards acetone-treated corpses of M. pharaonis. The Drosophila was eventually dragged towards the inside of the nest.

The same aggressive behaviour was shown towards corpses of the ant Plagiolepis. They were grasped by many ants and pulled towards the interior. Comparing behaviour towards acetone-treated Plagiolepis and acetone-treated M. pharaonis, it was found that Plagiolepis elicited much higher levels of aggression and was examined for a shorter period of time before being picked up (Fig. 6.6). This can partly be due to differences in shape and size.

6.4 Estimation of threshold concentrations

The emission of volatile substances by a fresh corpse can be represented by a model of a point source of continuous emission of chemicals in still air. This model is of course theoretical because we cannot be absolutely certain that the emission is continuous and that the air is still and without turbulence. However, for a limited period of 10 min. and for the small space where the observations take place, it can offer a good approximation of the actual sequence of events.

The theoretical model has been fully expanded by Bossert and Wilson(1963) and Wilson, Bossert and Regnier (1969) and it is this model that will be used here.

We consider the corpse to be at $x=y=z=o$ with a non-absorbent plane at $z=o$. The emission rate of chemicals is Q mol/sec, the threshold concentration below which there is no response by the workers is K mol/cm^3, the diffusion coefficient of the volatiles is D cm^2/sec and the radius of the sphere that contains the concentration of volatiles above the threshold after a time period t that tends towards infinity, is R max cm. Using the equation $\frac{Q}{K} = 2\pi DR\max$, we can calculate the ratio $\frac{Q}{K}$ and also estimate K in terms of Q.

Using also the equation $t(0,5) = \left(\frac{Q}{4\pi KD^{3/2}}\right)^2$ we can calculate the time it takes the threshold sphere to reach half the maximum radius.

In the case of a fresh corpse, deposited outside the nest on a food trail, the above variables for repellency were calculated assuming that $D = 0.05\ \mathrm{cm}^2/\mathrm{sec}$ (the D for monomorine III is $0.0482\ \mathrm{cm}^2/\mathrm{sec.}$) and having observed Rmax = 1 cm:

$$\frac{Q}{K} = 2 \times 3.14 \times 0.05 \times 1 = 0.314\ \mathrm{cm}^3/\mathrm{sec.}$$

and $K = 3.183\ Q\ \ \mathrm{mol/cm}^3$

$$t(0.5) = \left(\frac{0.314}{4 \times 3.14 \times 0.05^{3/2}}\right)^2 = 5.12\ \mathrm{sec.}$$

The observations show that Rmax decreases with time reaching 0 at approximately 20h after death. Since K and D are constant, Q decreases proportionally according to the equation $Q = 2K\pi D\ \mathrm{Rmax}(1)$.

From the results with corpses on a trail, the repellency Rmax follows approximately the equation $\mathrm{Rmax} = 9-7\log T$ where T is measured in hrs. Therefore:

$Q = 2K\pi D\ (9-7 \log T)$ and as $K = 3.183Q$ max where Q max is the maximum emission rate in the beginning of the experiment, then:

$$Q(f) = 6.4\ \mathrm{Qmax}\ \pi D\ (9-7 \log T).$$

If this equation is substituted in the density function equation, then we obtain the equation:

$$U(r.t) = \frac{1.6\ \mathrm{Qmax}}{\sqrt{\pi D}} \int_0^t \frac{9-7 \log T}{t^{3/2}}\ e^{\frac{-r^2}{4Dt}}\ dt$$

which is the density function equation for corpses of M. pharaonis aged 0-24 hr. After approximately 24h, other chemical volatiles with different diffusion coefficients and rates of emission appear.

Using equation(1) we can also calculate the threshold concentration for licking in terms of Q max:

$$K = \frac{Q\max}{2\pi D\ R\max} = \frac{Q\max}{2\pi \times 0.05 \times 0.07 \text{ cm}} = 45.5\ Q\max \ \ \text{mol/cm}^3$$

and $\frac{Q}{K} = 0.02 \text{ cm}^3/\text{sec}$.

6.5 Conclusions.

The results show a pattern of behaviour that consists of a skeleton of rigid and clearly definable behavioural units, including grasping of the corpse, increased linear volocity of locomotion and orientation towards the periphery of the foraging arena. These components are observed in all situations in which ants encounter corpses which can be assumed to have an odour different from that of the surroundings. However, the basic sequence is preceded by other changing sequences of behavioural units such as licking the corpse, intense antennal examination, repellency and a ranging latency for pick-up. These components change either in intensity or in their relative position in the sequence according to both age of corpse and location of the corpse. Table 6.12 summarizes some of the variables associated with these behavioural units.

In order to identify the stimuli involved, we shall discuss first what changes occur in corpses from the moment of death up to almost complete desiccation. A live ant contains in its poison sac and the Dufour's gland a considerable quantity of pheromones which it uses for trail following (Ritter et al, 1973 ; Ritter et al, 1977), alarm, recruitment and defence against intruders (Blum, 1966). Some of these have been identified, such as 5-methyl-3-butyl octahydroindolizine or monomorine I and 2-(5-hexenyl)-5-pentyl pyrrolidine or monomorine III (Ritter et al, 1973; Talman, Ritter & Verwiel, 1974). These pheromones are discharged in small quantities whenever necessary. However, when large quantities are present, as in the case of a crushed ant presented to foraging workers, we have found that repellency and alarm are elicited. The emission of the contents of the poison sac in the Myrmicinae is controlled by two muscles, one of which hermetically closes the poison duct and one which opens it. At rest, the walls of the duct are in contact, thus preventing the contents from leaking out (Janet, 1898). When the ant dies, it can be assumed that the muscles slacken and a much larger amount of the pheromones is discharged. Since the pheromones are volatile substances, they eventually volatilize completely and leave the ant with other odours which are less volatile. These substances can be either natural odours i.e. contact pheromones or other non-volatile odours, or they can be internal fluids and products of decomposition. After a further period, the decomposition products and the fluids predominate in the external volatile odours. Finally, as the products of decomposition and the fluids have volatilized, the corpse may absorb the odour of its surroundings.

These postulated changes in the odour of the corpse could explain changes in behaviour. Fresh corpses emitting large amounts of pheromones should elicit behaviour similar to the behaviour elicited by crushed ants. The behaviour is indeed similar, although not so intense. Repellency occurs but the alarm is not so high. This may be due to the fact that a crushed ant emits a far greater quantity of pheromones in a brief space of time than a fresh corpse.

Inside the nest, the motivation of ants that protect and feed the brood and the queens, and control conditions within the nest differ from that of hungry foragers. On meeting an ant strongly emitting repellent pheromones, ants in the nest will not flee but may act to stop the emission. Licking of the corpse may serve either for the removal of exudates from the body surface or for appeasement of the emitter by offering it saliva or other secretions. Such licking behaviour towards returning nestmates has already been observed in the ant Formica fusca (Wallis, 1962). The ants prefer to lick the abdomen and also give more attention to it when it is placed on the trail. If licking of the abdomen and licking of the head and thorax are compared, it can be observed that licking the abdomen is twice as intense and reaches a maximum much earlier than licking the head and thorax. This may be because the abdomen contains the pheromones which are far more volatile than other substances on the body.

As the pheromone emission is presumed to be decreasing, licking, repellency and the number of ants licking the corpse before it is picked up also decrease simultaneously (Fig. 6.8) and in parallel. The regression coefficients of the curves in fig. 6.8 do not differ significantly at the 5% level and correlation coefficients are close to 1.

When the corpses are about 12h old, they probably have an odour which contains traces of pheromones, decomposition products, internal fluids and natural short range external volatiles. This mixture may be unfamiliar to the ants inside the nest. It is at this stage that the characteristic shape and size of the corpse may be important in the recognition as a familiar object. When an object with a familiar shape and an unfamiliar odour - a whole corpse - was introduced the ants soon carried it outside, but when an object with an unfamiliar shape and unfamiliar odour - head and thorax or abdomen - was introduced, they took much longer time examining it before they carried it outside.

This could have been due to the fact that some internal fluids came out at this point from the cut surface between thorax and abdomen, but when whole corpses with partially severed abdomens were introduced into the nest, they were treated in the same way as the whole corpses.

As the pheromones disappear and more decomposition products appear, the odour of the corpse becomes more unfamiliar to the ants inducing them to pick the corpse up and remove it from the nest or the trail in the typical necrophoric pattern. Whole corpses, heads and thoraces and abdomens are treated in the same way.

When the corpses have dried and lost all familiar odours, there appears to be differential treatment. Whole corpses are recognized by their shape alone and do not elicit aggression, Plagiolepis with somewhat similar shape and similar odour, the odour of the environment, are taken apart, while heads and thoraces with half-familiar shape and same odour provoke an intermediate level of aggression. The abdomens are detected as simple round objects. They do not receive any aggression, they are not removed to the periphery but simply set aside and are readily picked up without much examination. The greatest interest is shown towards the whole corpses, probably because they are the ones with most familiar shape. The hypothesis that the typical necrophoric behaviour is elicited by chemicals is supported by the fact that the acetone-treated corpses which are believed to have the odour of the surroundings are not transported in the typical necrophoric pattern.

Table 6.12 Correlation between corpse age, changes in the corpse, probable stimuli and behavioural patterns.

(No of + = Intensity of behaviour pattern)

			Outside nest			Inside nest	
Corpse age	Changes in corpse	Probable stimuli	Repellency	No of encounters before pick-up	Licking	Duration of examination	Typical necroph. behaviour
0 - 6h	pheromone emission	volatile pheromones(alarm recruitment etc)	+++	+++	+++	+++	+
9 - 24h	decomposition	contact pheromones, internal fluids, decomposition products	+	++	+	++	
30 - 120h	decomposition	decomposition products	0	+	0	+	
30 days	desiccation	odour of the environment	0	+	0	+	0

CHAPTER 7

EXPERIMENTAL INDUCTION OF RESPONSES

7.1 Introduction

The behavioural responses of M. pharaonis workers were studied in various experimentally induced situations, first in order to observe and describe particular behaviour patterns in more detail and secondly, in order to elucidate the factors involved.

7.2 Recruitment to a food source

The first series of experiments dealt with observations of the behaviour of the scouts as they search for food and as they return to the nest after finding it. For these observations, a large piece of wood covered with filter paper was used as a foraging area. A paper bridge connected it with the nest arena (fig 10.1, ch.10).

The first scouts that found and passed the bridge, moved cautiously and slowly, turning their antennae right and left. In the beginning, they moved in their immediate environment near the bridge, but after they covered a part of it, they moved further. The first ants that found the food that was placed 2.5 cm from the end of the bridge, stayed near it for a while and then started returning to the nest. This time, the behaviour was totally different. They moved faster in a straight line and their abdomen was touching the ground. When they met ants coming from the nest, they exchanged some antennal beatings and then moved on. After a while, the food was surrounded by a group of ants, and the circulation on the bridge had increased in both directions.

In order to find during which part of their journey, the ants deposit the odour trail, the following experiments were made:

a) The ants were allowed to pass the bridge and find the food but they were not allowed to return to the nest. As soon as they reached the end of the paper strip they were picked up with a brush and thrown away.

b) The ants were allowed to pass the bridge and return but there was no food.

c) The ants were allowed to pass the bridge, find the food and then return to the nest.

The number of ants that passed the bridge in the direction of the food per minute, was counted (Table 7.1).

From fig 7.1 we can see that when the ants do not return, the number that pass the bridge per minute is constant. Therefore, there is no factor causing recruitment. So we can conclude that either the scouts searching for food do not leave any trails or that the trail they leave is not perceptible because it has not been preceded by a certain behavioural pattern inside the nest.

In the second case, in which there is no food, the number of ants per min. increases slowly. This suggests the presence of both alerting and orientational signals since those ants that are alerted move towards the bridge and not towards other directions outside the nest. Therefore, there must be an odour trail from the nest to the new unexplored area. The behaviour of the first scouts is similar to that of the scouts in the third case, namely fast running, rapid movements of the antennae etc.

In the third case, where ants are allowed to forage and return to their nest, the number of ants per min. rises steeply. Obviously, we have here both trail laying and excitement in the nest. However, the level of one or both must be different from that in the previous case. There seems to be a transfer of qualitative differences. The behaviour of the scouts and the foraging ants is similar to that described for the normal situation in foraging.

Mass recruitment has been described as recruitment where the workers at the food increase gradually to an asymptote, contrary to

Table 7.1. Number of ants/min crossing bridge from nest to foraging arena (n = 5)
(A = No food on arena, ants do not return
B = No food on arena, ants return
C = Food on arena, ants return)

	Minutes									
Exper.	1	2	3	4	5	6	7	8	9	10
A	7.3	4.6	3.3	4.5	3.7	3.1	3.9	3.4	3.7	2.9
B	7.8	6.7	7.9	6.5	8.2	10.7	11.2	15.4	16.4	17.0
C	8.5	6.8	9.7	13.5	13.8	19.5	24.2	26.3	29.2	44.5

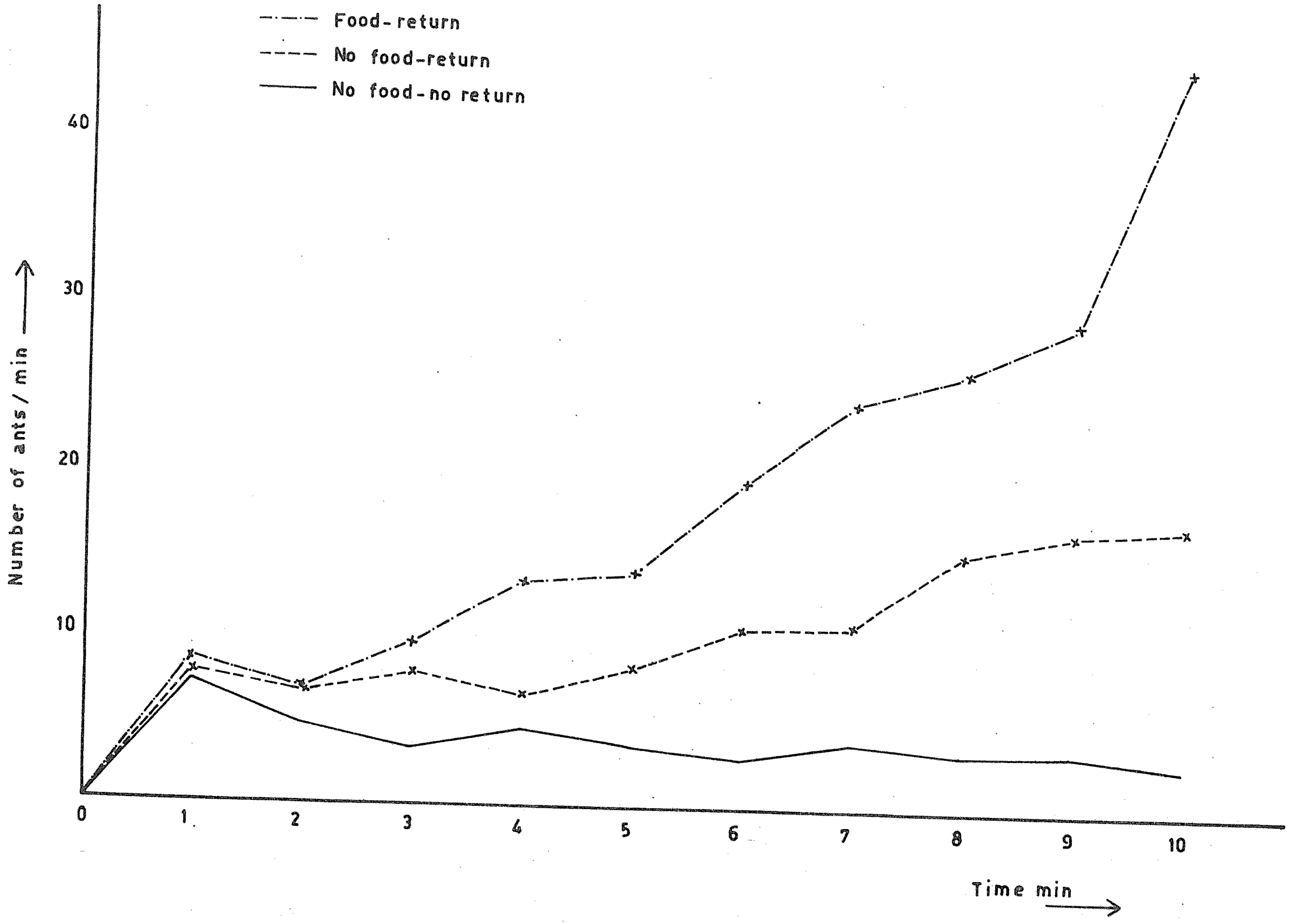


Fig. 7.1. Number of ants/min crossing bridge from nest to foraging arena.

group recruitment where a pulse of workers arrives and there is likely to be little later recruitment (Carroll and Janzen, 1973). The results suggest mass recruitment in M. pharaonis. They also suggest that at least in the phase of the development of recruitment, the presence of returning scouts is necessary. There is also the possibility of variation of information transmitted by the returning workers. Workers in the nest are able to distinguish between a new food source and a new unexplored area without food only from the messages transmitted to them by the scouts, whether they are tactile or chemical.

The results are not, however, helpful in distinguishing whether each ant secretes the same amount of pheromone, regardless of the amount present on the trail, or whether it regulates emission. Also, they are not helpful to distinguish, in the third case, recruitment for food and exploration of territory which may be occurring together.

7.3. Responses to possible sources of pheromones

In this set of observations, the responses of workers towards various glands and parts of the body were studied. The parts of the body that were used, were the head, containing the mandibular glands, the thorax, containing the metapleural glands, and the whole abdomen with the Dufour's and poison glands. Also tested, were live ants and monomorine I, one of the principal pheromones of M. pharaonis.

7.3.1. Materials and methods

Pieces of filter paper (Whatman No.1, area 1 cm^2) were placed on the ants' foraging arena, carrying for each experiment one of the following items:

1. A live ant fixed on the paper with soft paraffin wax.
2. 5 crushed heads.
3. 5 crushed thoraces
4. 5 crushed abdomens
5. 5 poison glands
6. 5 Dufour's glands

All came from the same colony, the one being tested and were all placed on the filter paper immediately after dissection except for the glands which were placed two minutes after their excision.

The controls used were blank pieces of filter paper. The control filter paper was placed and tested before the test filter paper.

The filter paper with the item being tested, was placed in the middle of a circle with radius of 4cm. (fig 7.2)

In every experiment, the number a_t of ants present inside the circle at time 0 and at every minute up to the twelvth, was counted (t from 0 to 12). Every experiment was repeated 10 times. A series of measurements a_{tn} was thus obtained (n from 1 to 10).

For every minute (t), the results of the ten experiments were added. So, for each of the 13 values of t, the $\sum_{n=1}^{10} a_{tn}$ was calculated.

The average number of ants present at every t was then calculated

$$\bar{a}_t = \frac{1}{10} \sum_{n=1}^{10} a_{tn}$$

The total number of ants for every experiment (n) was also calculated

$$\sum_{t=0}^{12} a_{tn}$$

Finally, for every item tested, the average number of ants for the total period of observation is calculated

$$\sum_{t=0}^{12} \bar{a}_t$$

Because the experiments were carried out with a varying number of ants due to different levels of activity and in order to be able

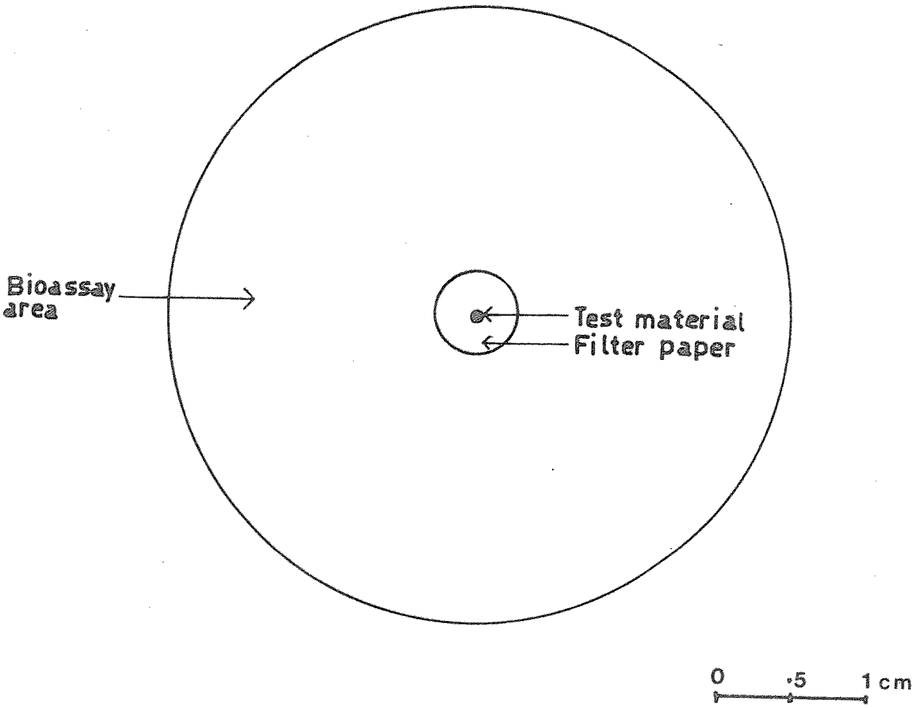


Fig 7.2. Diagram of bioassay area for the observation of responses to possible sources of pheromone secretion.

to compare the results, the measurements were changed by a factor K_n so that each quantity a_{tn} was replaced by X_{tn} according to the formula:

$$\frac{a_{tn}}{Kn} = X_{tn}$$

Where $$Kn = \frac{\sum_{t=0}^{12} a_{tn}}{\sum_{t=0}^{12} \bar{a}_t}$$. All results that appear, are

expressed as X_{tn}.

7.3.2. Results

a) Live ants. Live ants fixed on the filter paper were the most attractive to the workers on the foraging arena. The number of ants arriving on the filter paper per minute reached a maximum, 4 or 5 min. after the introduction of the ant, and throughout the observation period remained at these levels (fig 7.3.). The workers that approached the fixed ant were only slightly alarmed but were repelled at short range. They usually stopped at a minimum distance of 1 cm from the centre of the filter paper. Here, there is therefore, evidence of long-range attractancy and short range repellency.

During the period of observation, the fixed ant, apart from trying to disentangle itself from the parrafin, was also lifting its abdomen slightly upwards in a way similar to that observed when ants are emitting an alarm secretion.

b) Heads. Crushed heads were not as attractive as the live ant and their attractancy was not significantly different from that of the controls (fig 7.3.) The workers that gathered around them were slightly alarmed and were not repelled, but examined the heads with their antennae. In some cases grasping the head between the mandibles and dragging it towards the nest was observed. However,

Table 7.2 Number of ants present in observation area each min. (average of 10 experiments)

Experiments													
Controls	4.3	4.7	6.1	6.4	5.7	5.4	6.2	5.9	5.6	6.5	5.6	6.2	5.6
Live ant	4.6	12.0	16.3	18.9	19.3	21.9	20.7	19.3	21.7	20.8	22.4	23.9	23.8
Controls	4.5	4.5	5.5	5.9	6.0	6.2	6.3	6.3	6.7	6.0	6.7	6.0	5.4
Crushed heads	4.3	4.5	5.3	6.6	6.5	5.9	6.8	6.7	7.2	7.4	7.1	7.2	7.8
Controls	5.3	5.7	5.8	5.8	6.0	6.3	5.8	6.3	6.0	6.3	6.1	6.8	6.4
Crushed thoraces	5.6	7.4	7.1	6.4	7.3	8.6	7.4	8.1	7.7	7.0	6.7	7.0	6.9
Controls	6.5	7.3	8.3	6.0	6.3	4.7	8.0	6.0	5.7	4.5	5.7	6.5	6.7
Crushed abdomens	5.5	9.2	9.2	14.5	20.0	18.2	19.2	18.2	16.2	17.0	17.5	20.7	20.2
Controls	4.2	6.0	5.2	6.8	4.8	4.4	4.8	6.4	6.8	6.0	5.0	5.4	6.0
Crushed glands	5.2	10.0	11.4	12.2	14.0	13.8	13.0	16.8	16.8	16.6	15.8	15.4	16.0

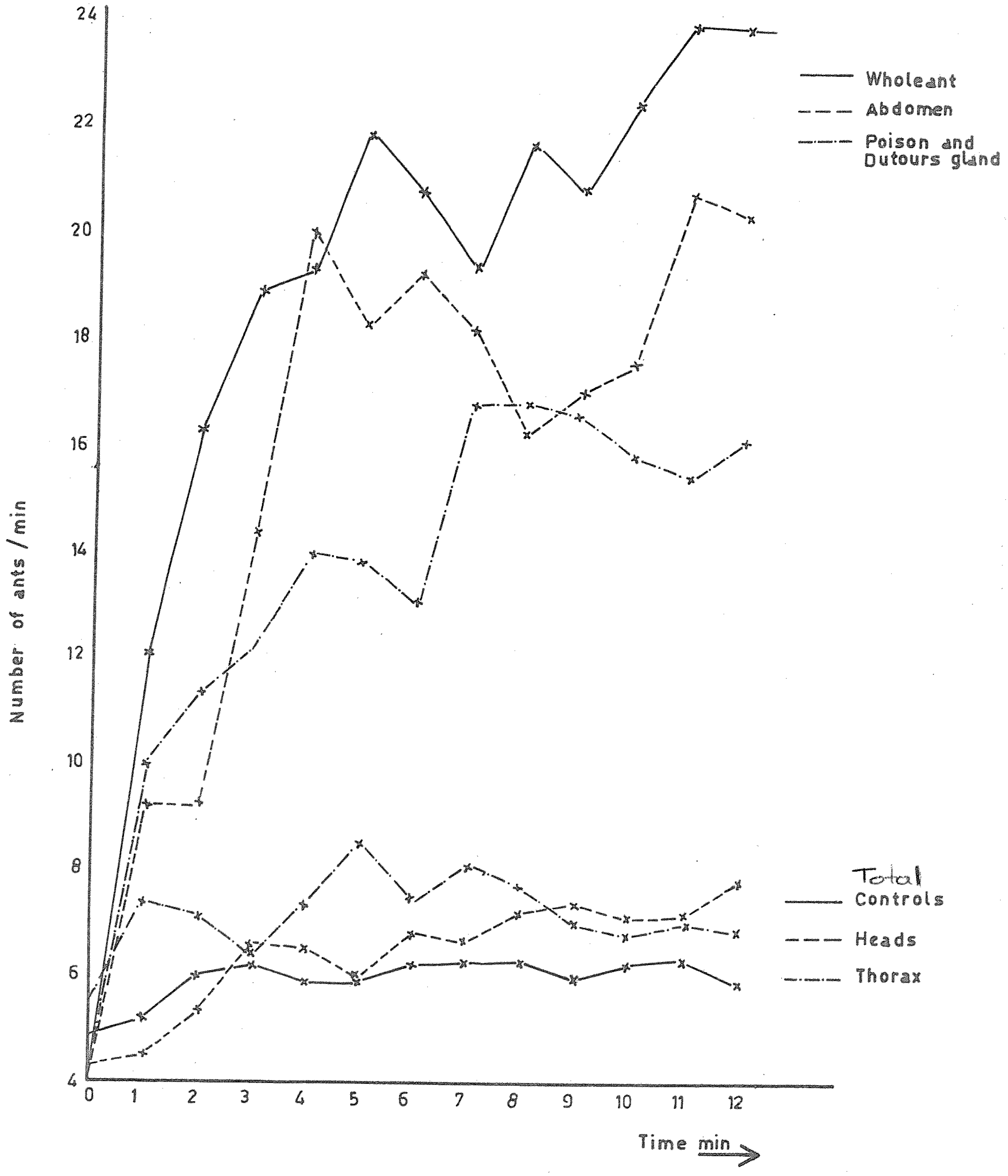


Fig 7.3. Number of ants present in observation area each min.

after a few centimetres, the head was abandoned.

c) Thoraces. The crushed thoraces were not attractive to the workers and their "attractancy" again was not significantly different from that of the controls (fig 7.3). However, repellency was observed among the workers that approached, did not touch the thoraces at all with their antennae and stayed at a minimum distance of 0.5 cm away.

d) Abdomens. Crushed abdomens were strongly attractant to the workers that encountered them. The number of ants arriving at the filter paper per min. was slightly less than when a live ant was present (fig 7.3). However, contrary to the live ant, they elicited very strong alarm behaviour. Ants were running in circles around the filter paper, standing on their hind legs and oscillating their antennae, or keeping their abdomens in touch with the ground. Their running was so fast and uncoordinated that they sometimes fell over. In addition to the alarm behaviour, repellency was also observed among those workers approaching the filter paper.

e) Dufour's gland. The reaction of workers towards crushed Dufour's glands was significantly different from the reactions towards the crushed abdomens. No alarm behaviour was observed and the ants approached the filter paper without being repelled. The attractancy of the glands was high, almost as high as that of the abdomen although the maximum number of ants per minute was reached approximately 7 min. after the introduction of the filter paper while abdomens reached a maximum attractancy at about 4 min. after the introduction (fig. 7.3).

f) Poison gland. The crushed poison glands elicited a different behaviour from that of the Dufour's gland, a behaviour that is very similar to that elicited by the abdomens. The glands produced strong alarm behaviour as described previously and were also repellent to the workers that encountered them. The long range attractanty was also present at levels similar to those of the crushed abdomens (fig 7.3). The hind gut which was also tested, elicited behaviour similar to this. It is possible that the hind gut was contaminated from the contents of the poison gland.

g) Monomorine I. Some experiments that were carried out with the pheromone monomorine I, in this bioassay, showed strong attractancy without alarm behaviour.

7.3.3 Conclusions

Table 7.3 summarizes some of the general results obtained by the bioassay. From these results it is evident that the chemicals that cause attractancy are in the abdomen, but it is not evident in which gland since both the Dufour's and the poison gland are attractant. On the contrary, strong alarm behaviour is elicited only by the poison gland, while substances in the head may play some role in the induction of alarm.

Repellency is observed in the same cases as alarm, apart from the thorax which either is genuinely repellent or was contaminated by the secretions of the poison gland.

Finally dragging, a purely aggressive pattern of behaviour, is elicited only by the heads. That may be because of two reasons:
a) the head does not have specific volatiles and is not recognized as being part of an ant of the same species, but as a possible food source, b) the head has specific volatiles that elicit aggression and slight alarm.

Table 7.3. Summary of responses towards various objects placed in the foraging arena.

Object \ Response	Attractancy	Repellency	Alarm	Dragging
Live ant	+	+	+	−
Heads	−	−	+	+
Thoraces	−	+	−	−
Abdomens	+	+	++	−
Dufour's gl.	+	−	−	−
Poison gl.	+	+	++	−
Monomorine I	+	−	−	−

CHAPTER 8

RESPONSES TO THE PHEROMONES

8.1 Introduction

Although the chemical structure of the major and some minor components of the volatiles in the *M. pharaonis* abdomen has been fully elucidated, their exact biological role is still a matter of controversy. Blum (1966) reported the poison gland as being the source of the trail pheromone while Hölldobler (1973) reported that the Dufour's gland contained the trail pheromone while the poison glands were used for defensive purposes. Ritter et al (1975) reported trail following activity in monomorine I and III and monomorene, while Ritter et al (1977) concluded that faranal from the Dufour's gland is the main trail pheromone. A more detailed study of the biological effects of various amounts of all the components of the glands both isolated and in various mixtures was considered necessary having in mind, the multicomponent nature of many pheromones in other ant species (Bradshaw et al, 1975).

8.2 Materials and methods

The bioassay apparatus is described in Ch.10. Fifteen ants were placed in the bioassay arena and allowed to settle for one hour. The amount of pheromone to be tested was deposited with a microsyringe on a piece of filter paper (Whatman No.1, r=2mm) which was placed in the middle of the arena. The number of ants entering a circle of 2.5cm diameter, the number of ants that touched the filter paper with their antennae and the time each ant remained in the circle were recorded for a period of 10 min. After this period of observation, the ants were allowed to settle for 30 min. before another observation took place. The bioassay of each amount of pheromones was repeated five times. In each series of observations of different amounts of the same pheromone, the sequence of observations was changed each time, because it was observed that in the first bioassay with a new set of ants, there was always increased activity because of exploratory behaviour.

The synthetic pheromones were obtained from various sources. The monomorine I precursor and trans-monomorine I were synthesised by Dr. C. Broomfield and Dr. G. Jackson of the Wolfson Unit of Chemical Entomology. The monomorine I isomers were synthesised by Dr. P. Sonnet and obtained through Mr. J.P. Edwards (PICL, Slough). The all-cis monomorine I, the trans-monomorine III and the cis/trans-monomorine III were obtained from Dr. F.J. Ritter (TNO, Delft Holland). All the pheromones were racemic mixtures.

The synthetic pheromones were diluted in solvents to obtain the desired concentration. The monomorine I precursor was diluted in diethyl ether, trans-monomorine III was diluted in both diethyl ether and hexane and monomorine I, cis/trans-monomorine III and monomorine I + III were diluted in hexane. One µl of each dilution was used in the bioassays.

8.3. Results

a) Monomorine I precursor 2- (3-hydroxy heptyl),6-methyl piperidine.

Various amounts of this synthetic precursor of monomorine I, (precursor 3, Oliver & Sonnet, 1974) ranging from 1 ng to 10 µg were bioassayed. None had any attractant effect on the ants. The results are summarised in Table 8.1. 10 µg elicited repellency at approximately 1 cm from the source and the ants remained in the circle for a significantly shorter period of time.

b) Monomorine I(all-cis)

Amounts ranging from 1 ng to 10 µg were bioassayed. Amounts of 1 ng, 10 ng and 100 ng elicited a significant attraction to the source. Both the number of ants in the circle and the percentage of the ants touching the filter paper were higher. The time spent inside the circle was also significantly higher. 10 µg of the pheromone repelled the ants at a distance of 1 cm from the source and also had the effect of reducing the time spent in the circle. No alarm behaviour was observed.

Table 8.1. Responses of ants to the monomorine I precursor. (*: significance at the 10% level, **: significance at the 5% level).

	No. of ants in circle		% of ants touching f.p.		Time spent in circle(sec.)	
	mean	s.d. ±	mean	s.d. ±	mean	s.d. ±
Controls	21.6	8.1.	74.0	11.0	12.8	2.3
1 ng	19.8	9.1	72.0	10.9	14.2	1.3
10 ng	22.6	8.5	77.0	6.3	14.8	3.6
100 ng	25.0	9.4	70.0	8.1	11.1	1.7
1 μg	29.4	9.0	69.0	9.4	9.6*	1.3
10 μg	26.2	2.2	30.0**	14.7	5.2**	0.7

Table 8.2. Responses of ants to all-cis monomorine I.
(*: significance at 10% level, **: significance at 5% level).

	No. of ants in circle		% of ants touching f.p.		Time spent in circle(sec.)	
	mean	s.d.±	mean	s.d.±	mean	s.d.±
Controls	23.2	5.7	73.7	9.6	14.9	1.6
1 ng	34.3**	4.1	79.7*	5.3	17.1*	3.8
10 ng	36.6**	3.1	80.6*	4.7	17.7*	3.9
100 ng	35.0**	5.0	81.5*	7.2	16.9*	2.6
1 μg	29.0	6.0	65.1	8.9	15.4	3.1
10 μg	31.0	7.1	17.2**	17.6	9.0**	3.6

c) Monomorine Ib, c and d

Only one amount of these three geometric isomers of monomorine I, 100 ng, was bioassayed. None had any attractant or arrestant properties and none elicited any significant amount of alarm. These results are not in exact accordance with previous results (Edwards & Pinninger, 1978) where it was reported that isomers 1b and 1c had some arrestant effects. However, it was mentioned there, that the arrestant activity was due to one or two ants remaining over the target area.

d) Trans-monomorine III

Amounts of this pheromone ranging from 1 ng to 10 μg were bioassayed. Only 10 μg had a low but statistically significant attractancy in the circle, but they also repelled the workers encountering the source at approximately 1 cm and reduced the time spent within the circle. 1 μg of the pheromone repelled the workers that encountered the filter paper and also reduced the time spent in the circle. 100 ng did not repel but reduced the time spent in the circle, while 10 ng and 1 ng had no significant effect. 100 ng, 1 μg and 10 μg also elicited alarm responses. The ants that encountered the source, turned at an angle of 90°-135°; moved with an increased speed and also raised the front part of the body and the head and oscillated both antennae at the same time.

There was no significant difference between monomorine III diluted in diethyl ether and diluted in hexane.

e) Cis/trans-monomorine III

Amounts ranging from 10 ng to 10 μg of this mixture of the two geometric isomers of monomorine III, were bioassayed. None had any attractant effect. 10 μg repelled the ants at a distance of more than 1.5cm, while 1 μg and 10 μg repelled the workers that entered the circle, caused a reduction in the time spent within it, and also elicited alarm behaviour as mentioned before.

Table 8.3. Responses of ants to the monomorine I stereo-isomers

	No. of ants in circle		% of ants touching f.p.		Time spent in circle(sec.)	
Isomers	Mean	s.d.±	mean	s.d.±	mean	s.d.±
Controls	19.4	3.3	72.2	7.1	13.6	1.5
1b	24.0	7.2	70.9	8.3	15.3	1.8
1c	19.5	4.6	67.3	6.2	13.1	2.0
1d	17.3	5.1	65.2	6.9	10.9	1.9

Table 8.4. Responses of ants to trans-monomorine III (*: significance at the 10% level, **: significance at the 5% level).

	No. of ants in circle		% of ants touching f.p.		Time spent in circle(sec)	
	mean	s.d.±	mean	s.d.±	mean	s.d.±
Controls (dieth.ether)	21.6	3.5	71.3	8.9	12.8	1.9
Controls (Hexane)	23.5	6.5	73.7	11.1	15.3	1.5
1 ng	26.0	5.6	61.9	9.7	10.9	1.2
10 ng	25.5	6.3	67.2	5.0	11.5	1.7
100 ng	23.7	8.8	65.2	14.1	10.3*	1.8
1 μg	30.2	6.9	55.5*	11.3	8.9**	2.3
10 μg	32.0*	4.2	9.4**	3.8	4.9**	0.5

Table 8.5. Responses of ants to cis/trans monomorine III (*: significance at the 10% level, **: significance at the 5% level)

	No. of ants in circle		% of ants touching f.p.		Time spent in circle(sec)	
	mean	s.d.±	mean	s.d.±	mean	s.d.±
Controls	33.5	6.4	71.8	5.7	12.8	2.7
10 ng	31.0	9.9	81.1	7.2	13.6	1.2
100 ng	33.0	15.6	79.5	7.3	12.0	1.2
1 μg	28.0	6.1	6.3**	3.3	8.2**	0.7
10 μg	14.5*	6.4	0 **	0	0 **	0

f) Monomorine I and III

A mixture containing 10 ng of monomorine I (all-cis) and 100 ng of monomorine III (trans) was bioassayed. The mixture elicited significant attractancy, increasing the number of ants entering the circle. This number was also significantly higher at the 5% level than that elicited by 10 ng of monomorine I alone. The mixture also repelled the ants that had reached a distance of 0.5 cm from the source and also reduced the time spent within the circle. It also elicited alarm behaviour as described before.

g) Alarm and defence secretions

One droplet of each of the alarm and defence secretions mentioned in Ch.4 was collected with a piece of filter paper which was then placed on the bioassay arena.

The defence secretion elicited the same responses as the monomorine I + III mixture i.e. attractancy up to 0.5cm, repellency at that point, reduced time spent in the circle and also alarm behaviour. Statistical analysis showed no significant differences between the secretion and the mixture at the 5% level.

The alarm secretion had similar effects. However, the percentage of workers touching the filter paper was significantly much lower than that obtained by the defence secretion, the monomorine I + III mixture and also 100 ng and 1 μg of monomorine III(trans). It was similar only to the results obtained by 10 μg of monomorine III (trans).

8.4 Conclusions

The pheromones tested are present in large quantities in the poison sac of the worker of M. pharaonis and are emitted under varying circumstances in each of which there is a different chemical message transmitted by one ant to the others. So far, it is known that the poison gland contents are emitted in three different cases which are accompanied by certain behaviour patterns: in trail follow-

Table 8.6. Responses of ants to the monomorine I + III mixture, the natural secretions and trans-monomorine III.

	No. of ants in circle		% of ants touching f.p.		Time spent in circle (sec.)	
	mean	s.d. ±	mean	s.d. ±	mean	s.d. ±
Controls (1)	25.3	7.4	71.8	5.7	12.8	2.7
Mon.I+III (2)	45.0	11.5	53.1	20.7	7.3	1.0
Defence secr. (3)	41.0	10.4	41.4	9.8	8.3	1.4
Alarm secr (4)	28.7	5.0	9.1	10.1	7.8	1.5
Mon. III						
100 ng (5)	23.7	8.8	65.2	14.1	10.3	1.8
1 μg (6)	30.2	6.9	55.5	11.3	8.9	2.3
10 μg (7)	32.0	4.2	9.4	3.8	4.9	0.5

Levels of significance (*: 10% level, **: 5% level)

(1)-(2)	**	**	**
(1)-(3)	**	**	**
(1)-(4)	NS	**	**
(2)-(3)	NS	NS	NS
(2)-(4)	**	**	NS
(3)-(4)	**	**	NS
(4)-(5)	NS	**	*
(4)-(6)	NS	**	NS
(4)-(7)	NS	NS	**

ing, in defence and in alarm. Each of these three behaviour patterns is composed of several responses and it is not impossible that each component of the poison gland emission elicits one or more responses.

The defence secretion which is associated with the specific response mentioned in Ch.5 has the role of recruiting a small number of workers at the entrance of the nest where the disturbance occurs, which then repels the intruder, causing other workers inside the nest to pick up the brood which lies near the entrance and transfer it to deeper parts as well as alarming and repelling the workers that are outside, away from the source of disturbance. The alarm secretion, which is associated with a different behaviour pattern, causes higher repellency and alarm among those workers that are outside the nest, where it is mainly used.

The mixture of monomorine I and III does provide a good approximation to these responses. Monomorine I elicits limited recruitment even in small amounts of 1 ηg. Monomorine III elicits alarm but the threshold amount is much higher. The ratio of monomorine I to monomorine III in the poison sac is about 1:5 and it was also calculated that the emitted droplet may contain from 5 to 150 ηg, therefore one droplet of emission that contains 5 ηg of monomorine I and 25 ηg of monomorine III would attract and also cause a limited amount of alarm, something much similar to that described above for the defence secretion. It was also calculated that the alarm secretion contains 5 times the amount of volatile components. A droplet containing 5 times more (25 ηg of monomorine I and 125 ηg of monomorine III) would still attract but it would cause much higher level of alarm. The possibility that monomorine III may be a component of an alarm pheromone is supported by the fact that it is more volatile than monomorine I (see Ch.4). Monomorine III is supposed here to be only one component of the alarm pheromone because at the amounts usually used by the ants in the situations mentioned above, it did not reduce the time spent within the circle by as much as the natural mixture. Therefore, there is a possibility that the minor components of the poison sac have very important roles.

In the trail, the amounts deposited by the ants are very low, usually less than 10 ng/cm. At these levels, monomorine I is still active, possibly orienting or attracting the ants to the trail, but monomorine III is below its threshold amount. However, very near the trail, the concentration may be above threshold and it may function to keep the workers excited while following the trail, a hypothesis which is supported by the fact that amounts of 10 ng/cm are active in inducing trail following (Ritter et al, 1975). Without underestimating the role that monomorines I and III may play in trails, it seems from other researchers' results (Ritter et al, 1977) that faranal has a much more important role in trail following, than the other components of the trail mixture. The importance of monomorine I may also lie in its higher persistency. Traces are still active after 24h (Ritter et al, 1975) while faranal evaporates within 1h (Ritter, unpublished data). Distant or long-lasting food sources may still be reached after 24h and it is known that scout ants use well defined routes whose position changes only slowly, over periods of some weeks (Sudd, 1960). This can only be achieved by a persistent pheromone such as monomorine I.

CHAPTER 9

SUMMARY OF FINDINGS AND CONCLUSIONS

Chemical communication between two or more organisms, involves various phases and various factors, each one of which is intimately connected with the others. First, it involves an organism that emits the message or messages. Each message may be closely associated with a behaviour pattern of the emitter. Since the message consists of one or more chemical substances, they must be produced by a group of cells, either a specific gland or another non-specific tissue and they must be stored some place, either in the cells themselves or in special containers. Their emission must be controlled if the message is not a continuous one. They must be able to travel short or long distances and they must be qualitatively and quantitatively modifiable in order to cope with the complexity of the social life of the organism. The organism that receives the message or messages must posess organs of chemical reception and a system for the interpretation of the messages. Finally, it must respond in a specific way to a specific message under specific circumstances and conditions.

In the present work, there was an attempt to elucidate some aspects of all these factors of chemical communication in _M_. _pharaonis_, and their synthesis can lead to the identification of the unanswered questions and to further detailed studies.

Volatile compounds that play a role in chemical communication are situated in two glands in the worker of _M_. _pharaonis_, the poison and the Dufour's gland. This does not exclude the possibility of other glands such as the mandibular and metapleural glands, producing active pheromones (Chs. 4.7), but the bulk of volatile chemicals, at least 95%, is present in these two glands (Ch.4). They are both situated in the posterior part of the abdomen and both lead into the sting bulb of the sting apparatus and through the sting itself, to the exterior (Ch.3). The sting in _M_. _pharaonis_, is not used as an injection apparatus but only to emit pheromones and defensive secretions into the air. The apparatus for controlling its movement is significantly reduced, and this can be correlated with a) the presence of only one abdominal ganglion in the gaster (Ch.2), b) the small size and complexity of the furcula which is connected with muscles important for the movement of the sting apparatus, and c) the almost complete absence of barbs in the lancets (Ch.3).

The secretions of the poison gland are stored in the poison sac and consist of two major components, all-cis 3-butyl-5-methyl-octahydroindolizine (monomorine I) and trans 2-(5-hexenyl)-5-pentyl-pyrrolidine (monomorine III) as well as some minor components, three of which have been identified (Ritter et al, 1975). The secretion of the Dufour's gland, which does not have a differentiated reservoir, consists mainly of an unsaturated hydrocarbon with the formula $C_{18}H_{30}$ (monomorene) (Ritter et al, 1975). One of the minor components that has also been identified is (6E, 10Z) 3,4,7,11-tetramethyl-1-6,10-trideca trienal-1(faranal) (Ritter et al, 1977).

The emitting ant utilizes these secretions in various circumstances, each of which is related to a specific behaviour pattern e.g. lowering the abdomen, extruding the sting and periodically touching the substrate with it, as in the case of trail laying, lifting the front part of the body and placing the abdomen between the legs in response to a disturbance at the entrance of the nest, or lifting the abdomen and extruding the sting in response to intense disturbance (Ch.5). In each of these circumstances a different message is emitted as witnessed by the different responses of other workers that encounter the signal.

The signal can be modified to suit each case in many ways. One way is by controlling the source of the chemicals. In the sting bulb, where both glands lead, there are two muscles that can close or open one or both glands. In this way, the ant can control which gland it will use and it can also control the quantity of the contents of each gland that it will use (Ch.3). In the three cases mentioned above, both procedures are used. The trail pheromone contains secretions from both the Dufour's and the poison gland in a ratio of 15:1, while in the other two cases only the poison gland secretions are emitted but in different quantities (5-30 ng in the first case, 30-150 ng in the second) (Ch.4).

The physical properties of the chemicals themselves can play a role in the kind of response that they may elicit. Faranal and monomorine III are much more volatile than monomorine I (Ch.4). When placed on a trail, faranal will evaporate within one hour and therefore must be constantly reinforced to provide continuous strong attraction (Ritter, unpubl.). Monomorine I on the other hand, lasts up to 24 hrs. and can be used by the ants to identify and follow long-lasting trails, such as trunk routes from the nest to foraging areas. The high volatility of monomorine III makes it a good candidate for alarm pheromone since alarm pheromones must disperse and fade out quickly.

The use of a mixture of compounds with different properties ensures the coordination of more complicated behavioural patterns than a single compound could elicit, thus increasing the information content of the pheromone. The presence of an intruder near the entrance of a nest calls for a limited recruitment of some workers for the defence of the entrance but also necessitates the removal of brood from the vicinity of the disturbance and the dispersal of younger, more vulnerable workers (Ch.5). A multicomponent mixture of pheromones containing one component that attracts and one that alarms ensures the coordination of these functions. Monomorines I and III are two possible candidates for these two roles. Monomorine I elicits limited attractancy and arrestancy while monomorine III produces alarm. (Ch.8).

The modification of a signal consisting of a mixture of various compounds produces an effective multicomponent pheromone system especially if the components have different threshold concentrations. A small amount of secretion may contain one component over its threshold and another below the threshold. In this case, only one kind of response would result, and in the specific case of a monomorine I and III mixture, there would be attraction and arrestancy. When the quantity of the mixture which is emitted increases, the second component exceeds its threshold and is activated as well (Ch.8).

The information content of one compound can increase if it elicits different responses or different levels of responses depending on its concentration. For example, monomorine III, when present in very small quantities (less than 10 ng/cm in the trail) does not alarm the workers but may keep them sufficiently activated to continue following the trail. When emitted by a returning scout, it may also serve to activate the workers inside the nest so that they leave it and follow the trail it has laid. It is known in other *Monomorium* species that scouts excite workers by specific antennal oscillatory displays (Szlep & Jacobi, 1967), but it is not impossible that in *M. pharaonis*, either the display has been replaced by a chemical message, or it is used in conjunction with it. At much higher quantities, 10µg, monomorine III is also a strong repellent, but it is doubtful if such large amounts are used by the ants. The chemoreceptors of *M. pharaonis* are situated on the last segments of the antennae. The chemoreceptors are of several types including sensilla trichodea, basiconica and placodea (Ch.2). The nervous impulses travel from the receptor sites to the antennal lobes of the insect which in the case of *M. pharaonis*, are relatively larger than in other ant species (Ch.2). From the antennal lobes, in which primary integration takes place, the signals travel to other parts of the brain where they are further integrated with signals from other receptors. Especially important for olfactory signals, are the corpora pedunculata that lie in the posterior part of the head and are known to receive neurons from the antennal lobes and also play a role in olfactory memory (Howse, 1975). In *M. pharaonis*, the part of the corpora pedunculata which is supposed to be related to olfaction, the lip of the calyx, is much relatively larger than in other ant species, indicating the increased role of chemical communication in this species (Ch.2). It is known that *M. pharaonis* uses vision only for general orientation purposes when scouts return to the nest from foraging (Sudd, 1960).

The integrated signals become specific motor patterns and the receiving ant responds in a particular way. There are many behaviour patterns of *M. pharaonis* that can possibly be mediated by pheromones. For example, trail following consists of a complex series of behavioural responses including activation of workers, orientation, attraction and other responses. Therefore, it is not surprising that many compounds are involved. From the results so far obtained, it seems that faranal is the main trail pheromone as it is the most active in eliciting attraction and trail following (Ritter et al, 1977). In quantities of 1 ng, monomorine I elicits attraction and arrestancy and is an important and persistent component of the trail. As mentioned above, monomorine III may induce workers to follow the trail and monomorene has also been reported to be active in trail following tests. The returning foragers seem to be able to communicate qualitative differences between food and new territory to those inside the nest. It is not known, however, whether this is achieved by the antennal display, the amount of volatiles secreted in the nest or the amount or quality of chemicals on the trail. If the latter, it is possible that faranal is the most important compound being both active and volatile. Also, it is not known whether each ant secretes the same amount of pheromone on the trail or whether it controls its emission according to the quantity already present (Ch.7).

Alarm behaviour is elicited by monomorine III but the natural poison gland secretion is more repellent to workers (Ch.8). Some alarm behaviour is also elicited by crushed heads (Ch.7) but the exact role of cephalic compounds of *M. pharaonis* has never been studied in detail.

Another behaviour pattern that may be mediated by chemicals is corpse carrying, or necrophoric behaviour, which involves grasping and lifting the corpse, moving directly towards the entrance of the nest and towards the periphery of the foraging arena with a linear speed greater than when transporting other materials and depositing the corpse on the periphery of the arena. Freshly killed ants, severed heads and thoraces and abdomens elicit licking when inside the nest but are repellent when outside on a trail. Oleic acid, which has been reported to elicit necrophoric behaviour in other ant species, also elicits licking and repellency. Older corpses are immediately transported. Acetone-extracted corpses and heads and thoraces elicit aggressive behaviour while acetone-extracted abdomens are ignored. The acetone extract elicits responses similar to those elicited by 6h old corpses. The evaporation of pheromones in corpses up to 24h old and the presence of decomposition products or other non-volatile chemicals in older corpses, may explain these changes in behaviour (Ch.6). It is not certain if we can use the term necrophoric pheromones to describe these chemicals. Can we accept a decomposing corpse as an individual? After all, it is not the corpse but the bacteria that produce decomposition. And can we speak of communication between a corpse and a live animal? We can only use this term if the emission of such chemicals is adaptively advantageous to the ants. If we take into account the decomposing organisms, it is difficult to distinguish who is communicating with whom and who acquires an adaptive advantage, the bacteria or the ants?

There are still many unknown aspects of chemical communication in M. pharaonis. The exact role of the minor components of the pheromones must be elucidated as well as the neural mechanisms which control their emission. Also, the chemicals that play important roles in caste and colony recognition, in trophallaxis, cleaning and other behaviour patterns must be identified. More general research into sensory and nervous physiology of ants must be carried out before we can try to elucidate those factors in such a small insect as M. pharaonis. The differences between castes, as far as communication is concerned, must also be studied, as well as the evolution of the signals in relation to other species. We must also not forget that the chemical signals are not the only signals that play a role in communication. Other possible means of communication such as optical, acoustic etc. must also be studied.

The control of this pest using pheromones is not an impossibility. Strong natural attractants such as faranal can be used to increase the attractancy of baits containing insecticides. If a sex pheromone, the presence of which has been postulated, is identified, it may be used to attract sexuals to baits or to confuse mating. Even a substance eliciting necrophoric behaviour could be useful. Spraying foraging ants with it could induce workers inside the nest to reject them as "corpses". However, the use of pheromones without prior complete knowledge of the biology and ecology of this species as well as any pest species, can be a fruitless activity.

CHAPTER 10

EXPERIMENTAL

10.1 Culture of ants

a) *M. pharaonis*

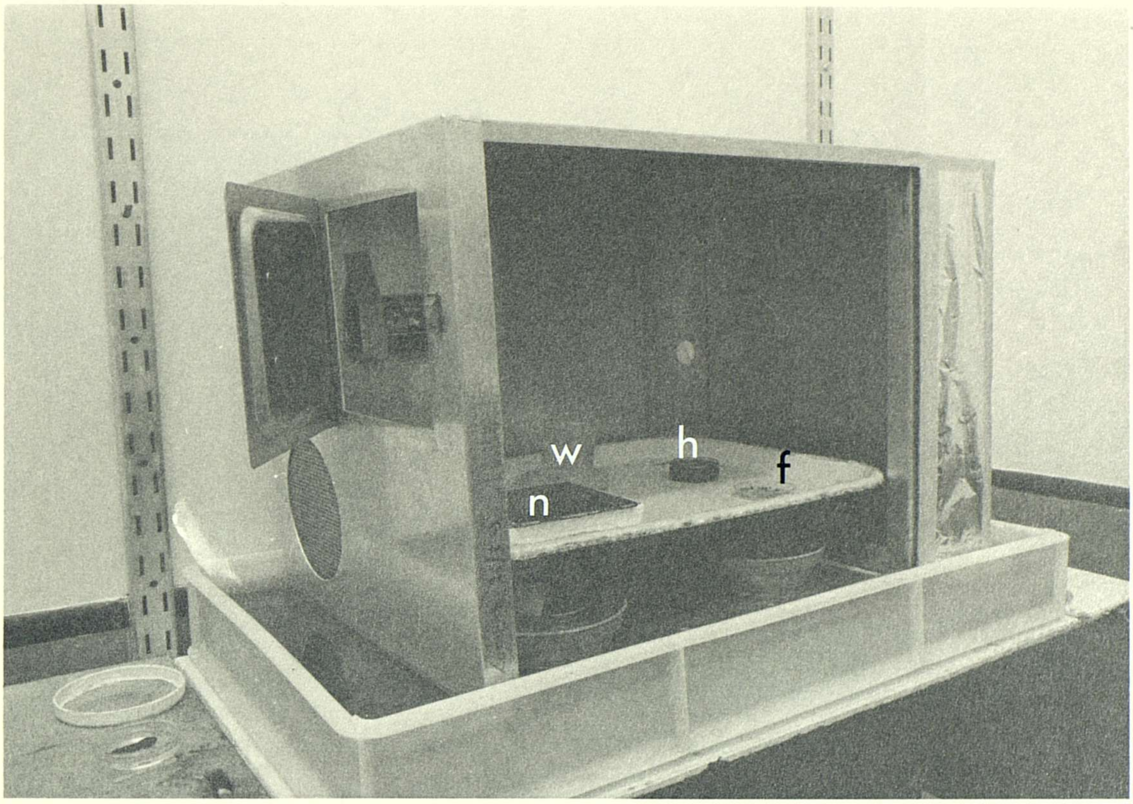
The original colonies came from the Pest Infestation Control Laboratory of the Ministry of Agriculture, Fisheries and Food in Slough, Berkshire. They were housed in wooden boxes without tops, with dimensions 13 x 8.5 x 1.5cm. The boxes were covered with glass so that it would be possible to observe the inside of the nest without disturbing the ants. The glass itself was covered by a piece of wood to protect the nest from intense light.

Care was taken during transportation to prevent the ants from escaping from the nest. The whole nest was placed in a plastic bag which was then sealed hermetically.

The nests were placed on large trays (51 x 38 cm). These trays contained a dish with watered cotton to keep the appropriate humidity and another dish with a solution of honey in water as a source of carbohydrates (pl 10.4). The food used was cut pieces of liver, locusts, crickets and *Tenebrio* larvae. It was changed every three or four days because moulds would appear after a while. The water in the cotton dish was also changed every week. Later, an inverted 50 ml beaker containing water was placed on the cotton dish in order to provide a larger supply. The edges of the tray were covered with soft paraffin wax to prevent the ants from escaping. The whole tray was placed over a solution of soap in water so that the ants that escaped would drown. The temperature of the culture room was 27°-30°C and the relative humidity around 70%. Temperature was kept constant with a heater fitted with a thermostat. Humidity was provided by a 'Defensor' humidifier and later by a water bath fitted with a heating element, both controlled by a hygrostat. Light was maintained on a 12 hours light, 12 hours dark cycle.

pl. 10.1. Culture box of *M. pharaonis*
(n: artificial nest, f: food, h: honey, w: water)

pl. 10.2. Culture boxes of *F. rufa*.
(n: nest material, g: glucose, w: water)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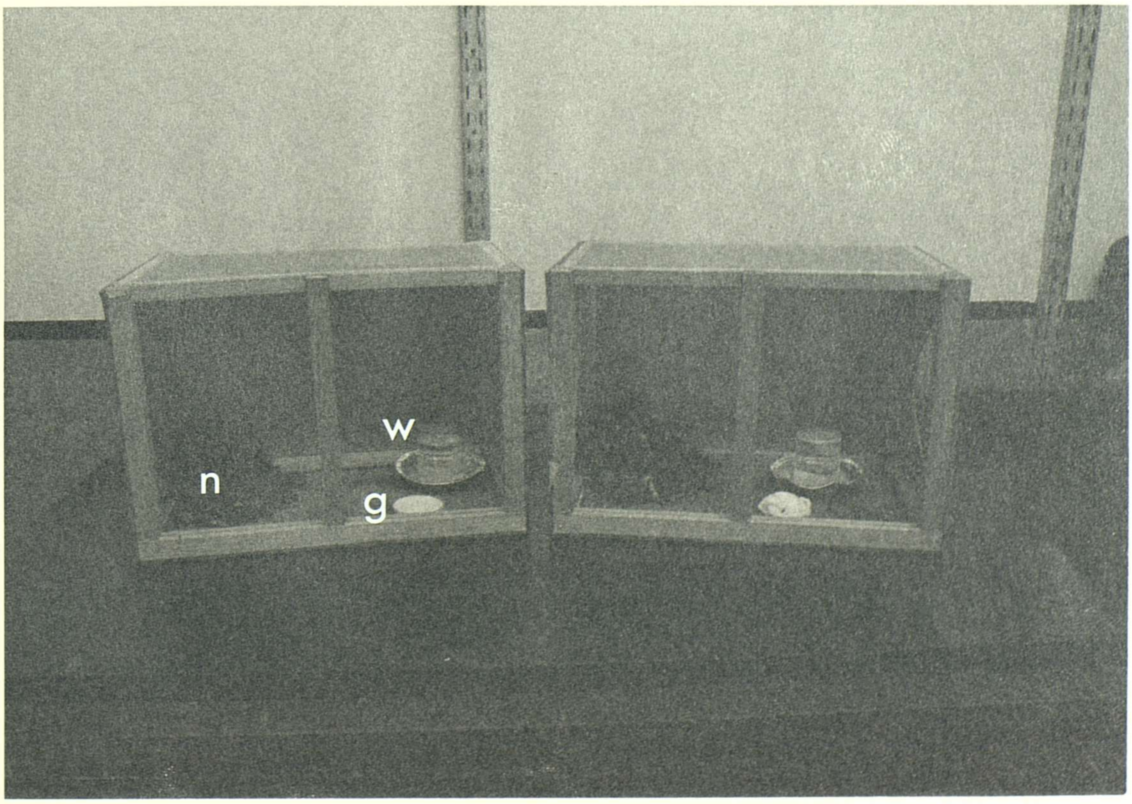
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When, due to technical difficulties, the humidity of the culture room fell to 50% the nests were covered with a transparent perspex cover with holes for ventilation which increased the humidity up to 70%. Later, the tray was placed inside a large aluminium box (56 x 40 x 40 cm) with holes for ventilation and a porthole for putting new food and water. The front side of the box was covered with perspex to enable observation and all the other holes were sealed. The box was placed on a tray full with water. Humidity inside the box was 75% (pl 10.1).

After arrival in the laboratory, the colonies were left to adapt to their new environment for approximately 10 days before any experiments were carried out. The colonies showed very good growth and multiplied sufficiently so that more colonies could be established.

The new colonies were placed in nests built from plaster of Paris and having the same specifications as the wooden nests. They too were placed on trays and kept in the same culture room. Despite their good performance, the first new colonies died after 5 months because of a breakdown of the thermostat that raised the temperature to very high levels.

In general, the humidity in the room showed high fluctuations at times, ranging from 45% to 80%. For this reason, the number of ants available for bioassays and especially for chemical analysis never reached ideal levels.

b) *Plagiolepis sp.*

The ants of this species nested in a glasshouse situated next to the building where the laboratories of the Chemical Entomology Unit were. The glasshouse contained tropical plants such as banana plants, and it is believed that the ants were introduced with them since they are not native to the U.K. They formed very long (25m) and persistent (6 months at least if undisturbed) trails. They fed on dead moths, *Musca*, cockroaches, sugar and honey solutions.

One banana plant containing a large nest of *Plagiolepis sp.* was cut and transferred to the culture rooms where it was kept on a tray. The ants were provided with water, honey and dead locusts. The room was kept at 24-29°C and RH 65-85% on a 12 hours light, 12 hours dark cycle.

c) *Formica rufa*

The original colony of *F. rufa* was a large nest collected in the New Forest, Hampshire, consisting mainly of pine leaf litter. The nest was mound shaped with a diameter at the base of 60cm and a height of 30cm. It was placed in a large dustbin and provided with honey, water and food.

The subcolonies that were formed from the original colony, were placed in rectangular boxes (40 x 28 x 25 cm) with removable perspex covers on one side and a fine mesh on the other. The boxes contained 1000 cm^3 of pine leaf litter, water, glucose and *Tenebrio* larvae (pl 10.2). They were kept in 25°C temperature and 50% relative humidity. The subcolonies containing 25 workers each, lasted an average of 8 days.

10.2 Observations

Observations of the inside of the nest, of the foraging arena and the trails, were made with the help of a travelling microscope. It consisted of a 'Watson' microscope fitted on a long horizontal arm (pl. 10.3).

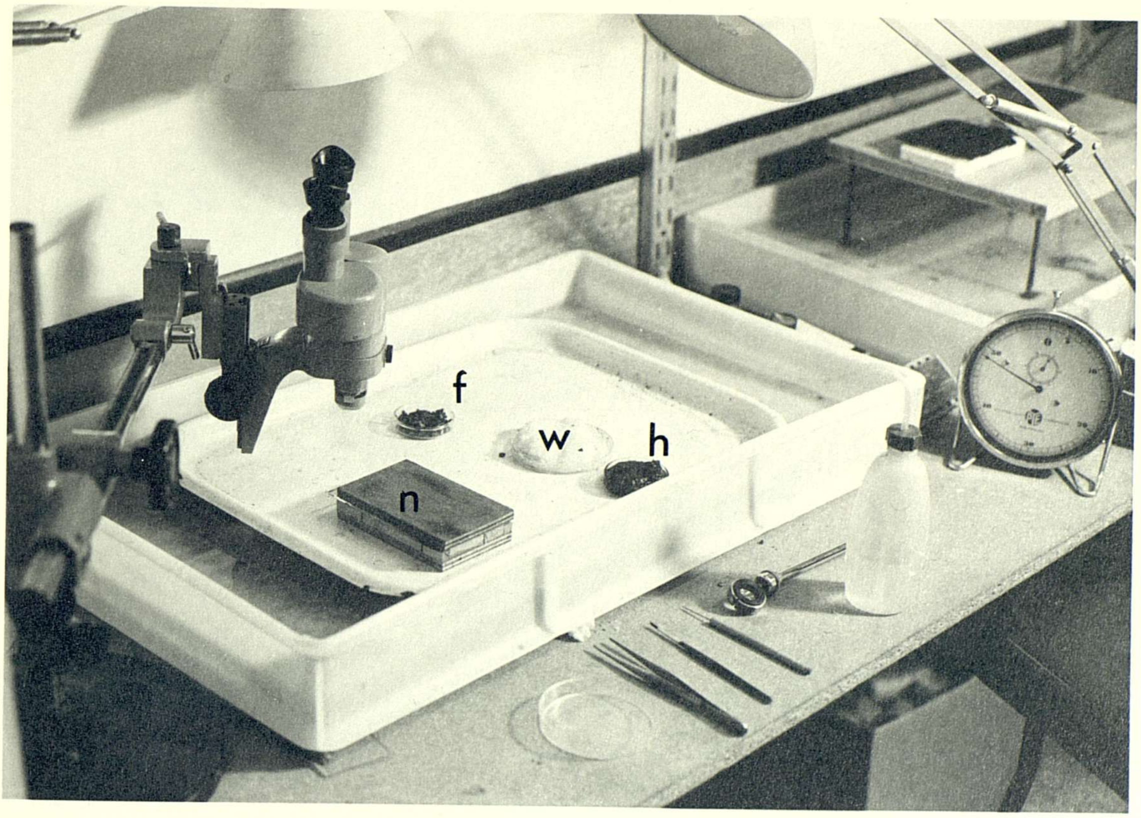
Observations of the bioassays involving a standard number of ants were made with a 'Nikon' zoom binocular dissecting microscope.

Observations of whole mounts and excised parts were made with a 'Vickers Instruments' binocular dissecting microscope with a maximum magnification of 140x.

Observations of sections were made with a 'Wild' high power optical microscope fitted with a camera lucida and a 'Nikon' high power optical photo-microscope.

pl. 10.3. Experimental set-up for observation of *M. pharaonis* (n: nest, f: food, h: honey, w: water)

pl. 10.4. Artificial nest and foraging arena of *M. pharaonis* (n: nest, f: food, h: honey, w: water, fa: foraging arena)


f
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h
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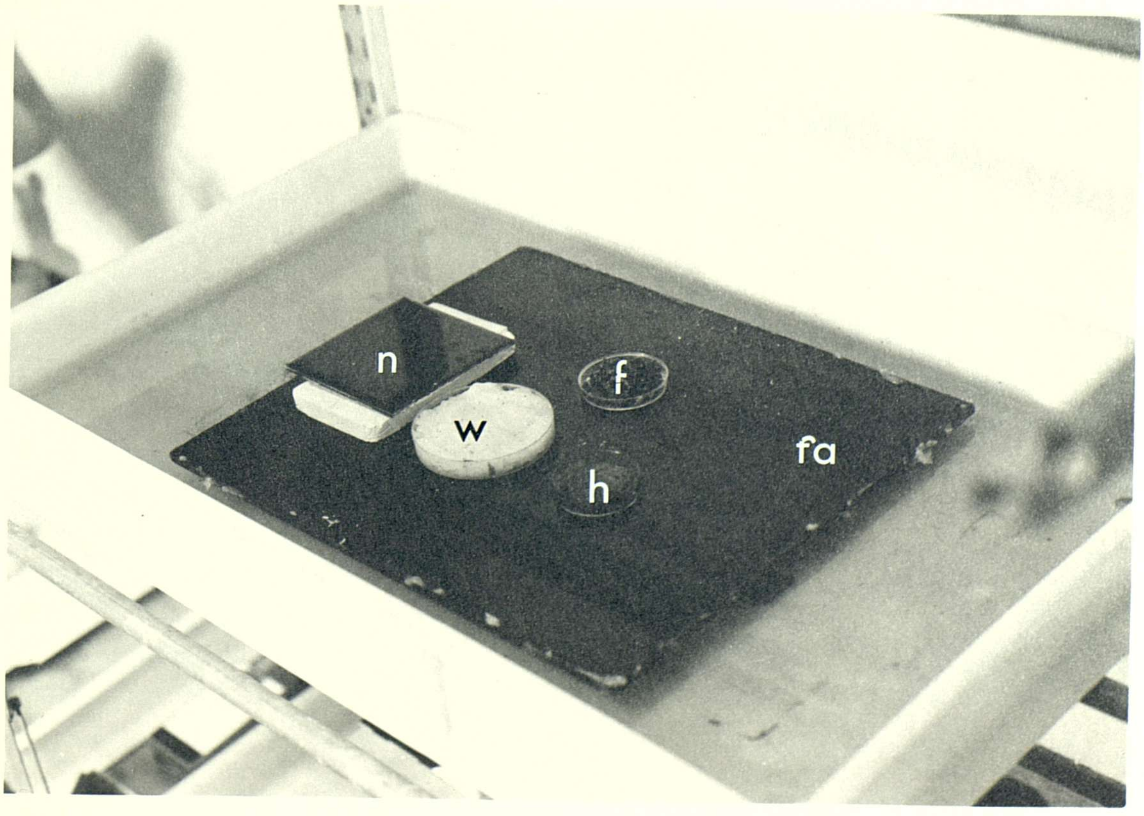
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Scanning electron photographs were taken with a 'Jeal' scanning electron microscope. The specimens were first covered with a gold-palladium film.

10.3 Whole mounts of sting apparatus, dissection of glands.

The ants were placed on petri dishes containing dry ice and left for 15 min. to die. They were then placed on a wax tray under the dissecting microscope. For the excision and the study of the poison and Dufour's glands and the other parts of the abdomen, very fine entomological pins were used, inserted into matchsticks. The abdominal segments were removed and the glands excised. The results were not successful because it was very difficult to distinguish the glands after the cuticle was removed. Also, the high temperature caused by the strong lights evaporated a significant amount of the substances present in the gland. For this reason, instead of the ordinary tungsten lamp, an optical fibre was used, connected to the lamp. The optical fibre did not produce any heat and the evaporation of the volatiles was averted. In order to overcome the difficulty of distinguishing the glands, the wax tray was replaced by a petri dish containing water. This method proved more successful because the glands could be clearly distinguished once they were in the water.

For the study of the skeletal components of the sting, the abdomen was placed on a wax tray and the abdominal segments were carefully removed one by one with the help of fine watchmakers forceps and entomological pins. The remaining sting apparatus together with the hind gut, was left in a 5% solution of KOH for one day to dissolve all the non-chitinous parts. The remaining sclerites were then placed in ethylene glycol and observed under both the dissecting and the high power microscopes. Care was taken to use diffused light in the dissecting microscope by placing a translucent plate between the light source and the mirror of the microscope.

10.4 Fixing, sectioning and staining sections.

Specimens were fixed in Carnoy's solution, washed in 80%

alcohol for 24 hrs, dehydrated via 90% alcohol (2 changes), equal parts of absolute alcohol and ether 24 hrs. Specimens were transferred to 2% low viscosity nitrocellulose (LVN) for three days and left for a further three days in 6% LVN. Specimens were then placed in fresh LVN in a suitable mould and the celloidin hardened in chloroform vapour. After hardening, the blocks were trimmed and cleared in a mixture of chloroform 30 mls, origanum oil 30 mls, cedarwood oil 30 mls, absolute alcohol 75 mls, phenol 7.5 gms. The cleared blocks were placed in benzene for 24 hrs, transferred to molten paraffin wax (MP 58^{o}C), infiltrated, embedded and sectioned at 7μ . Sections were stained in Ehrlich's haematoxylin and counterstained in eosin, in 90% alcohol, dehydrated, cleared in xylol and mounted in Canada balsam. The technique is described in detail in Corrington (1941).

10.5 Chemical analysis

a) Columns

All chemical analyses of samples were carried out on a Pye-Unicam 104 gas chromatograph with dual flame ionisation detectors (FID), injection port heaters and a detector oven. The chromatograph was coupled to a potentiometric recorder. All-glass columns were used, fitted with 6 mm o.d. ground glass ends. The columns were connected to the detector, with a Pye-Unicam column connector with a viton O-ring.

The columns used were the following:

4 mm(i.d.) x 1.5 m 5% Carbowax 20M/Diat C/HMDS treated.
4 mm(i.d.) x 1.5 m 5% OV-1 on 85-100 mesh Diat C/HMDS.
2 mm(i.d.) x 3m 5% OV-101 on 100-120 mesh CLQ.

The columns were packed by connecting the detector end to a pump and applying reduced pressure. A funnel was fitted on the other end and the packing was slowly dropped in the funnel. The column was tapped until all the packing was well pressed. The ends were sealed with glass wool. The columns were conditioned overnight at temperatures higher than those used and the flow rates of the carrier gas were reduced to half the normal.

Nitrogen (BOC high purity) was used as carrier gas with flow rates 25 ml/min for the 2mm i.d. column and 50 ml/min for the 4 mm i.d. columns. The flame for the ionisation detector was provided by air (BOC) and hydrogen (BOC high purity).

In order to obtain experience using gas-liquid chromatography, a pine oil extract was analysed using first a silica gel chromatographic column which separated it into two fractions, one containing only hydrocarbons and the other only oxygenated compounds, and then using the 5% 20 M Carbowax/Diat C/HMDS column at 70°C for the hydrocarbon fraction and 130°-180°C, 4°C/min, for the oxygenated fraction. Mass spectra were also obtained on an AEI MS 12 spectrometer coupled to a Pye-Unicam 104 system via a Watson-Biemann glass-frit separator. The spectre were analysed by a V.G. Digispec 16 data system.

b) Solid sample gas chromatography

The solid sample injector of Morgan and Wadhams(1972) was used for the gas chromatographic analysis of whole ants, heads, thoraces, abdomens, individual glands and filter papers containing extracts. The ants were killed with dry ice and the parts to be examined were placed inside glass capillary tubes, 1mm(i.d.) x 5cm, made from Pasteur capillary pipettes, sealed at the bottom. The material was pushed to the sealed bottom of the tube and the other end was quickly sealed. The tubes were inserted into the solid sample injector and were heated up to 150°C. After 5 min. they were crushed and all volatiles were taken up by the carrier gas into the column. When the temperature of the oven reached 100°C, the injector heater was turned off to avoid burning the material. After the run was finished, the injector was disconnected and the glass fragments and the pieces of material left, were removed.

10.6 Bioassays

a) Recruitment to a food source

The observations of recruitment to a food source were

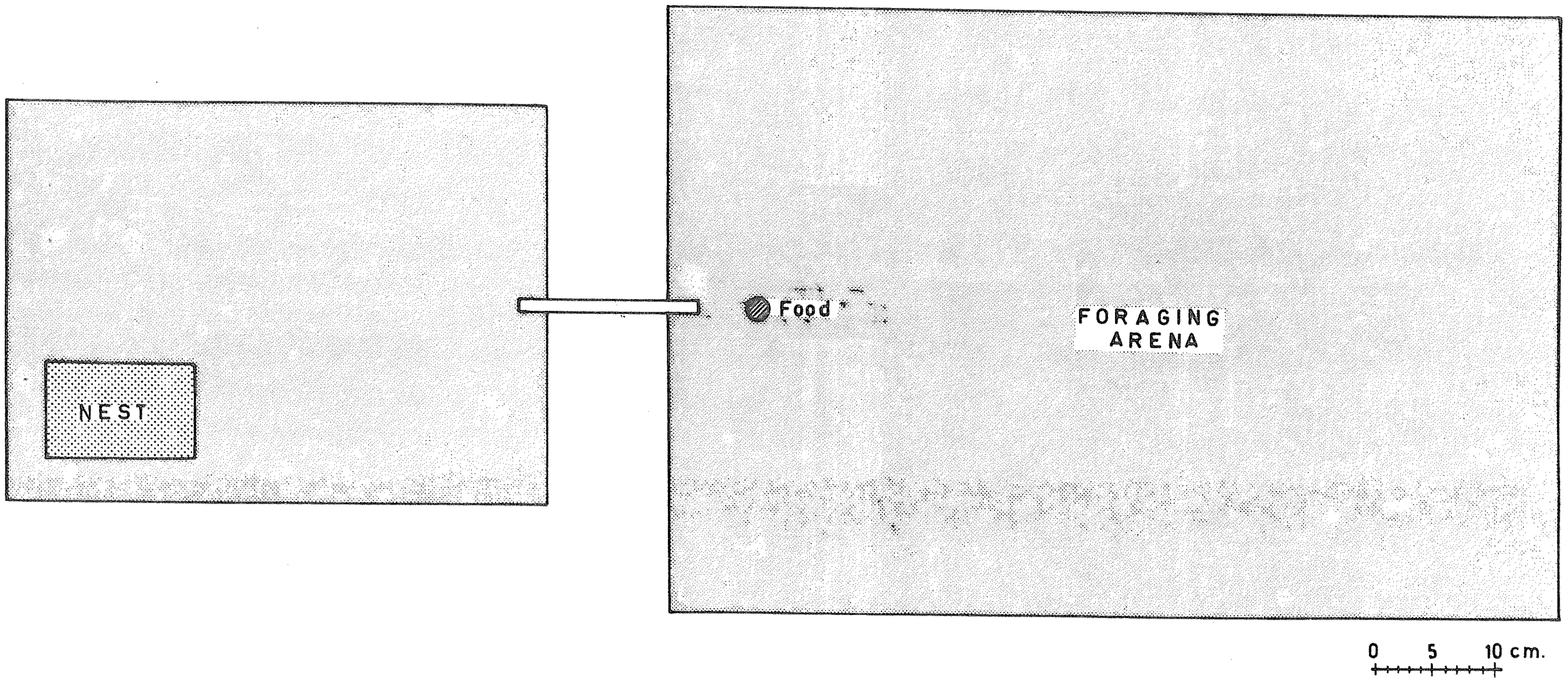

Fig. 10.1. Layout for the observation of recruitment to a food source.

carried out on a large foraging arena placed 10 cm away from a nest tray. The nest tray was similar to that described in section 11.1a. The foraging arena consisted of a table, the surface of which was at the same level with the level of the nest tray. The dimensions of the top of the table were 75cm x 50cm. A large sheet of white filter paper with the same dimensions, was placed on the top and its edges were covered with paraffin soft white to prevent the ants from escaping. The foraging arena and the nest tray were connected with a strip of filter paper, 1 x 15 cm. After each observation, the large sheet of filter paper and the bridge were replaced by new ones to prevent contamination. Temperature and humidity were the same as for the cultures(fig 10.1).

b) Pheromones

The bioassays for the evaluation of the activity of pheromones were carried on a petri dish under a dissecting microscope. A round filter paper (9cm diam.), marked with concentric circles at 5mm intervals, was placed under the petri dish. The edges of the petri dish were covered with Fluon to prevent the ants from escaping. 15 ants were placed in the dish and left for 1hr to adapt to the environment. The small piece of filter paper containing the substance to be tested was placed in the middle of the petri dish. Lighting was provided by a 40w lamp situated left and above the microscope. Temperature and relative humidity were the same as for the cultures (fig 10.2).

10.7 Statistics

Statistical analysis was carried out on the results obtained from the study of necrophoric behaviour, the recruitment to a food source, the responses to possible sources of pheromones and the responses to the pheromones. The basic methods used were t-tests and analysis of variance between the means of the various treatments. Whenever a significant difference between the means appeared, the multiple-range test of Newmann was used in order to arrange the treatments into groups with no significant differences inside them and

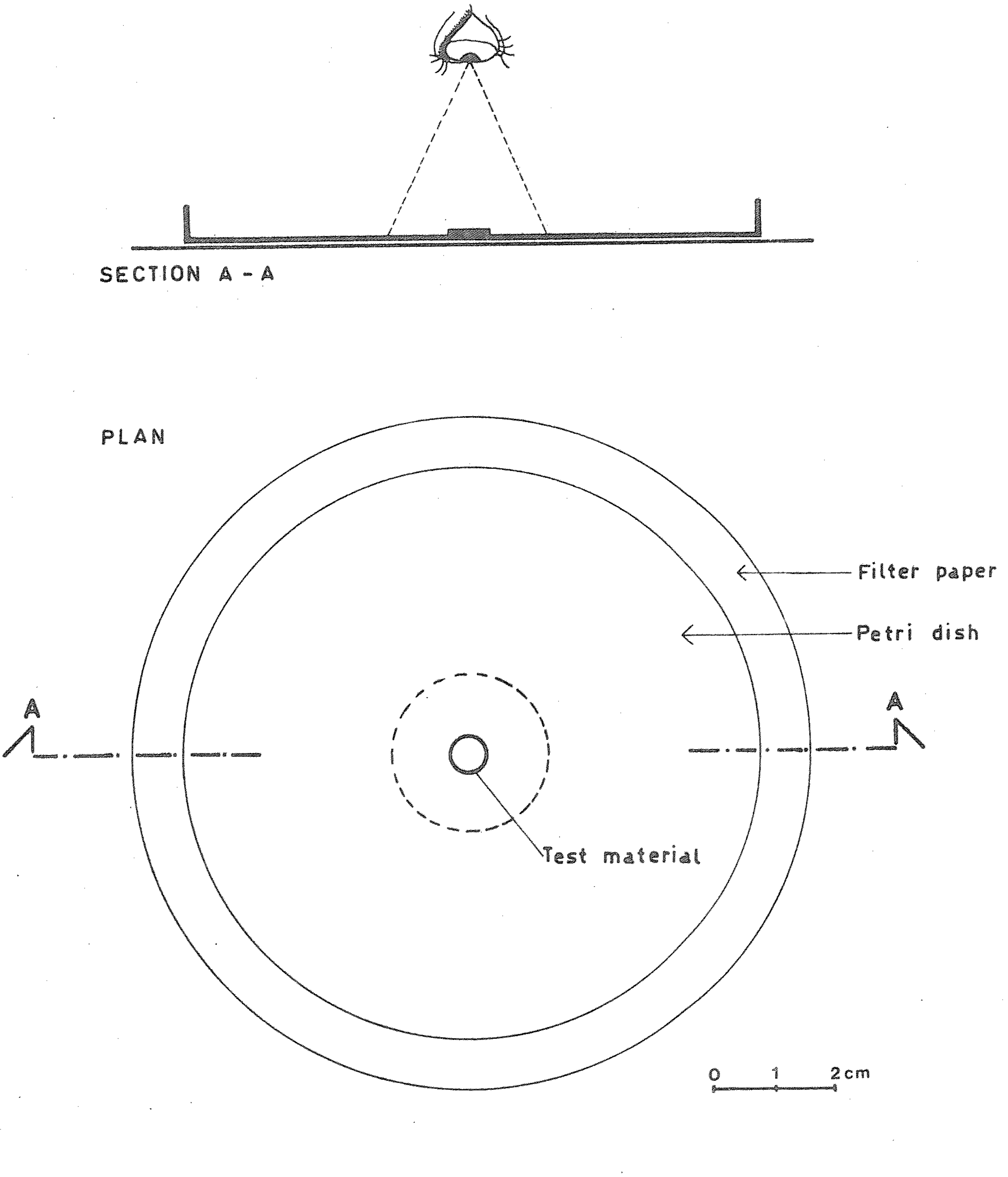

Fig 10.2. Layout for the bioassay of pheromones.

also to compare them with the controls. The method is described in detail in Snedecor (1967).

For the comparison of regression lines, their regression coefficients were compared in pairs.

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