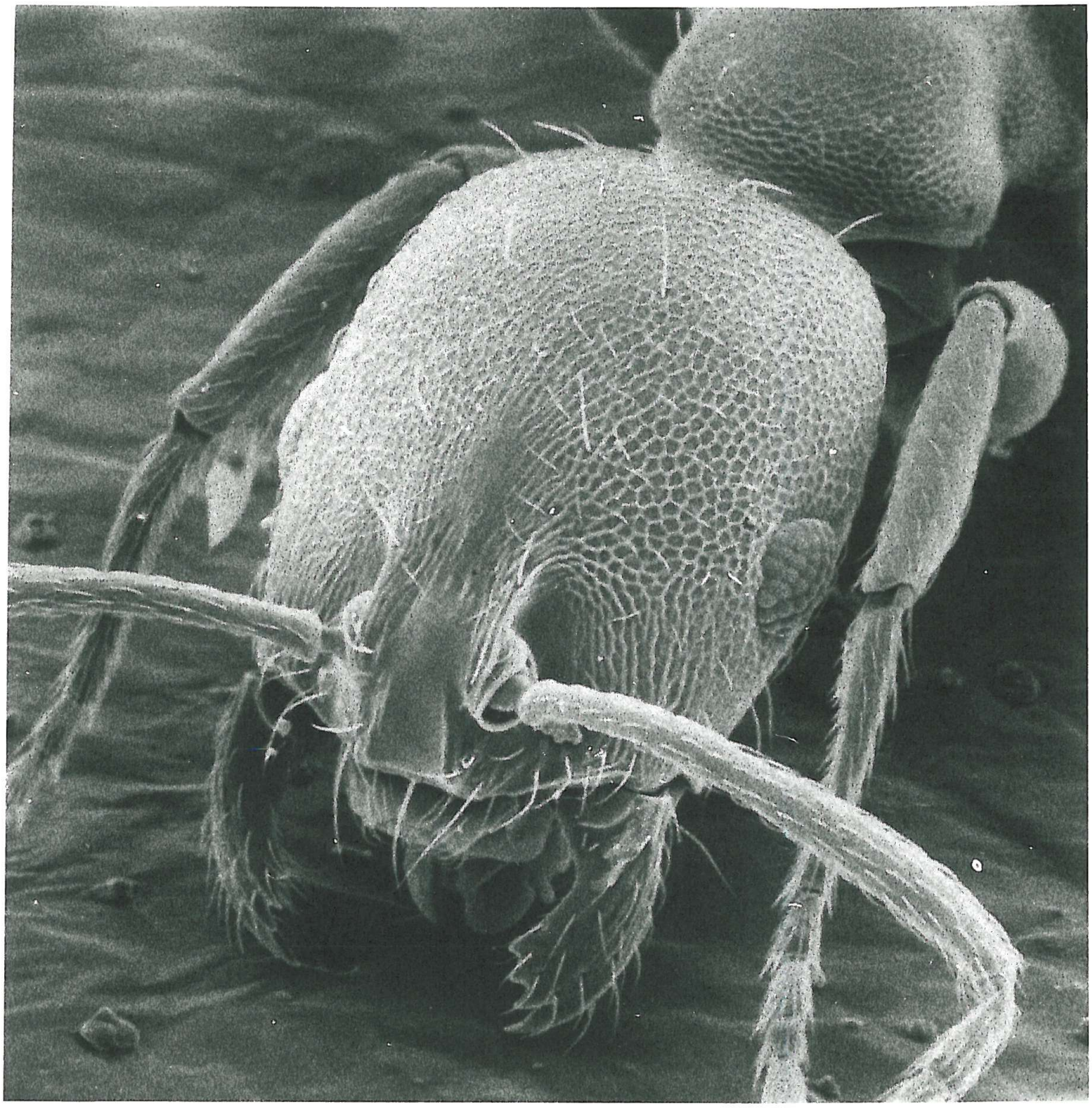

UNIVERSITY OF SOUTHAMPTON

CASTE REGULATION AND DETERMINATION
IN THE PHARAOH'S ANT,
MONOMORIUM PHARAONIS (L.).

Thesis submitted to the University of
Southampton for the degree of
Doctor of Philosophy
by
John P. Edwards
1984

To my Mother.

"There are more things in heaven and earth, Horatio,
than are dreamt of in our philosophy".

Hamlet, Act I. Scene 5.

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UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

BIOLOGY

Doctor of Philosophy

CASTE REGULATION AND DETERMINATION IN THE PHARAOH'S ANT *MONOMORIUM PHARAONIS* (L.).

by John Patrick Edwards.

The presence of fertile queens in *M. pharaonis* colonies inhibits the appearance of new sexual forms (males and queens). This inhibitory effect is maximal in mature fertile queens, less in young (newly-fertilized) queens, and absent in young alate (virgin) queens. To exert their inhibitory action, queens must have physical contact with other members of the colony. Dead queens and queens rinsed in organic solvents lose their ability to inhibit the production of sexuals.

The Dufour's gland of fertile queen *M. pharaonis* contains a cast specific chemical which has been identified as 1-isopropenyl-4,8,12-trimethylcyclotetradeca-3,7,11-triene (neocembrene). This material is not responsible for the ability of fertile queens to inhibit the production of sexuals, but may serve as a queen-recognition pheromone.

Mature fertile queens of *M. pharaonis* lay about 24 eggs/day, but egg-production is markedly less in young queens and in senile queens. Virgin queens lay few eggs and spend less time on the brood pile than fertile queens. The inhibitory effect of the presence of fertile queens on the production of new sexual forms can be substituted by the artificial introduction of eggs. Moreover, queens sterilized by exposure to a juvenile hormone analogue lose their ability to inhibit the production of sexuals. This suggests that workers recognise the presence of eggs as a signal of the presence, and fecundity, of queens.

Eggs with the potential to form sexuals are always present in the nest. The caste of female *M. pharaonis* is determined at an early stage in development, probably in the egg. Workers of *M. pharaonis* show age-polyethism, and probably use topographical, rather than chemical, cues to recognise the brood stages. Sexual larvae are almost hairless, but worker larvae are covered with rows of bifurcate setae. Workers may use these morphological differences to distinguish between worker and sexual larvae.

In queenright colonies of *M. pharaonis* workers will cannibalise introduced sexual larvae. By contrast workers in queenless colonies will accept and rear sexual larvae. Thus, in the presence of fertile queens (signalled by the presence of their eggs) workers prevent the appearance of new sexuals by destroying them as eggs or newly-hatched larvae. However, as the egg-producing capacity of the colony declines (e.g. as older queens die), workers will rear, rather than destroy, sexual brood to produce the next generation of sexuals.

ACKNOWLEDGEMENTS.

Firstly, I wish to thank Dr John Chambers (M.A.F.F., Slough Laboratory) for patiently guiding me through the complexities of chemical idientification, and for his interpretation of N.M.R. data and his elegant synthesis of neocembrene. I also wish to thank Dr G. Jamieson and the late Dr J.B. Siddall (Zoecon Corp.), Dr. C.S. Creaser (Physico-Chemical Measurements Unit, Harwell) and Messrs D.F. Lee and J.P. Wilkins (M.A.F.F. Harpenden Laboratory) for providing various mass spectra. Dr N.F. Janes and Dr J.A. Pickett (Rothamsted Experimental Station) kindly provided and discussed the Fourier-transformed N.M.R. spectrum. Profs. T. Kato (Japan) and R. Baker (Southampton) and Drs P. Sonnet (U.S.D.A. Gainesville) and J. Newton (Rentokil, U.K.) all generously provided samples of various chemicals.

For scanning-electron micrographs I am indebted to Mrs V. Ely and Miss B. Higgins, and for photographs to Mr J. Hammond and Mr M. Brown, all of the M.A.F.F. Slough Laboratory.

I also wish to thank Miss J.E. Short and Mrs L. Abraham M.A.F.F., Slough) for maintaining the stock cultures of *M. pharaonis* during this study, and Mrs Dorothy Nolan for patiently typing this thesis.

Finally, I wish to express my gratitude to Dr P.E. Howse (Southampton) for acting as my supervisor during this project, and to Mr C.E. Dyte (M.A.F.F., Slough) who remains an inspiration and whose helpful discussions of this work have greatly improved it.

CHAPTER 1 INTRODUCTION.

1.1. *Monomorium pharaonis* (L.) - the Pharaoh's ant.

Pharaoh's ant *(Monomorium pharaonis)* is probably the most widely distributed of all the ant pest species. The three adult forms (male, queen and worker) are shown in Figure 1. The monomorphic, wingless workers are small yellow-brown ants (2 mm) with a noticeable lightening of an area at the dorsal, anterior part of the gaster. The gastral portion of the body is smooth but the rest of the body is finely reticulate-rugose. Queens are larger than workers (4mm) but are otherwise similar in general shape and colouration. Queens and males have 3 light-sensitive organs (ocelli) at the posterior apex of the head but these organs are absent in workers. Queens are winged when they emerge from the pupal stage but the wings are lost soon after mating. It is not clear whether the wings are shed by queens or bitten off by workers. The distinctive, black, winged, males (Figure 1) are only rarely present in a colony and are seldom seen in natural infestations.

Within the family Formicidae (ants) the genus *Monomorium* is placed in the sub-family Myrmicinae (tribe Solenopsidini) along with closely related genera like *Solenopsis* (BOLTON and COLLINGWOOD, 1975). Many species within the tribe Solenopsidini are lestobiotic (i.e. feed on the brood of other ant species) and DUMPERT (1981) states that *M. pharaonis* lived originally as a thieving ant species in its natural habitat. The taxonomy of the genus *Monomorium* is rather poorly studied. ETTERSHANK (1966) recorded about 300 named forms, but it is likely that there are only about 250 valid species of *Monomorium* world-wide (B. BOLTON, pers. comm.) Within the genus, very few species have been studied and *M. pharaonis* is certainly the best known and most economically important species.

The type specimen described by LINNAEUS (1758) was from Egypt and it is probable that the species originated in the North African region. However, in the last 200 years, with increasing world trade, the species has been distributed worldwide and is now a truly cosmopolitan species. The ant is widespread in Europe including the USSR, (EICHLER, 1978) is found in the USA, Canada, South America, Africa, Australia and Japan, and is probably endemic throughout the tropics. In tropical regions, the species lives both outdoors and in human dwellings (SUDD 1962). In temperate regions, where the species is dependant on artificial heating to survive the winters, infestations are invariably associated with human habitation. In Europe and North America infestations are frequently associated with hospitals, bakeries, factories, offices and large domestic apartment blocks, and the insect is more common in urban than in rural regions. In the U.K. the species was first recorded, in London, in 1828 (DONISTHORPE, 1927) and since that time has become widely distributed throughout the country. In a recent survey it was estimated that more than 10% of English hospitals were infested by this ant, and that in urban areas (e.g. London) the figure was closer to 20% (EDWARDS and BAKER, 1981).

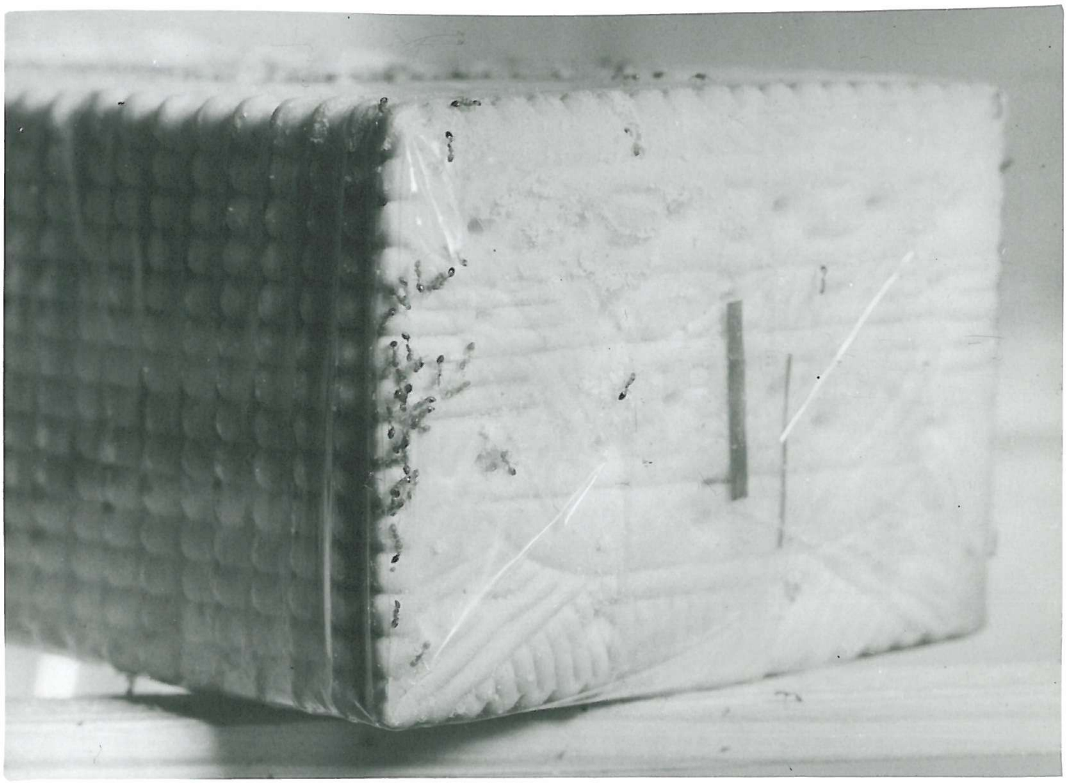
Before 1972, infestations of *M. pharaonis* were regarded mainly as a nuisance. However, BEATSON (1972) demonstrated that the species was able to transmit a variety of pathogenic organisms including *Salmonella, Pseudomonas, Staphylococcus, Streptococcus, Klebsiella* and *Clostridium* spp., and ALEKSEEV *et al.* (1972) demonstrated the mechanical transmission of the plague organism, *Pasteurella pestis,* by *M. pharaonis* after ants had fed on carcasses of animals which had died of the disease. Although other insects (e.g. houseflies and cockroaches) are able to carry and transmit pathogens, pharaoh's ants present a particularly serious infection hazard. The small size of foraging workers enables them to pass through the smallest gaps to invade wounds and sealed chambers, and they readily chew through paper, plastic and rubber to gain entry into packages (Figure 2). Foraging workers have been found in sterile supplies, in equipment for giving intravenous fluids and under wound-dressings on post-operative patients (ANON, 1974; BEATSON, 1973; CARTWRIGHT and CLIFFORD, 1973). Often the presence of these small insects goes undetected and, once a food source has been located by a foraging ant, many others will be recruited to feed at the site, thus increasing the chance of disease transmission. Because the species has a high requirement for moisture (SUDD, 1962) ants are often found feeding at sluices, drains, toilets and other areas likely to harbour disease organisms. Thus, it is clear that infestations of *M. pharaonis* in hospitals and domestic residences constitute a serious threat to health.

Colonies of *M. pharaonis* are generally polydomic (i.e. comprise of several nests) and there is free interchange of all stages between nests. In indoor infestations, nests are often located in inaccessible sites like foundations, wall-cavities and underground ducting systems. Nests are unstructured (i.e. not built) and occupy any suitable crack or crevice. In the laboratory, the size of nests appears to be limited only by the space provided (PEACOCK *et al.*, 1955). Within the nests there are the developing brood stages (eggs, larvae, pre-pupae and pupae) numerous workers and several queens. In laboratory colonies the number of queens per nest is variable, ranging from a few individuals to several hundreds. Queens generally remain inside the nest and are solely responsible for egg-laying since the workers are sterile. Workers perform the 'housekeeping' duties within the nest (feeding and rearing brood stages and queens) and forage outside the nest for food and water.

As in the majority of the social Hymenoptera, eggs laid by *Monomorium* queens may give rise to males or queens or workers. Males are produced from haploid (unfertilized) eggs, but both queens and workers are produced from diploid (fertilized) eggs (SMITH and PEACOCK, 1957). As a result, unmated queens can lay eggs which can, under appropriate circumstances, give rise to males (PEACOCK *et al.*, 1954). The majority of eggs laid by fertilized queens give rise to the most numerous morph, the workers. The small, translucent, spheroid eggs (Figure 3) hatch after about 7 days (PEACOCK and BAXTER, 1950). Eggs tend to be placed together in bunches and newly-hatched larvae probably obtain much of their nourishment by feeding on unhatched eggs in their immediate vicinity. As the larvae grow they are moved by workers to a separate 'larval' part of the nest. This tendency to segregate brood at different stages of development

Figure 1. *Monomorium pharaonis* (L.). Male (left) queen (centre) and worker (right). (Mag. x 20).

Figure 2. Workers of *Monomorium pharaonis* entering a cellophane biscuit packet.



into distinct areas of the brood pile is clearly seen in Figure 4. The number of larval instars in *M. pharaonis* is not known, but by analogy with other Myrmicine species, it is probably 3 or 4. The larvae are typical soft-bodied, apodous grubs (Figure 3) similar to many other ant larvae. The larval stage takes about 17 days (PEACOCK and BAXTER, 1950) and at the end of larval development a distinct pre-pupal (pharate pupa) stage is formed (Figure 3). Workers appear to be essential for the formation of the pre-pupal stage as they are responsible for removing the sac containing the larval 'meconium' which they grasp with their mandibles and pull away from the larva, depositing it on a rubbish pile outside the nest. This is necessary because the passage between the larval midgut and hind gut is occluded until the end of larval development, and solid faecal material is excreted by larvae only at that time. This is an adaptation to social life, and precludes the constant fouling of the nest area by developing larvae. The pre-pupal stage lasts for about 3 days and is followed by the pupal stage (approx. 9 days) from which the adults emerge (PEACOCK and BAXTER, 1950). Newly-formed pupae are white and not enclosed in a silken cocoon, but as they age they become brown as the adult cuticle is laid down beneath the pupal cuticle (Figure 3). In some ant species, especially those in which the pupa is enclosed in a cocoon, adult emergence is aided by workers. However, in pharaoh's ant, workers can emerge from the pupal stage without the assistance of other workers. PEACOCK and BAXTER (1950) have measured the longevity of adult stages and record that queens may live for at least 39 weeks, workers for 9-10 weeks and males, depending on whether or not they mate, for 3-8 weeks. Observations at this Laboratory confirm that these values are reasonably accurate although, in laboratory colonies, some queens have remained alive for over 1 year.

From time to time, new sexual forms (males and queens) are reared by the workers and appear in the nest, although the factors which initiate such a phase of sexual production are not fully understood. However, it is clear that the presence of existing queens in a colony inhibits the appearance of new sexual forms (PEAKCOCK *et al.*, 1954; 1955a). Males and queens take about 5 days longer than workers to complete development and PEACOCK and BAXTER (1950) suggest that this difference is mainly due to a longer period of larval growth. Fully-grown sexual larvae are about twice the size of the corresponding worker larvae, and PEACOCK and BAXTER (1950) have reported that there are differences in the colour of the gut contents of the two types of larva. This would suggest that there may be differences in nutrition between worker and sexual larvae. It is not possible to differentiate between male and queen larvae (except by examining chromosomes). However, preliminary studies (EDWARDS, unpublished) have provided data which allow the pre-pupal stages of sexuals to be separated on the basis of their relative weights (Figure 5). Sexual pupae are distinctive and can be identified on the basis of size, weight and gross morphological features (Figure 6).

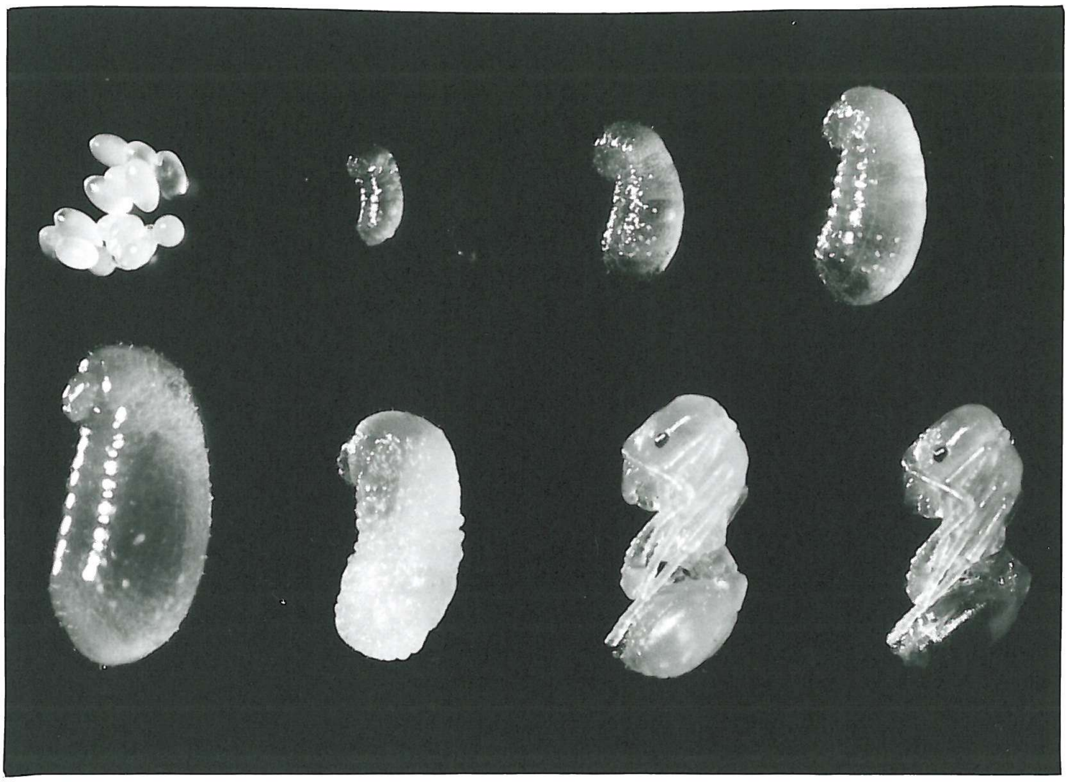
In the majority of ant species, new nests are founded by single fertilised queens following the 'nuptial flight'. In temperate regions this process usually occurs at the end of the summer and is preceeded by the production of large numbers of new reproductive forms (sexuals) in the nest. Typically, these sexuals leave the nest on their mating flight at a time which is synchronous for many nests distributed over a large geographical area. In this way, a certain amount of cross-fertilization between sexuals from different nests is ensured. In *M. pharaonis* this claustral foundation of new nests does not occur and there is no nuptial flight. Instead, fertilization of newly-formed queens takes place in or near the nest and, although newly-emerged males and queens are winged, neither sex is capable of flight. Males often emerge from the pupal stage a day or so before the queens and are strongly attracted to them. Virgin queens are thought to produce a sex-pheromone in their Dufour's gland and bursa copulatrix which attracts the males and acts as a stimulant to mating (HOLLDOBLER and WUST, 1973). Mating lasts for 5-15 minutes and queens mate only once whereas males may mate upto 4 times (PETERSEN and BUSCHINGER 1971).

The dissemination of the species and the foundation of new nests occurs by budding (sociotomy) when workers, carrying brood stages with them, emigrate to a new nest site (PEACOCK *et al.*, 1955). Although queens sometimes accompany these emigrant groups, they are not essential for the survival of the new colony, since workers can rear new queens and males from the transported brood. Isolated queens are unable to found new colonies, as are groups of queens and workers without brood stages (PEACOCK *et al* ., 1955a). These authors record that the smallest natural nest observed comprised of one queen, thirty five workers, twelve pupae and "a small number" of eggs and larvae, and state that the minimum number and composition of budding groups which can successfully establish a new nest is about 100 workers and a similar number of eggs. These mobile groups often form temporary nests while searching for a more permanent abode. Such transitory nests are probably the main method by which infestations are spread as a result of the inadvertant transportation of such nests to previously uninfested premises. The budding process may occur at any time and the factors which precipitate colony division are not understood. PEACOCK *et al.* (1955a) have indicated that overcrowding in the nest or changes in the humidity of the local environment may lead to partial or total dispersal of the nest. From the practical view-point, it is of considerable interest that the presence of insecticides may also serve to induce the dispersal of nests to untreated areas (GREEN *et al.,* 1954). The polydomic habit of *M. pharaonis,* coupled with a lack of aggressive behaviour between occupants of different nests, enables the species to respond rapidly to, and move away from, adversely changing environmental conditions. This adaptability, together with the lack of a complex nest structure, has enabled the species to adopt a synanthropic existence and become a pest. Another factor which has contributed in this respect is the omnivorous nature of *M. pharaonis*. In laboratory colonies these ants will feed on a wide range of foodstuffs - fats, proteins and carbohydrates - and they will also take and kill small insects. In their natural habitat, small insect prey, especially the brood of other ant species, probably constitutes the bulk of their food (SUDD, 1962).

When a food source is located by a foraging worker, the worker returns to the nest laying a chemical trail (trail pheromone) on the ground. The returning worker enters the nest and 'excites' other workers to leave the nest and follow the trail to the food source (SUDD, 1957; 1960). This exitation (resulting from rapid movement

Figure 3. Developmental stages of Monomorium pharaonis (worker). Top (left to right) eggs, very small larva, small larva, medium larva. Bottom (left to right) fully-grown larva, pre-pupa (pharate pupa), white (young) pupa, dark pupa (pharate adult). (Mag. x 20).

Figure 4. Part of the brood area in a nest of *Monomorium pharaonis* showing segregation of large larvae



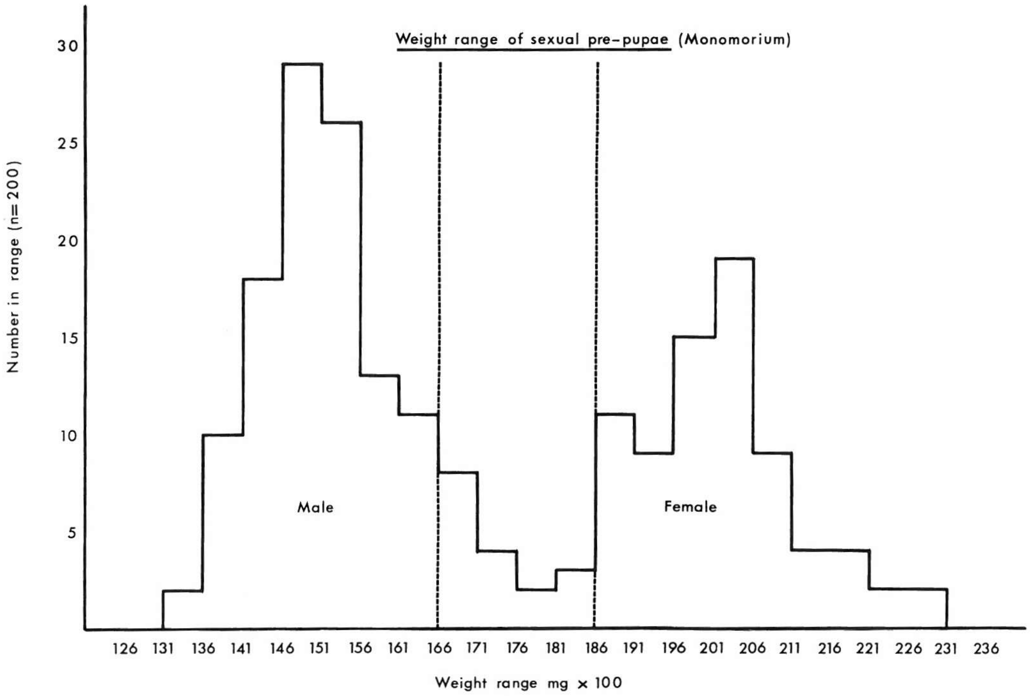
and physical contact) of nestmates by returning, foraging workers has also been observed in other *Monomorium* species (SZLEP and JACOBI, 1967). As more workers use the trail and deposit more trail pheromone, the trail becomes reinforced. These chemical trails can be many metres long. The active principle (faranal) is produced in the Dufour's gland of workers (RITTER *et al.*, 1977) and deposited on the ground by the sting. Workers forage by day and night and do not avoid light, even strong sunlight. When workers bring food or water back to the nest they often feed other workers, brood and queens. However, when food is plentiful a large proportion is stored in the nest in discrete food piles which act as a reserve. Subjective observation suggests that this reserve food is part-chewed by workers prior to storage on food piles. This chewing may serve to partly digest the food before it is fed to the brood, or, in some way, to protect the food against mould growth and decomposition.

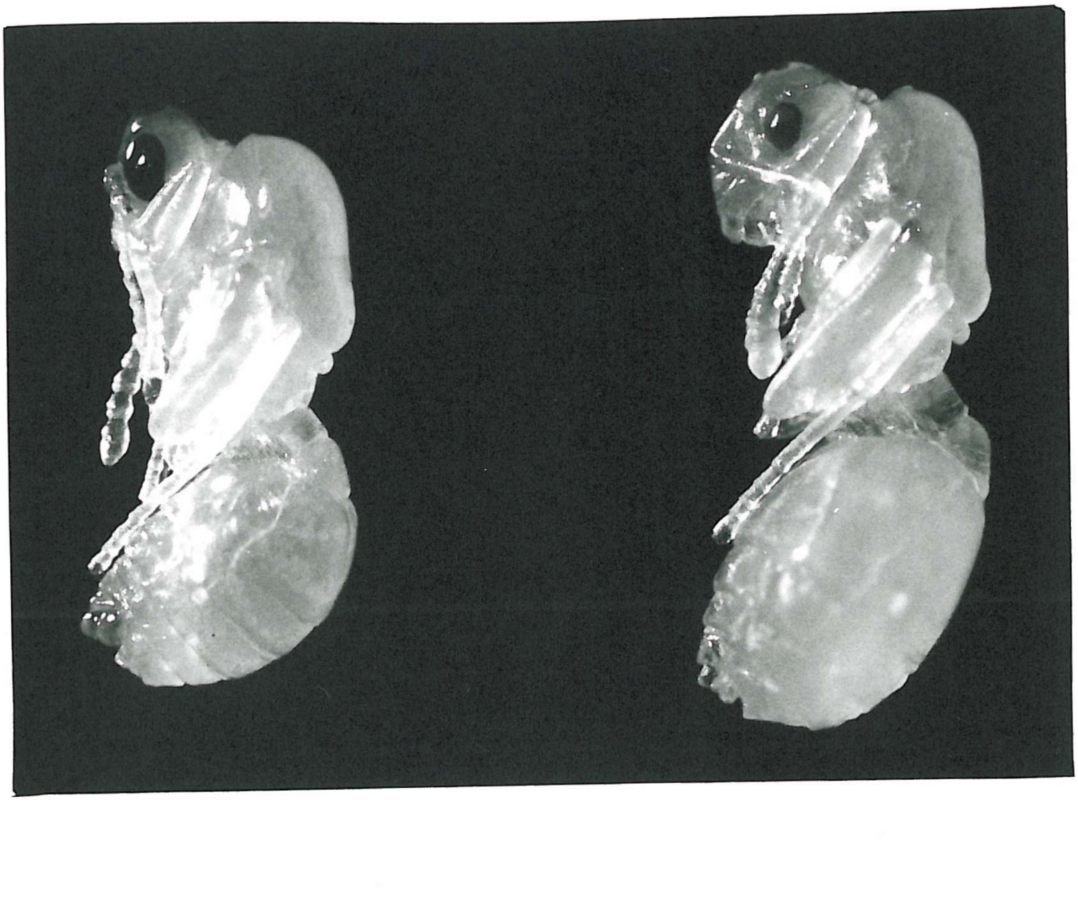
In addition to the trail pheromone, workers produce other chemicals (TALMAN *et al.*, 1974; RITTER *et al.*, 1975) which act as alarm and defense secretions or as additional components of the trail pheromone (EDWARDS and PINNIGER, 1978). These compounds, mostly pyrollidines, are produced in the poison gland of workers and are also dispensed with the sting. The sting and associated glands have become adapted for trail-laying etc., and pharaoh's ants are not able to sting, neither are their jaws capable of piercing human flesh.

Colonies of *M. pharaonis* remain active and viable over a relatively wide temperature range. PEACOCK *et al.* (1955b) reported the lower threshold for colony survival, over a protracted period, to be about 18°C. At lower temperatures, the insects become sluggish or immobile, and when diurnal and nocturnal temperatures fluctuate between 11 and 6°C, death of the colony occurs within 7 days. However, small colonies may survive even lower temperatures for a few days and subsequently can be revived when restored to favourable conditions. There are no data available on the high temperature threshold for the species but laboratory colonies flourish between 27 and 31°C and, in domestic infestations, the ants are found in areas with even

Figure 5. Bimodal weight distribution of queen pre-pupae of *Monomorium pharaonis*. (From unpublished data, EDWARDS J.P.)

Figure 6. Male (left) and queen (right) pupae of *Monomorium pharaonis*. (Mag. x 25).


Weight range of sexual pre-pupae (Monomorium)
Number in range (n=200)
30
25
20
15
10
5
Male
Female
126 131 136 141 146 151 156 161 166 171 176 181 186 191 196 201 206 211 216 221 226 231 236
Weight range mg × 100



higher temperatures (> 40°C). Survival at high temperatures is probably dependent upon a good supply of water, since the species appears to require high humidity, especially in the nest. Thus, SUDD (1962) studying this species in Nigeria, reported that dense trails were formed to raindrops and other sources of water, and that water appeared to be collected more avidly during the dry-season. In domestic infestations in the U.K. , ants are often most numerous in areas where high temperature and humidity prevail (i.e. boiler houses, kitchens and laundries). Away from such areas these insects are most often found in the vicinity of hot pipes, radiators, sinks, drains and toilets.

The history of the use of control measures against pharaoh's ant can be traced back almost 100 years and, in that time, the variety and toxicity of the materials used is an indication of the past difficulties of controlling this species. Control measures used against pharaoh's ants show the complete picture of the development of insect control techniques ranging from early attempts where sugar-soaked sponges covered with foraging workers were dropped into boiling water (RILEY, 1889) through the use of highly toxic inorganics like arsenic and thallium sulphate, and later persistent organochlorine compounds, to modern methods utilizing juvenile hormone analogues (EDWARDS and CLARKE, 1978) and bacterial pathogens (VANKOVA *et al.,* 1975).

1.2 Caste determination and regulation in the Formicidae.

In ants, as in many other social insects, adult polymorphism is extended further than the normal morphological and physiological separation of the male and female sexes. Sex determination in ants is a result of haplodiploidy whereby males develop from haploid This mechanism seems to be the main method of sex determination throughout the social Hymenoptera (WILSON, 1971). It has been demonstrated to be the case in *M. pharaonis* where male cells have 11 chromosomes, and female cells, 22 (SMITH and PEACOCK, 1957).

In the majority of ant species, the female sex can be separated morphologically, physiologically and behaviourally into two or more recognisable types or castes e.g. queens and workers. These castes usually have different functions within the social organanisation of the colony. Thus, in *M. pharaonis,* queens are large females with fully functional ovaries and are soley responsible for egg-laying. They generally remain inside the nest and take little or no active part in the care of the brood or in foraging for food. By contrast, workers are small, sterile females who perform the day to day tasks associated with foraging for food, cleaning and defending the nest, and feeding and tending the brood and queens. This pattern of two distinct female castes (queens and workers) is generally the rule for the social Hymenoptera. However, in some ant species, the worker caste is further sub-divided into sub-castes. For example, in the genus *Pheidole,* two distinct worker types are found *viz,* minor workers and major workers (soldiers). The large, soldier workers have disproportionally large heads and mandibles, comensurate with their role as defenders of the colony. Thus, the soldier sub-caste is distinct both morphologically and behaviourally from the minor workers who are responsible for colony duties other than defence. Such morphologically distinct sub-castes may represent the most advanced point in the evolution of social organization based on division of labour. However, even in species like *M. pharaonis* with monomorphic workers (i.e. without morphological division of the worker caste) there is often a functional division of labour within the caste. This is illustrated by the temporal polyethism present in some species whereby the duties of workers change as they grow older. Such age-related division of labour is also present in those species with a continuous polymorphism within the worker caste. In such species there is continuous variation in size between small and large workers and this condition may represent a transient evolutionary stage between species with monomorphic workers and species with two, morphologically distinct, worker sub-castes.

The evolution of eusociality and the development of polymorphism based upon division of labour between co-habitants within the social unit (colony) were probably closely related events. It is certainly true that the evolutionary success of social insects, especially ants, in terms of the number of species, diversity of habitat and ecological impact, is largely due to the efficiency with which these insects can utilize resources and adapt to changing environmental conditions. In this respect, some authors have suggested that the evolution of two or more female castes has increased the behavioural repertoire of eusocial species by allowing specialization of some individuals for particular tasks. However, it is more likely that division of labour on a caste (or sub-caste) basis is advantageous, not because it increases the total behavioural repertoire, but because it allows all the basic behavioural tasks to be carried-out simultaneously. Thus, co-operation and division of labour between individuals increases the efficiency with which they, as a group, are able to cope with the demands inherent in the survival of the species (e.g. feeding, reproduction, defence and dispersal). Once co-operative division of labour has become established, it no longer becomes necessary for all members of the socially organized unit to be competent at all tasks. Thus, we may view the evolution of caste as a natural consequence of the evolution of eusociality - from the aggregation of solitary forms into collaborative groups wherein the demands of increased ergonomic efficiency led to a division of labour among individuals, and finally to specialization and polymorphism.

This study, is concerned with two aspects of the production of the two female castes in the ant *Monomorium pharaonis*. First, is the question of how two morphologically, physiologically and behaviourally distinct female types are produced from diploid eggs - that is, what determines the caste of a female ? Second, we shall consider the mechanisms whereby the relative numbers of each caste present in the colony at any given time are regulated - that is the method of caste regulation. These problems, in particular the question of caste determination, have interested students of social insects and other biologists for many years. Several theories of caste determination have been proposed and the major theories are considered below. However, since *Monomorium pharaonis* is a species with a monomorphic worker caste, this consideration of caste determination and regulation will not be concerned with the development of sub-castes (e.g. soldiers) which may develop as a result of other determining or regulating factors (e.g. allometric growth patterns). The development of sub-castes is discussed by WILSON (1971) and is briefly considered later in the present study (Chapter 11). Caste determination has been the subject of a number of recent reviews, including WEAVER (1966), WILSON (1971), LUSCHER (1976), BRIAN (1979) and PASSERA (1982).

It is generally accepted that caste determination in ants is basically either genetic (i.e. irreversibly determined by the genes at the egg stage), or blastogenic (i.e. a function of some pre-determination occurring within the embryo), or trophogenic, i.e. the influence of some environmental factor (e.g. nutritional or temperature) upon a plastic or bi-potent larval developmental programme. These latter two mechanisms of caste determination are epigenetic. Obviously, there may be variations or even overlap within these basic mechanisms. However, the fundamental questions remain largely unanswered in the majority of ants . Thus, in most species it is not known whether queens lay two types of diploid egg, i.e. queen-determined eggs and worker-determined eggs, or whether any diploid egg laid by a queen may develop into either caste depending upon how, or when, or by whom they are reared. This is, essentially, the problem of caste determination. The ultimate appearance in the colony of adult forms arising from these eggs (be they pre-determined or bi-potent) is not a function of the determination of adult morphs, but rather of the regulation of the numbers of each caste able to complete development at a given time. This is the phenomenon of caste regulation and its importance, as distinct from caste determination, has not, in the past, been fully recognised. Indeed, the two have so often been confused that this had led to misunderstandings surrounding the possible mechanisms of caste determination in ants. Few detailed studies have been made on caste determination and regulation in ants. The exceptions being the studies of Bier and co-workers on *Formica* spp and those of Brian and his colleagues on *Myrmica* spp.

In *Formica polyctena,* queens lay two types of egg - winter eggs and summer eggs. Winter eggs produce queens and workers, while summer eggs produce only workers (GOSSWALD and BIER, 1957). Winter eggs contain more RNA around their nuclear membranes and have a larger polar plasm (BIER, 1953; 1954). These observations suggest that cytological differences in the eggs contribute to the later biasing of larval development of bi-potent, winter eggs. Final determination in *F.polyctena* occurrs during the first 72 hr of larval life (GOSSWALD and BIER, 1957). The biasing of eggs in relation to caste determination led FLANDERS (1945) to propose that caste determination was simply a result of the amount of yolk present in the egg. Thus, he proposed that a faster oviposition rate gave eggs with less yolk which, in turn, were less likely to produce queens as opposed to workers. This is, essentially, the blastogenic theory of caste determination.

Undoubtedly, the most detailed study of caste determination has been made over a period of 30 years by M.V. Brian and his colleagues in the ant *Myrmica rubra* (= *Myrmica ruginodis*). These studies have identified at least six interacting factors which are responsible for determining and regulating caste in this species. These factors include larval nutrition, the effect of temperature on eggs, presence of queens in the nest, response of workers to sexual larvae, egg size and the age of queens. In *M. rubra,* differentiation between queen and worker larvae occurs during the final (3rd) larval instar of bi-potent larvae (BRIAN 1962; 1963). As in *Formica polyctena,* bi-potent larvae are those which overwinter as larvae (diapause larvae) and it is from such larvae that the majority of queens develop. By contrast, non-diapause (summer) larvae produce mostly workers. The factors contributing to the differentiation of queens and workers in overwintering larvae are several. The predominant factor appears to be the ability of potential queen larvae to gain weight whilst inhibiting the growth of adult rudiments (imaginal discs) inside them (BRIAN, 1965). This led BRIAN (1968; 1976) to suggest that workers could be regarded as neotenic or aborted females which metamorphose to adulthood before completing full (sexual) development. Interestingly, these differences have been linked with the effects of the two hormones controlling moulting and metamorphosis in insects. Thus, ecdysteroid (moulting hormone) treatment of sexual larvae hastens metamorphosis and results in the formation of some intercastes and workers (BRIAN, 1974). By contrast, treatment of larvae with juvenile hormone slows the development of larvae and results in the formation of more, larger, queens (BRIAN, 1974). A further control on the type of adult female produced in colonies of *M. rubra* is exerted by the presence or absence of queens (BRIAN, 1958; BRIAN and CARR, 1960; BRIAN and HIBBLE, 1963) and by the age of extant queens (BRIAN and HIBBLE, 1964). In the absence of fertile queens, *M. rubra* colonies will tend to rear new sexuals. However when queens are present, they inhibit the development of sexual larvae in two ways. First, the queen's presence may directly inhibit the growth and development of sexual larvae through the action of chemical secretions produced in the head glands of queens (BRIAN and BLUM, 1969). Second, the presence of queens may change the behaviour of workers towards developing queen larvae such that workers attack these larvae by biting them. This attack causes some larvae to moult prematurely (to workers) and others to die (BRIAN, 1973).

Obviously, workers must be able to recognise the presence of fertile queens, and some evidence has been presented to indicate that they do this both by recognising the shape of queens and by recognising the chemical signals which they produce. For example, the inhibition of sexual production in *M. rubra* colonies can be accomplished by substituting freshly-killed queens for live ones. However, inhibition is not maintained when such dead queens are a) infertile (virgin), b) rinsed in organic solvents or, c) presented after dissection and rearrangement of the major body portions (head, thorax and gaster) (BRIAN, 1973). Moreover, in *Myrmica rubra,* where workers have functional ovaries and can lay either non-viable, trophic eggs or viable (haploid) male eggs, the presence of fertile queens in a colony can suppress the egg-laying activity of workers, thus preventing the production of males (BRIAN, 1970). It is therefore clear, that in some ant species the presence of queens in a colony has an inhibitory effect on the production of new sexual forms. In some species the caste-regulating action of the queens appears to be directly, chemically mediated, whereas in others, queens may influence the behaviour of workers towards developing larvae. An alternative mechanism to explain the inhibitory effect of queens on sexual production has been proposed by several authors. This theory suggests that the development of sexual larvae is dependant on the availability of special 'pro-fertile' food. This food is also required by queens and, in queen-right colonies, queens compete for the pro-fertile food at the expense of developing larvae which are thus unable to complete full (sexual) development and only develop into workers. The 'pro-fertile food' theory is closely allied to the theory of trophogenic caste determination and implies that some, or all, diploid eggs are bi-potent with respect to caste. There is considerable evidence linking nutritional factors with the development of sexual larvae in *Leptothorax* (BIER 1954) *Formica* (GOSSWALD and KLOFT, 1960) and in *Myrmica ruginodis* where larvae reared on a low-protein diet produced a higher proportion of workers than those reared on a protein-rich diet (BRIAN, 1973b). Moreover, as discussed later, it has been proposed that caste determination in *Monomorium pharaonis* is also a result of the mutually-exclusive competition for pro-fertile food between queens and larvae (BUSCHINGER and KLOFT, 1973).

One of the major problems with the trophogenic theory of caste determination is that it is not always clear whether sexual larvae develop because they are fed certain food types or whether sexual larvae are fed more (or differently) because they are sexual larvae. In essence, the question is whether nutrition is a caste-determining or a caste-regulating factor? The answer to this question depends upon the definition of the term caste determination, and, since this term means different things to different people, it is appropriate to consider here what the term 'caste determination' means.

At the most fundamental level, the two female castes (queens and workers) are both phenotypic expressions of the female rather than the male genotype. In this sense, all castes are genetically determined. The main question surrounding the phenomenon of caste in ants and other social insects is concerned with whether there is one plastic (bi-potent) female genotype or several female genotypes. If there are several female genotypes and queens lay either queen eggs or worker eggs, then caste determination *per se* is genetic (or blastogenic), and the subsequent survival of queen-determined developmental stages is solely dependent on caste-regulating factors like the presence of queens or the behaviour of workers towards such stages. If there is a single female genotype which is plastic (bi-potent) and female eggs may give rise to either workers or queens depending on circumstances, then the ultimate developmental fate of each egg may be dependent on 'caste-determining' factors such as nutrition (trophogenic determination) as well as caste-regulating factors such as the presence of queens etc. It is these differences that have, in the past, led to a certain inexactitude in the use of the term 'caste-determination'. For example, a nutritional factor may be caste-regulating but not caste-determining in one species but the same factor may be caste-determining but not caste-regulating in another. Thus, it is evident that 'caste determination' is not an appropriate term to encompass all factors governing the development of the two female castes, especially in ants. This is because, used in its broadest sense, caste determination encompasses both caste-determining factors and caste-regulating factors, and it is misleading not to distinguish between the two. It would be more appropriate to use the term caste-determination in the restricted sense of the potential for the phenotypic expression of the primary genotype. Thus, we might use the term 'monomorphogenic' when referring to caste determination which is fixed and where eggs (and larvae) are either queen-determined or worker-determined, and 'polymorphogenic' to indicate those situations where eggs (and larvae) are able to form either queens or workers. In each case, the eventual appearance of sexual forms in a colony would depend on a variety of caste-regulating factors which may include nutrition, the presence of queens, the behaviour of workers, seasonal influences etc. Using this scheme, it is possible to differentiate between caste determination and caste regulation.

1.3. Caste determination and regulation in *M. pharaonis*.

As well as providing much useful basic biological data on *M. pharaonis*, A.D. Peacock and his co-workers were the first to make observations on caste regulation in this species. PEACOCK *et al.* (1945; 1955a) reported that removal of brood stages and workers from a queenright (queen-containing) colony resulted in the appearance of new sexual forms in the queenless colony whereas no sexuals were produced in the original queenright colony. This strongly suggests that the presence of queens inhibits the formation or development of new sexual forms. PEACOCK and BAXTER (1950) also noticed certain differences in the colour of the gut contents of worker and sexual larvae and this, together with the discovery of ergatogynes (queen-like workers) in some colonies, led HALL and SMITH (1953) to conclude that differences in nutrition were mainly responsible for determining caste in this species.

Further evidence in support of a trophogenic basis for caste determination in *M. pharaonis* was presented by BUSCHINGER and KLOFT (1973) who found that queens were fed 'glandular' (i.e. not raw) food during their period of intensive egg-laying. By contrast, newly-emerged queens and senile queens were fed mainly raw food. These observations led BUSHINGER and KLOFT (1973) to suggest that queens consume the bulk of the colonies glandular food, which is in some way 'pro-fertile', at the expense of developing sexual larvae. More recently, ERPENBECK (1981) has demonstrated that colonies of *M. pharaonis* consume more protein-rich food during periods when when sexuals are being produced than when only workers are produced. Thus, he concluded that it was the quantity and not the quality of food which determined the development of larvae into sexuals. However, there is no conclusive evidence that sexual larvae develop because they are fed protein-rich food or even, because they are fed more food than worker larvae.

PETERSEN-BRAUN (1975) identified three phases of life for queens of *M. pharaonis* - a juvenile phase (0-4 weeks after fertilization), a fully fertile phase (the majority of the queens lifetime when oviposition is maximal), and a senile phase (a period of a few weeks before the death of the queens during which the new sexual brood is reared). These three phases correspond to the feeding states identified by BUSHINGER and KLOFT (1973). Thus, juvenile and senile queens are fed raw food whereas, fully fertile queens are fed glandular food. Moreover, the different phases are correlated with the dilation of the thoracic crop such that only in fully fertile queens is this organ developed to its maximum size (PETERSEN-BRAUN and BUSCHINGER, 1975). These authors cite this observation as further evidence that it is the demands of fully fertile queens for pro-fertile (glandular) food which diverts this food material from larvae, and prevents their development into queens. However, it is possible that the increase in size of the thoracic crop merely compensates for the reduction in the size of the gastric crop which is compressed by the ovaries of fully-fertile queens (PETERSEN-BRAUN and BUSCHINGER, 1975). PETERSEN-BRAUN (1977) concluded that caste determination in *M. pharaonis* occurs predominantly on a trophogenic basis. However, because this author was unable to rear sexual animals from eggs laid by young queens (0-4 weeks old) she concluded that there was, in addition, a blastogenic factor and that mated queens are able to lay eggs of different developmental capacities. Thus, young queens lay eggs which can only give rise to workers. Older queens, in the fully fertile phase, lay eggs which may develop into either workers or sexuals depending upon how they are fed by workers. PETERSEN-BRAUN (1977) further states that the different developmental capacity of eggs is reflected in the fact that juvenile queens lay larger eggs than do fully fertile queens. However, this may simply reflect the fact that egg size may be related to the rate of oviposition, as may be the case in *Solenopsis invicta* (CHERIX and FLETCHER 1982). It is clear therefore, that despite the concensus that caste determination in *M. pharaonis* is a result of trophogenic determination, the evidence for this belief is mostly circumstantial.

The factors controlling the regulation of caste in *M. pharaonis* have also been ascribed on the basis of inconclusive evidence. On the one hand, the proposed trophogenic determination of caste implicates queens as caste regulators by way of their consumption of pro-fertile food material (which is thus not available for the rearing of sexual forms). On the other hand, the fact that the presence of queens inhibits the development of sexual forms has also led to the suggestion that queens may produce an inhibitory pheromone which prevents the development of sexuals in a queenright colony (PETERSEN-BRAUN, 1975). Some evidence for the existence of an inhibitory pheromone was reported by BERNDT (1977) who found that rinsing queens in organic solvents apparently removed their ability to suppress the development of sexuals. On the basis of similar, independent experiments, the possible existence of an inhibitory pheromone was also proposed by EDWARDS (1978) cited by KING (1979).

The present study has been undertaken to investigate aspects of caste determination and regulation in *Monomorium pharaonis* in order to gain a better understanding of these processes.

CHAPTER 2. MATERIALS AND METHODS.

Specific details of special materials or methods used are given at the appropriate points in the experimental sections. This chapter outlines a number of general techniques and materials which have been used throughout the study.

2.1 General culture methods for *Monomorium pharaonis*.

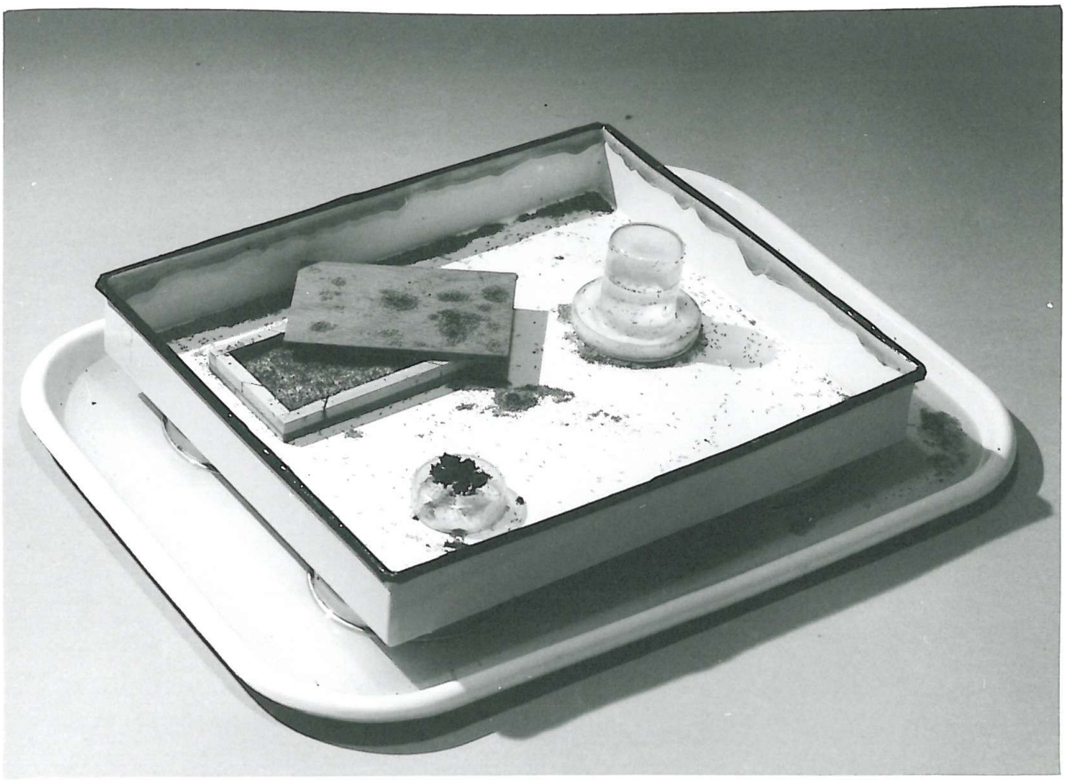
Unless otherwise stated, all experiments were carried out with the standard laboratory strain of *Monomorium pharaonis* (L.) maintained at the Slough Laboratory of the Ministry of Agriculture, Fisheries and Food. These insects have been in culture at this Laboratory for approximately 30 years and, as far as is known, the strain is the same as that studied by A.D. Peacock and his colleagues at the University of St Andrews (Dundee). During this long period of culture there has been no apparent diminunition of the viability nor any apparent changes in the form or behaviour of these insects.

Laboratory colonies of *M. pharaonis* were reared in small wooden nest-boxes (12.5 x 8.5 x 2.0 cm) with removable lids (Figure 7). The sides of the boxes were furnished with 8 entrance slits, 2 mm wide, to allow the ants to enter and leave. Nest-boxes were placed, singly or in pairs, in enamelled surgical trays (35 x 25 x 5 cm) the sides of which were coated with an aqueous suspension of polytetrafluoroethylene (Fluon) to prevent the ants escaping. Initially, the top edges of the trays were covered with a thin layer of insect trapping-grease (Stiktite) but later this procedure was discontinued. The enamelled trays were, themselves, placed on 4 rubber stands (5 cm high) which supported the trays in larger metal trays containing a 1 cm depth of mineral oil (Figure 7).

The colonies were given access to food and water *ad libitum*. Food was placed on an inverted petri dish lid (5.5 cm dia) and water was provided in a 50 ml glass beaker which was inverted into a petri dish containing a pad of cotton wool (Figure 7). The food was a mixture of dried powdered ox-liver, honey and sponge-cake in the ratio 2:1:1

Figure 7. Laboratory colony of *Monomorium pharaonis* (L.).

Figure 8. Worker ants feeding on food mixture in a laboratory colony of *M. pharaonis*.

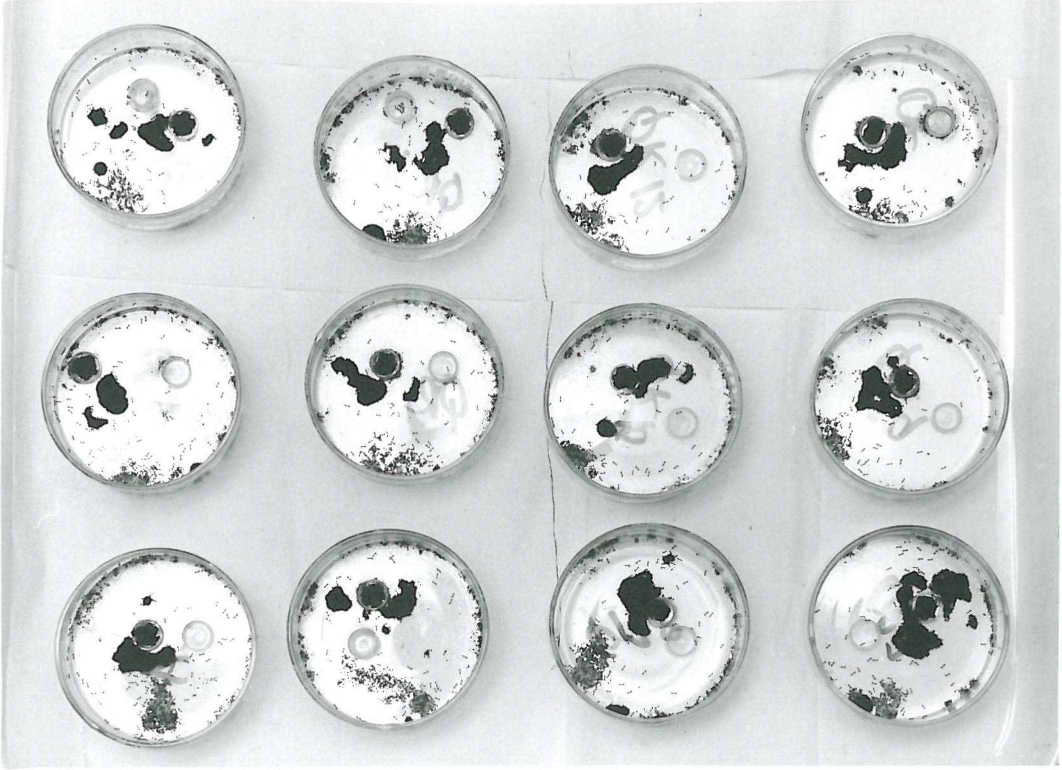


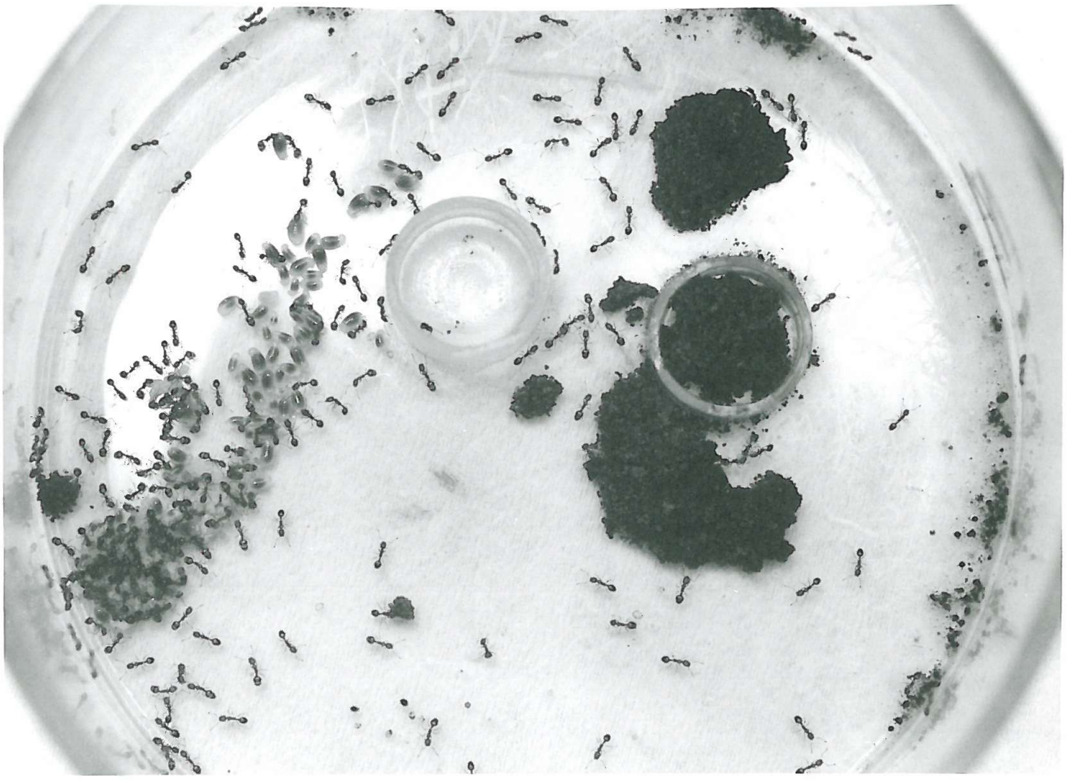
(by weight) to which a small quantity of distilled water (< 10%) was added to achieve a smooth moist consistency. This food mixture is highly attractive to foraging *M. pharaonis* workers (Figure 8). Food material was stored in sealed glass jars at -20°C to prevent decomposition and mould growth.

Colonies of *M. pharaonis* were kept in subdued (red) light at 27°C and 70% r.h. throughout the experiments. Such colonies contained many thousand workers and developing stages (brood) and usually, several hundred queens. From such large colonies, small 'mini-colonies' (Figures 9 and 10) were set-up for detailed observation or manipulation. These colonies were housed in glass crystalizing dishes (8 cm dia.) the sides of which were coated with Fluon. Such mini-colonies were set-up by transferring appropriate numbers of adults and developing stages from the large laboratory colonies. Thus, several hundred mixed brood stages were removed with a spatula from the brood area of a laboratory colony nest cell. This brood was placed in a small petri dish lid, the sides of which were coated with Fluon. The dish was transferred to the stage of a binocular (zoom) microscope and appropriate brood stages transferred to the mini-colony dishes with a fine, moistened, paint brush. Mini-colonies typically comprised of 100 mixed brood stages (larvae, pre-pupae and pupae), 50 to 100 workers, 50 eggs and a known number (usually 1) of queens. With the exception of the number of queens, these values are approximate (because of the difficulty of counting developmental stages) particularly in the case of eggs. Unless otherwise stated, these mini-colonies were set up with insects which were removed from large colonies which contained fertile queens and which were not producing a new batch of sexuals. All mini-colonies were given food and water *ad lib.* which were provided in small glass dishes (Figure 7). Mini-colonies were examined with a binocular microscope with zoom lens (10x to 70x) and illumination was provided by a single, variable intensity, light source equipped with a red filter. The use of a red light source minimised the disturbance caused to mini-colonies while they were being examined under the microscope. Such mini-colonies appeared to thrive and behave normally under such conditions for at least 100 days.

Figure 9. A series of mini-colonies used in experiments with *M. pharaonis*.

Figure 10. Close-up of a mini colony of *M. pharaonis* showing brood aggregated into 'nest-area'.





However, most experiments with mini-colonies were completed before the colonies were 50 days old. Although no nesting place was provided in the dishes containing mini-colonies, the ants moved brood to a discrete area (usually near the side wall) of the dish (Figure 10) and this 'nest area' (comprising brood, queens and food-pile) once established, was seldom moved. Unless otherwise stated mini-colonies were examined at 2-3 day intervals throughout the experiments. The appearance and number of sexual brood stages was recorded at each examination, and the sex of developing sexuals was recorded at the pupal stage, at which point they were usually removed from the mini-colonies.

Sexual stages (males and queens) for use in mini-colonies or for rinsing in organic solvent were obtained by removing all queens from one of the large laboratory colonies such that a new batch of sexual brood was produced. Virgin queens were obtained by isolating queen pupae, obtained in the above manner, with workers, food, etc. in mini-colony dishes such that adult queens emerged in the absence of males. It was not possible to distinguish between male larvae and queen larvae, even when these were fully-grown. However, male and queen pre-pupae could be separated on the basis of their relative weights (Figure 5). Male and queen pupae are easily distinguishable on the basis of gross morphological characteristics (Figure 6).

2.2 Dissection of exocrine glands.

Dissection of adult queens (for removal or examination of internal organs) was made by immersing the insect in a small dissecting dish filled with sterile insect Ringer solution. The insects were held in place with small entomological pins and dissection was performed, under a binocular microscope, with a variety of fine forceps, pins and fragments of razor blades mounted in a stainless steel handle.

2.3 Rinses of ants in organic solvents.

Known numbers of insects, removed from large laboratory colonies or from mini-colonies were rinsed in organic solvents in the following way. Insects were gently agitated in solvent (methylene chloride or diethyl ether, both "AnalaR" purity) for a known period of time - usually 4 or 5 min. Preliminary investigations had shown that longer periods of rinsing did not increase the efficiency with which certain materials (see Chapter 4) were extracted but did increase the quantities of high molecular weight lipids in the rinse. Initially, methylene chloride was used as the solvent, but this material was found to be unsuitable for use with the Varian 3700 gas chromatograph (see below) and subsequently, the rinses were made with diethyl ether.

After rinsing, the solvent was transferred to a small glass volumetric flask (2 to 10 ml) and reduced to dryness under a stream of dry nitrogen at 25°C. This process was stopped as soon as the solvent had evaporated to minimize the loss of highly volatile materials. After reduction, the residue was taken up in a known volume of solvent (50 to 100 μl) and aliquots were removed for analysis by gas-liquid chromatography (GC).

2.4 Analytical and preparative gas-liquid chromatography (GC).

Analyses of rinses were performed on a Varian gas chromatograph (Model 3700) equipped with a flame ionization detector and a thermal-conductivity detector. In general, analyses were performed on aliquots (1 to 10 μl) of the solvent rinses dissolved in diethyl ether. The use of methylene chloride as a solvent was discontinued after it was found to cause (conductive) carbon deposits on the ceramic insulation of the flame ionization detector. Analyses were run on 5% OV 101 on acid-washed diatomite (80 - 100 mesh) in a packed glass column (1m x 2 mm id.). Helium (30ml/min.) was used as the carrier-gas. Three temperature programmes were used depending upon the particular requirements of the analysis:-

Programme I	(For preliminary investigation of the components of solvent rinses) : temperature 60-230°C at 10°C/ min; initial time, 8 min; final time, 4 min; injector and detector temperature 240°C.
Programme II	(For general and comparative analysis of rinses) : temperature 100-250°C at 10°C/min; initial time 6 min; final time, 6 min; injector and detector temperature, 260°C.
Programme III	(For rapid identification and measurement of GC peak d from queen rinses) temperature 120-200°C at 5°C/min; initial time, 4 min; final time, 10 min; injector and detector temperature, 280°C.

Injection of a sample aliquot was made using a Hamilton gas-tight syringe (10µl capacity). Although the concentration of various chemicals in the solvent rinses varied depending on the number of insects rinsed and the volume of the solvent, most aliquots contained between 0.1 and 1 insect equivalents per injection. The sensitivity of the detector was adjusted (range and attenuation) to give reasonably sized peaks at such concentrations. Unless otherwise stated, the chart speed of the pen-recorder used to monitor the analyses was 5mm/min and the sensitivity (input) set for 1mv.

Preparative gas chromatography of peaks from insect rinses was performed on the Varian 3700 GC using the thermal conductivity detector. This detector is non-destructive and allows measurement and location of peaks which subsequently can be trapped in glass tubes inserted into the outlet port of the GC. Preparative GC was run on 5% OV101 on acid washed diatomite CLQ, 80-100 mesh in a 1m column (id. 4mm). Column temperature was 200°C (isothermal), detector current 203-205 mA, detector temperature 200°C, injector temperature 210°C and filament temperature 260°C. Samples of rinses (10-100µl) were injected with a Hamilton gas-tight syringe and relevant peaks were trapped in clean glass tubes inserted into the outlet port of the GC. Subsequently, the collection tubes were thoroughly washed in "AnalaR" diethyl ether and the combined washes, reduced to an appropriate volume, were stored at -20°C in sealed flasks.

2.5 Mass spectrometry and Nuclear Magnetic Resonance spectrometry.

Mass spectrometry was performed on whole rinses or constituents of rinses using a variety of machines. These analyses were kindly performed by the late Dr J. Siddall and Dr. G. Jamieson (Zoecon Corp. Palo Alto, California) and, Mr D. Lee and Mr J. Wilkins (Ministry of Agriculture, Harpenden Lab.). In addition, high resolution mass spectrometry of peak d from queen rinses was performed by Dr C. Creaser (Physico Chemical Measurements Unit, Harwell). Nuclear magnetic resonance spectrometry was kindly provided by Dr N.F. Janes (Rothamsted) in conjunction with Dr J. Chambers (Slough Laboratory). I am indebted to these colleagues and to Dr. J. Pickett (Rothamsted) for their interpretation of the NMR data.

2.6 Scanning electron microscopy.

Scanning electron micrographs of various stages of *M. pharaonis* were kindly provided by Mrs V. Ely and Miss B. Higgins (MAFF, Slough Lab) and were obtained using a Cambridge Stereoscan 250 machine. Specimens for electron microscopy were dehydrated and coated with gold using standard procedures.

CHAPTER 3 THE INFLUENCE OF QUEENS ON THE PRODUCTION OF SEXUALS.

3.1 Introduction.

As previously indicated (section 1.3) the presence of queens in a colony of *M. pharaonis* normally prevents the appearance of new sexual forms. The following experiments were made to examine the nature and extent of this inhibitory effect.

3.2 Isolation of queens from contact with workers.

Preliminary studies were made with fertile queens enclosed in short sections of glass tubing (2 cm long) sealed at each end with a double layer of brass gauze. These tubes prevented physical contact between the queens within the tubes and the workers in the colonies in which such tubes were placed. Four mini-colonies, each containing 100 eggs, 100 mixed brood stages and 150 workers were set up. Into two of these were introduced 4 queens confined in glass tubes. The remaining 2 mini-colonies were kept without queens. In these experiments, all 4 mini-colonies produced sexual pupae (males and queens) suggesting that queens are unable to suppress the development of sexuals if physical contact between them and workers is prevented. This result would also seem to preclude the existence of a volatile inhibitory pheromone produced by queens as the factor responsible for inhibiting sexual production. However, because queens kept isolated from contact with workers might have behaved abnormally (because they would have been isolated from normal social interaction e.g. feeding, grooming etc.) a further experiment was conducted to investigate the effect of isolating queens from workers.

In these studies, pairs of mini-colonies were set up in a covered, concentric glass container (Figure 11) such that the two nests were connected by their common atmosphere but no contact between insects in adjacent nests was possible. The composition of these colonies was 100 eggs, 100 mixed brood stages and 150 workers. In 3 of

Figure 11. Concentric mini-colonies (with glass lid removed).

Figure 12. Queen 'guillotine' apparatus. Queens held in place by the indented 'guillotine' blade (b).

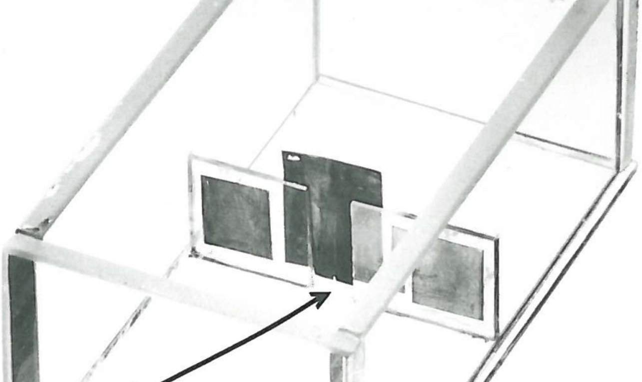

b

Table 1 Production of sexual forms in mini-colonies in which contact with queens was either direct or only through a common atmosphere.

No.	type	contact with queens (+ or -)	production of sexual larvae (+ or -)	No. of sexual pupae produced	
				Male	queen
1	outer	+	-	0	0
	inner	-	+[a]	0	0
2	outer	+	-	0	0
	inner	-	+	7	5
3	outer	+	-	0	0
	inner	-	+	3	9
4	outer	-	+	11	8
	inner	-	+	9	3
5	outer	+	-	0	0
	inner	+	-	0	0

[a] Only one sexual larva produced, which subsequently failed to pupate.

these concentric colonies, the outer nests were each given 5 fertile queens whilst the inner nests were kept queenless. In another concentric colony, both inner and outer nests were given 5 fertile queens, and in a further concentric colony, both the inner and outer nests were kept queenless. The production of sexual larvae and pupae in both inner and outer nests was recorded. The results of this experiment (Table 1) clearly demonstrate that physical contact between workers and queens is essential for the suppression of sexual forms, and that there is no evidence for a volatile chemical signal emanating from fertile queens or queenright colonies which is responsible for supressing the development of sexual forms. That physical contact between queens and workers is necessary in order that queens can inhibit the development of sexual forms was further investigated in an experiment designed to examine the 'quantity' of contact required to inhibit sexual development. In these experiments, 16 identical mini-colonies, each containing 100 eggs, 120 mixed brood stages and 150 workers were each exposed to 3 fertile queens for periods ranging from zero to 7 days per week. The results of this experiment are given in Table 2. Three days per week (approx. 40% exposure) was sufficient exposure to completely suppress the production of sexuals, and with one or two days exposure per week, the production of sexuals was markedly reduced. This falling-off of response suggests that there may be a threshold level of contact between workers and queens above which the production of sexuals is completely suppressed.

3.3 Influence of age and physiological state of queens on their ability to inhibit the production of sexuals.

Four mini-colonies each containing 50 eggs, 100 mixed brood stages and 150 workers were set up. Each colony was given two queens of known age-range (initially 0-4 weeks). Three of the colonies were given fertilized queens and one colony was given virgin queens. These queens were kept in the mini-colonies for a period of 28 days whereupon they were transferred to newly set-up mini-colonies. In this way, colonies were reared in the presence of queens of known age. The production of sexual forms in these colonies was recorded during the period when queens were present. In addition, after the removal of queens, the colonies were kept for a further 28 days so that the subsequent production of sexuals in the absence of queens could be recorded. In these experiments one virgin queen died when it was 12-16 weeks old and the other when it was 20-24 weeks old. In two of the colonies containing fertile queens, both queens in both colonies died when they were 48-52 weeks old. In the remaining colony, one queen died when it was 52-56 weeks old and the other when it was 56-60 weeks old. Thus, these fertile queens had an average life-span of 52 weeks.

Table 2 Effect of amount of contact between queens and workers on the production of sexual forms.

No.	Time of worker contact with queens	Sexual brood produced	No. of sexual pupae		
	(days per week)	(+ or -)	male	queen	total
1	0	+	6	6	33
2	0	+	10	11	
3	1	+	1	2	11
4	1	+	7	1	
5	2	+[a] (1 larva)	0	0	2
6	2	+	1	1	
7	3	-	0	0	0
8	3	-	0	0	
9	4	-	0	0	0
10	4	-	0	0	
11	5	-	0	0	0
12	5	-	0	0	
13	6	-	0	0	0
14	6	-	0	0	
15	7	-	0	0	0
16	7	-	0	0	

[a] Only one sexual larva produced which subsequently failed to pupate.

The results of these experiments are presented in Table 3 and show that virgin queens, irrespective of age, are unable to suppress the development of sexual forms. By contrast, fertile queens are able to inhibit sexual formation throughout their lifetime. However, by measuring the time after removal of queens to the first

Table 3. Influence of age and physiological state of queens on their ability to inhibit the production of sexual forms.

Colony No.	age range of queens (weeks)	physiological state of queens	sexuals produced during exposure to queens		sexuals produced after removal of queens		Time (days) to first queen pupa
			male	queen	male	queen	
1	0-4	fertile	0	0	5	2	12
2		fertile	0	0	7	10	12
3		fertile	0	0	4	3	12
4		virgin	4	8	-	-	-
5	4-8	fertile	0	0	0	12	25
6		fertile	0	0	2	15	29
7		fertile	0	0	2	11	25
8		virgin	3	8	-	-	-
9	8-12	fertile	0	0	1	12	22
10		fertile	0	0	1	9	29
11		fertile	0	0	3	15	22
12		virgin	7	4	-	-	-
13	12-16	fertile	0	0	1	9	27
14		fertile	0	0	1	3	32
15		fertile	0	0	0	3	29
16		virgin	0	6	-	-	-

Cont.

Table 3. (Continued)

Colony No.	age range of queens (weeks)	physiological state of queens	sexuals produced during exposure to queens		sexuals produced after removal of queens		Time (days) to first queen pupa
			male	queen	male	queen	
17	16-20	fertile	0	0	2	22	25
18		fertile	0	0	0	3	27
19		fertile	0	0	2	11	29
20		fertile	2	7	-	-	-
21	20-24	fertile	0	0	1	3	32
22		fertile	0	0	0	3	36
23		fertile	0	0	1	2	32
24		virgin	2	3	-	-	-
25	24-28	fertile	0	0	0	7	31
26			0	0	0	4	31
27			0	0	0	1	34
29	28-32	fertile	0	0	3	7	25
30			0	0	10	11	29
31			0	0	0	3	32

Cont.

Table 3. (Continued)

Colony No.	age range of queens (weeks)	physiological state of queens	sexuals produced during exposure to queens		sexuals produced after removal of queens		Time (days) to first queen pupa
			male	queen	male	queen	
33	32-36	fertile	0	0	1	16	29
34			0	0	5	12	29
35			0	0	0	9	29
37	36-40	fertile	0	0	0	10	29
38			0	0	0[a]	0[a]	-
39			0	0	0[a]	0[a]	-
41	40-44	fertile	0	0	0	3	15
42			0	0	0	1	18
43			0	0	(one larva)		-
45	44-48	fertile	0	0	1	5	23
46			0	0	1	9	21
47			0	0	(1 pre-pupa)		-
49	48-52	fertile	0	0	0	0[a]	-
50[a]			0	0	4	6	23
51[b]			0	0	4	5	21
53[b]	52-56	fertile	0	0	3	5	7

[a] Colony lost through mould growth.

[b] One or both queens died during period.

appearance of new queen pupae (Table 3) and using this as a measure of the degree of inhibition exerted by queens, it is clear that the inhibitory effect of queens is less when they are 0-4 weeks old. By utilizing the same measure, there is also an indication that the inhibitory effect of old queens (i.e. > 40 weeks old) is also somewhat reduced (Table 3). These findings are in agreement with results obtained by PETERSEN-BRAUN (1975) and are further discussed in Chapter 7.

3.4 Artificial de-alation of virgin queens - effect on production of sexuals.

In the previous experiment, it was shown that virgin queens were unable to suppress the development of sexual forms. Since virgin queens are normally winged, it was decided to investigate whether artificial de-alation (removal of wings) might endow virgin queens with the ability to suppress the development of sexuals normally present only in fertile (i.e. wingless) queens.

Nine mini-colonies, each containing 100 mixed brood stages, 50 eggs and 100 workers were set up. After 3 days, 3 colonies were each given single fertile queens, 3 were each given a winged virgin queen, and the remaining 3 were each given a virgin queen whose wings had been surgically removed close to their attachment to the thorax. Subsequently the colonies were examined regularly for 45 days, and the numbers of sexuals produced in each colony was recorded (Table 4). None of the colonies given fertile queens produced sexuals. However, all mini-colonies containing either normal (winged) virgins, or artificially de-alated virgins produced sexual forms, although in one instance, sexual larvae did not survive to form pupae (Table 4). These results suggest that the presence or absence of wings is not important in relation to the ability of queens to inhibit the development of sexual brood.

Table 4. Effect of artificial de-alation of virgin queens on their ability to inhibit the production of sexuals.

Colony No.	type of queen	Number of sexual pupae produced		
		male	queen	total
1)	fertile	0	0	0
2)		0	0	0
3)		0	0	0
4)	winged virgin	4	3	7
5)		2	1	3
6)		0	0	0[a]
7)	wingless virgin	1	4	5
8)		0	2	2
9)		1	3	4

[a] Produced sexual larvae but these failed to form pupae

3.5 Exposure of workers to dead queens and parts of live queens - effect on inhibition of production of sexuals.

3.5.1. Exposure of colonieB to parts of live queens.

For these experiments, a piece of equipment was designed so that two colonies could be separated by a partition such that one colony had access to the head and thorax of a live queen while the other colony was exposed to the gaster only. Contact between workers in each colony was prevented by bands of Fluon. The apparatus used (Figure 12) was a glass container divided in the centre by a moveable partition which incorporated a "guillotine" which held the queen between the thorax and gaster. Four such experiments were set up, in each of which the two separate colonies contained 50 eggs, 100 mixed brood stages and 150 workers. Each colony was exposed to either head and thorax or gaster of a succession of live queens for 22-30 days. Dead queens were replaced daily.

The numbers of sexuals produced in these colonies is given in Table 5. In all 4 colonies sexuals were produced in both the colony with access to queen head and thorax and in the colony with access to the gaster only. However, the mortality of "guillotined" queens was high and the apparent conclusion that both parts of a queen are necessary to inhibit the production of sexuals may not be justified.

3.5.2. Exposure of workers to dead queens.

Ten mini-colonies, each containing 100 mixed brood stages, 50 eggs, and 100 workers were set up. Two colonies were given no queens and two colonies were given a new live queen each day for the duration of the experiment. Of the remaining six colonies, two were given a freshly killed queen, two were given a queen that had been dead for 24 hr and the remaining two were given a queen that had been dead for 48 hr. Queens were killed by exposure (15 min.) to ethyl acetate vapour and, in all cases, queens were replaced daily. The experiment was continued for 41 days during which time a record of the sexual forms reared in each colony was kept (Table 6). The results indicate that even the daily addition of freshly killed queens does not prevent the rearing of sexuals in colonies of *M. pharaonis* During these experiments it was noticed that workers appeared very 'interested' in freshly-killed queens when they were first introduced into the colony. The workers clustered around the dead queen 'licking' it and tourching it with their antennae. This behaviour diminished relatively rapidly and the dead queen was usually removed from the nest area after a few hours. When queens that had been dead for more than 24 hr were introduced into colonies, the response of workers was much less apparent and such queens were usually removed from the nest area immediately.

Table 5. Effect of limiting contact of workers to either head and thorax or gaster of fertile queens on the production of sexual forms.

Colony No.	total time of contact with queen part (days)	Sexual pupae produced in colony with gaster		head & thorax	
		male	queen	male	queen
1	22	5	12	0	5
2	25	4	9	4	10
3	27	5	5	2	8
4	30	3	13	7	10

Table 6. Effect of dead queens on the production of sexual forms.

Colony No.		type of queen (age after death)	sexual pupae produced male	queen
1	)	dead queens	1	2
2	)	(0-24hr)	1	1
3	)	dead queens	5	1
4	)	(24-48hr)	2	0
5	)	dead queens	1	0
6	)	(48-72hr)	3	4
7	)	live queens	0	0
8	)		0	0
9	)	no queens	3	1
10	)		3	4

3.6 Effect of rinsing queens in organic solvent on their ability to suppress the formation of sexual brood.

It has already been established that it is unlikely that queens of *M. pharaonis* produce a volatile chemical signal by which they exert their inhibitory effect on the production of sexual brood. However, the possibility that a non-volatile chemical signal, transmitted by contact with workers, is present cannot be excluded. For this reason an experiment was set up in an attempt to repeat earlier, unpublished tests by the author and to confirm the results of similar tests carried out independently by BERNDT (1977). Six mini-colonies containing 50 eggs, 100 mixed brood stages and 100 workers were exposed to 2 queens for 3 days per week over a period of six weeks. Two colonies were given fully fertile queens, two were given virgin queens, and two were exposed to queens which were rinsed, each day during exposure, for 7 sec in ether/acetone (2:3 v/v).

The results of these experiments are presented in Table 7 and demonstrate that rinsing queens in organic solvent removes their ability to inhibit the formation of sexuals. This observation suggests that there may be a non-volatile, lipid-soluble, chemical signal produced by fertile queens which is responsible for the inhibitory effect of queens on the production of sexual forms. During these experiments, the mortality of rinsed queens was rather high (approx 25% at each rinsing) and, non-quantitative observation suggested that, the rate of oviposition might have been reduced in rinsed queens. This last observation was in contrast to the results reported by BERNDT (1977) who noted no diminution of oviposition in queens rinsed in acetone for 15 sec. daily. However, preliminary attempts to repeat the rinsing regime of BERNDT (1977) resulted in much higher mortality than was the case when queens were rinsed for 7 sec in ether/acetone.

Table 7. Effect of rinsing queens in organic solvent on their ability to inhibit the formation of sexual forms.

Colony No.	type of queen	days exposed (per week)	sexual pupae produced		
			male	queen	total
1	fertile	3	0	0	0
2			0	0	
3	virgin	3	5	12	36
4			14	5	
5	rinsed	3	5	6	31
6			4	16	

3.7 Summary of the influence of queens on the production of sexual forms.

From the results of experiments described in this chapter, several conclusions can be made concerning the inhibitory effect of queens upon the production of new sexual forms. Firstly, it appears that only fertile queens are able to suppress the development of new sexuals. Virgin queens are unable to suppress sexual production irrespective of their age or of the presence of wings. Secondly, even in the case of fertile queens, young queens (0-4 weeks old) (and possibily old queens) are less able to inhibit the production of sexuals than are fully fertile (mature) queens. Thirdly, the inhibition of the production of new sexual forms by fertile queens requires that they have physical contact with other colony members (i.e. workers and brood) and that this contact be maintained for about 40% of the time. Lastly, freshly-killed, dead queens and queens that have been rinsed in organic solvents, lose their ability to inhibit the production of new sexual forms.

These results suggest that further information on the nature of the inhibitory affect of fertile queens on the production of sexuals, might be obtained by comparing the biology of virgin and fertile queens. Although the 'queen-rinse' experiments, suggest that a chemical signal (pheromone) may be responsible for the inhibitory action of fertile queens, it is not possible, at this stage, to exclude other factors. Thus, in the following chapters, several aspects of the chemistry, physiology and behaviour of virgin and fertile queens are investigated in an attempt to identify the factor(s) responsible for the queen-inhibition of sexual production.

CHAPTER 4. INVESTIGATION AND ANALYSIS OF SEMIOCHEMICALS PRODUCED BY WORKERS, VIRGIN QUEENS AND FERTILE QUEENS.

4.1 Analysis of solvent rinses from workers, virgin queens and fertile queens.

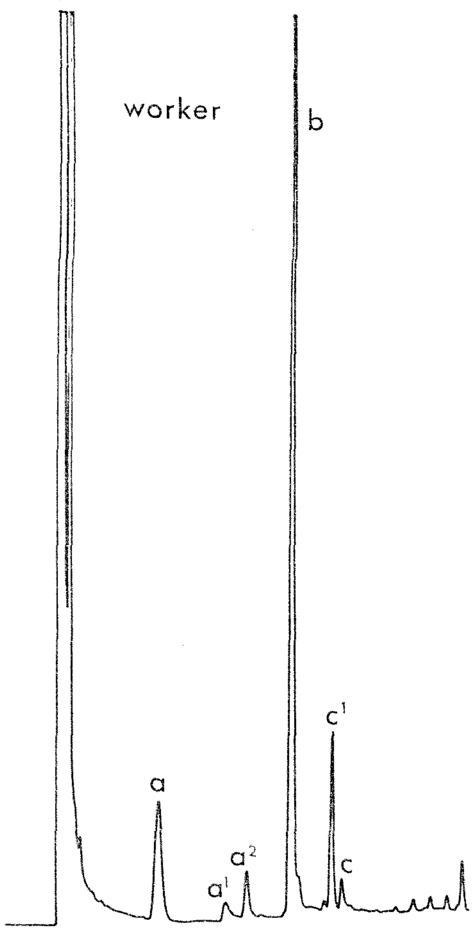
Worker ants, virgin queens and fertile queens were rinsed in organic solvents as described in the methods section, and the rinses analysed by gas-liquid chromatography (GC). Workers and fertile queens were randomly selected from established colonies. Virgin queens were obtained as described in Chapter 2. Several such analyses were performed on each type of female on different occasions and a typical set of chromatograms is presented in Figure 13. On all occasions, the three types of female produced the characteristic chromatographic patterns shown in Figure 13. Each of the early peaks on the chromatograms was given a letter of identification as follows: a, a^1, a^2, b, c^1, c and d. Peaks eluting after peak 'd' were not labelled and their intensity and presence was associated more with the time of rinsing than with the type of female. The retention time and occurence of each peak is given in Table 8.

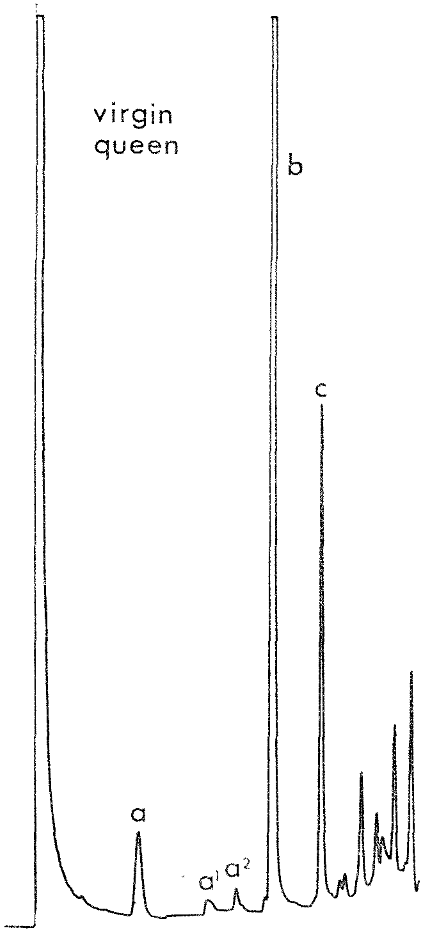
Table 8 Occurrence and retention time of peaks analysed by gas-liquid chromatography from rinses of worker and queen *M. pharaonis*.

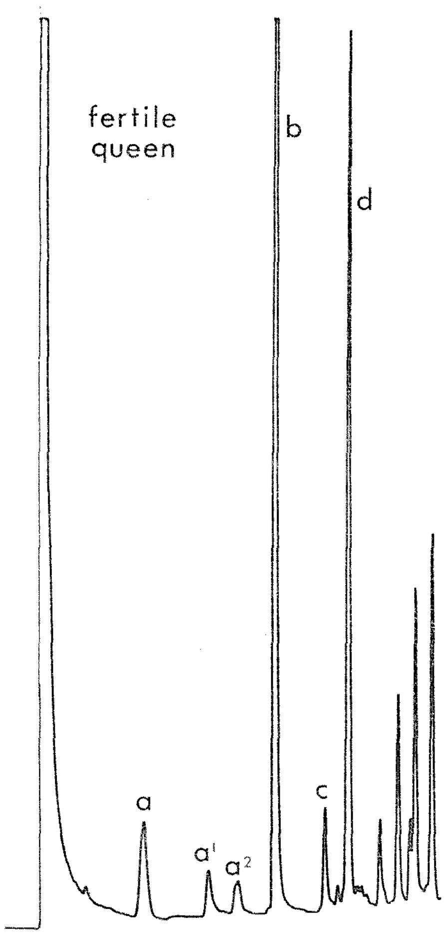
GLC peak	retention time (min)[a]	peak found in rinses of		
		workers	virgin queens	fertile queens
a	5.2	+	+	+
a^1	8.3	+	+	+
a^2	9.3	+	+	+
b	11.5	+	+	+
c^1	13.5	+	–	–
c	14.0	(+)[b]	+	+
d	15.1	–	–	+

[a] GC programme II. [b] Possibly present in trace amounts.

Figure 13. Chromatograms from GC analysis of solvent rinses of fertile queens, virgin queens and workers of *M. pharaonis*.


worker
b
a
a¹
a²
c¹
c


virgin queen
b
c
a
a¹
a²


fertile queen
b
d
a
a¹
a²
c

The results of these analyses indicate that peaks a, a^1, a^2 and b are common to workers, virgin queens and fertile queens. Peak c^1 appears to be present only in workers, and c appears to be much more abundant in queens (virgin and fertile) than in workers. However, of most interest was the fact that peak d, present in fertile queens, was absent from rinses of both workers and virgin queens. This material is therefore a prime candidate for the suggested 'inhibitory pheromone' which fertile queens may use to suppress the production of new sexuals.

4.2 Tentative identification of the chemicals found in rinses of queens and workers.

4.2.1. Introduction to the chemistry of *M. pharaonis*

Previous studies by several workers have demonstrated that workers and queens of *M. pharaonis* produce a number of semiochemicals (pheromones) with a variety of proposed roles. SUDD (1960) and BLUM (1966) investigated trail following in *M. pharaonis* and HOLLDOBLER (1973) investigated the foraging strategy of *M. pharaonis* workers. The latter author concluded that worker ants produce a trail substance in the Dufour's gland and a repellent substance, enabling workers to compete for Crey with other ant species, which is discharged from the poison gland. HOLLDOBLER and WUST (1973) reported the existence of a sex pheromone produced in the Dufour's gland and bursa copulatrix of virgin queens, which was absent in workers and in older, fertilised, females. RITTER *et al.* (1973) homogenized large numbers of workers in dichloromethane and analysed the samples by GC. Using other analytical techniques, these authors identified a substance capable of inducing trail following in workers as 5-methyl-3-butyl-octahydroindolizine. The structure of this material was subsequently re-examined and found to be 3-methyl-5-butyl octahydroindolizine and the compound was called monomorine I (RITTER *et al.*, 1975). In addition, RITTER *et al.* (1975) reported the structure of several other chemicals identified in workers and queens of *M. pharaonis* and the chemical names and source of these compounds are given in Table 9. The chemical structures of five of the six monomorines identified by Ritter and co-workers are given in Figure 15. It was originally thought that monomorine I and two of the pyrrolidine compounds, (monomorine II and III) were the major active components of the trail pheromone (RITTER *et al.*, 1973; 1975). Subsequently however, a further compound was discovered in the Dufour's gland of workers (RITTER *et al.*, 1977) which was much more active than the monomorines as a trail-initiating substance. This material was identified as 3,4,7,11-tetramethyl-6,10-tridecadienal-1 and given the trivial name faranal (Table 9). With the discovery and identification of faranal as the likely trail pheromone, the question arose as to the function of the other compounds identified by Ritter and his colleagues. Jones *et al.* (1982) in a study of the venom chemistry of several species of *Monomorium* (excluding *pharaonis*) found that several species produced a mixture of asymetrical *trans* -2,5-dialkyl pyrrolidines similar to monomorine V. These authors suggested that these pyrolidines were defence secretions or repellent substances utilized when these lestobiotic species are stealing the brood of other ants. It is possible therefore, that the monomorines produced by *M. pharaonis* have a similar function. Further support for this hypothesis was provided by BLUM, *et al.* (1980) who demonstrated that the thief ant, *Solenopsis fugax* utilizes 2-butyl-5-heptyl pyrrolidine to repel workers of other ant species, whilst stealing their brood. However, both RITTER *et al.* (1973) and EDWARDS and PINNIGER (1978) were able to induce trail following in *M. pharaonis* with deposits of all- *cis* monomorine I, so the possibility that these compounds are an important part of a multi-component trail pheromone cannot be excluded. Furthermore, since workers of *M. pharaonis* exhibit noticeable alarm responses when disturbed, characterised by raising of the gaster and emission of volatile material (LEGAKIS, 1980), it is conceivable that one or more of the alkaloids found in this species might act as alarm pheromones. In addition to the monomorines and faranal, RITTER *et al.*(1975) reported the occurrence of an unidentified bicyclic hydrocarbon ($C_{18}H_{30}$) found only in young winged queens (virgins) which they called monomorene (Table 9). These authors suggested that this material might be the sex pheromone previously reported by HOLLDOBLER and WUST (1973).

Table 9. Chemical name, source and occurrence of substances previously isolated and identified in *Monomorium pharaonis*.

Chemical name	Trivial name	Source (gland)		Occurs in		
		Dufour's	Poison	workers,	virgin queens,	fertile queens
5-butyl-3-methyl octahydroindolizine	monomorine I	–	+	+		+
2-butyl-5-pentyl pyrrolidine	monomorine II	–	+	+		+
2-(5^1-hexenyl), 5-pentyl pyrrolidine	monomorine III	–	+	+		+
2-(5^1-hexenyl), 5-heptyl pyrrolidine	monomorine IV			+		
2-(5^1hexenyl), 5-nonyl pyrrolidine	monomorine V			+		

Table 9 (continued)

Chemical name	Trivial name	Source (gland)		Occurs in		
		Dufour's	poison	workers	virgin queens	fertile queens
3-hexenyl-5-methyl octahydro indolizine	monomorine VI			+		
-	monomorene	+	+	-	+	-
3,4,7,11-tetramethyl-6,10-tridecadienal-1	faranal	+	-	+		

[a] Data from RITTER *et al.* (1973, 1975, 1977)

Thus, a considerable amount of information was available on the identity and source of chemical substances produced by workers and queens of *M. pharaonis*. It seemed possible that at least some of the compounds present in the rinses of queens and workers might correspond to faranal, monomorene or some of the six monomorines previously reported in this species. This possibility was therefore investigated.

4.2.2 Identification of the chemical structure of peaks a and b from queen rinses

4.2.2.i Comparison of GC retention times of peaks a and b with those of pure samples of monomorines I and III

Pure samples of monomorine I and monomorine III were injected onto a 1m, 5% OV101 packed column (programme II) as described in the methods section. The sample of monomorine I (all-*cis* isomer) was synthesised and provided by Dr. P. Sonnet (U.S.D.A. Gainesville) and the sample of monomorine III was a kind gift from Dr. J. Newton (Rentokil U.K. Ltd). The retention times of these materials were found to be the same as those of peaks a and b of the queen rinse respectively (Table 10) suggesting that these materials could well be identical.

Table 10 Retention times[a] of pure samples of monomorines I and III compared with peaks a and b from queen rinses

Compound	retention time (min)[a]
monomorine I	5.2
monomorine III	11.5
peak a	5.2
peak b	11.5

[a] 1m 5% OV101 programme II

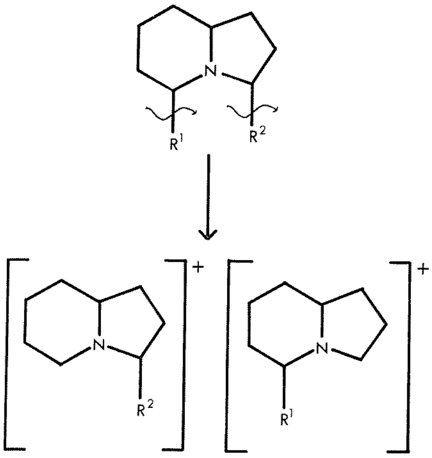
4.2.2.ii Mass spectrometry of peaks a and b from queen rinses

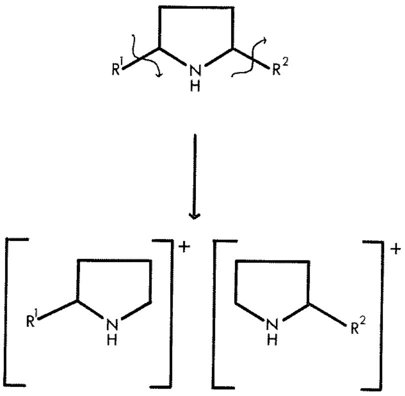
The fragmentation patterns of dialkyl indolizines and pyrrolidines subjected to mass spectrometric analysis are, fortunately, characteristic. This is because such molecules preferentially cleave at the alpha-carbon bonds. Thus, these compounds tend to fragment in the manner illustrated in Figure 14 whereby the alkyl chains are cleaved from the ring. The result of this preferred fragmentation is that mass spectra from such molecules show strong signals (^{m}z) for ions corresponding to the molecular ion minus R^1 and molecular ion minus R^2 (Figure 14). Thus for monomorine I, RITTER *et al.* (1973) reported strong signals at ^{m}/z = 180 and ^{m}/z = 138 and these values represent the mass of the indolizine ring plus the methyl group at carbon 3 and the indolizine ring plus the butyl group at carbon 5 (Figure 15). Given this characteristic fragmentation pattern, the structure of such compounds can be predicted with reasonable confidence.

Samples of rinses from fertile queens were subjected to combined GC/mass spectrometry. Initially these analyses were kindly performed by the late Dr. J.B. Siddall and Dr. G. Jamieson (Zoecon Corp. U.S.A.). Subsequently GC/mass spectrometry data was generously provided by Mr D. Lee and Mr J. Wilkins (MAFF Harpenden). Data obtained from these analyes are presented in Figures 16 to 21. Considering Figures 16 and 17, i.e. the mass spectra of peaks a and b from the queen rinse, the data is consistent with the structures of monomorine I and monomorine III respectively. In the case of peak a (Figure 16) there is a base peak (100% response) at ^{m}/z = 138, a major peak at ^{m}/z = 180, the molecular ion at ^{m}/z 195, and a peak at ^{m}/z 194 (M$^+$-H) the latter being characteristic of a nitrogen containing ring (RITTER *et al.*1973). This spectrum is entirely in accord with that obtained by RITTER *et al.* (1973) for monomorine I and, together with the chromatographic data, strongly suggests that peak a of the queen rinse is monomorine I (3-methy-5-butyl octahydroindolizine). The mass spectrum of peak b (Figure 17) shows two intense ions at m/z = 152 and m/z = 140 together with smaller signals at m/z = 223 (mass ion) and 222 (M+-H).

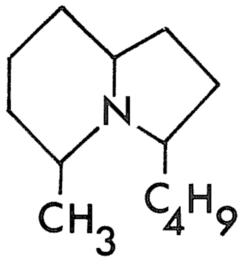
Figure 14. Typical fragmentation patterns of dialkyl indolizines (left) and pyrrolidines (right) subjected to mass-spectrometry.

Figure 15. Chemical structures of monomorines (I to V) previously identified in *M.pharaonis*.

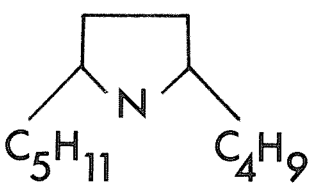

N
R1
R2
+


N
H
R1
R2
+

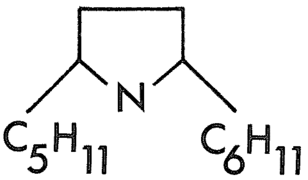
I


N
CH3
C4H9

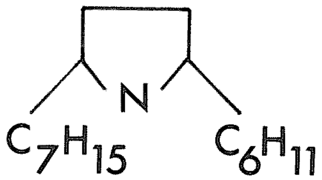
II


N
C5H11
C4H9

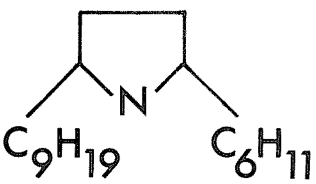
III


N
C5H11
C6H11

IV


N
C7H15
C6H11

V


N
C9H19
C6H11

These data are consistent with the expected fragmentation pattern of monomorine III and, together with the chromatographic evidence, indicate that peak b of the queen rinse is monomorine III (2-(5'-hexenyl) 5-pentyl pyrrolidine.

4.2.3 Tentative identification of peaks a^1 and c from queen rinses by GC/mass spectrometry

Mass spectra obtained for peaks a1 and c of the queen rinse are presented in figures 18 and 19 respectively. The data for peak a^1 (Figure 18) show strong signals at $^m/z$ = 126 and $^m/z$ = 140, with small signals at $^m/z$ = 197 (mass ion) and $^m/z$ = 196 (M^+-H). This suggests that compound a^1 is monomorine II (2-butyl-5-pentyl pyrrolidine). The mass spectrum obtained for peak e of the queen rinse (Figure 19) shows two strong signals at $^m/z$ = 152 and $^m/z$ = 168, with an indication of a molecular ion $^m/z$ = 251. These data indicate that peak c is monomorine IV (2-(5'-hexenyl),5-heptyl pyrrolidine. Unfortunately, synthetic samples of these compounds were not available so no comparative studies (eg. retention times etc.) were possible.

4.2.4 Possible structures of peak c^1 from the worker rinse and peak a^2 from the queen rinse

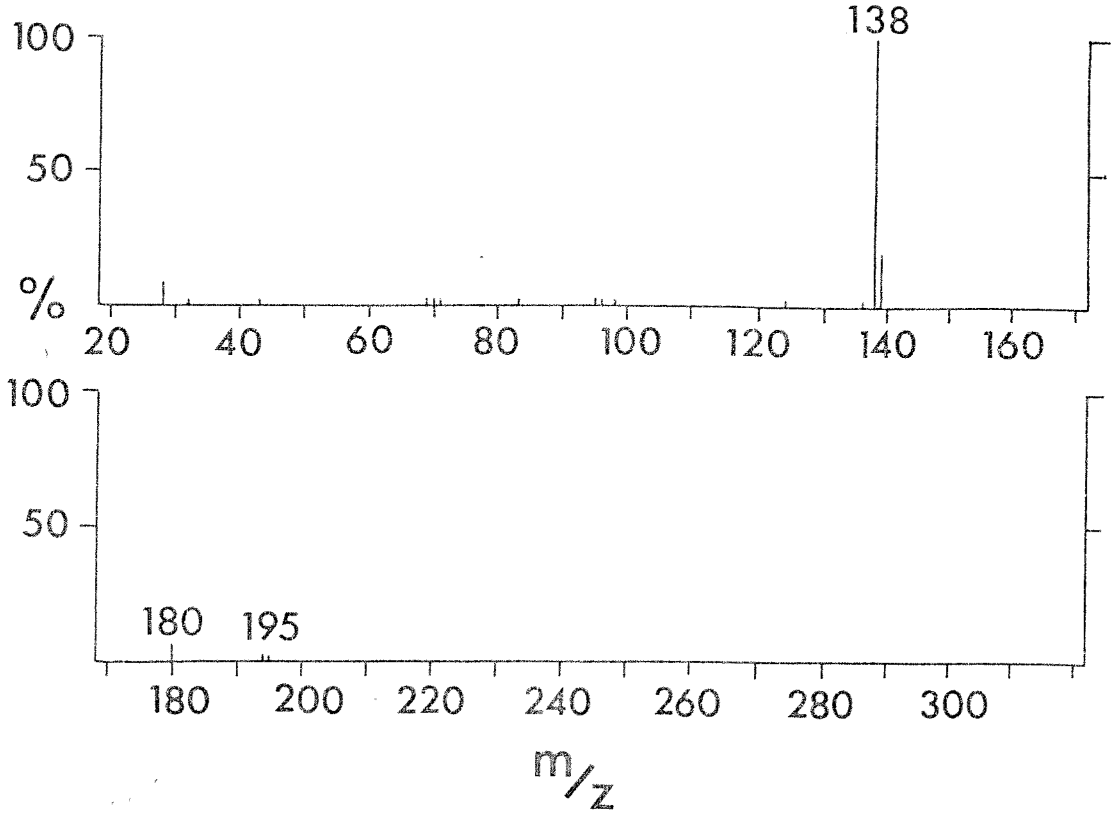
Peak c^1 was found only in workers and was not detected in rinses of virgin or fertile queens. Because it was felt that this material was unlikely, to play a major role in caste regulation (being absent in queens) only limited efforts were made to identify its chemical structure. No mass spectrometry was performed on this material. However, by comparison of the retention time on gas chromatographic analysis with that of a sample of faranal (kindly provided by Prof. R. Baker, Southampton University) some indication was obtained that the two compounds could well be identical.

Peak a2 from the fertile queen-rinses was also present in rinses of workers and virgin queens. Again, it was felt that this compound was probably not involved in the regulation of sexual produc-

Figure 16. Mass spectrum of GC peak a from fertile queen rinses.

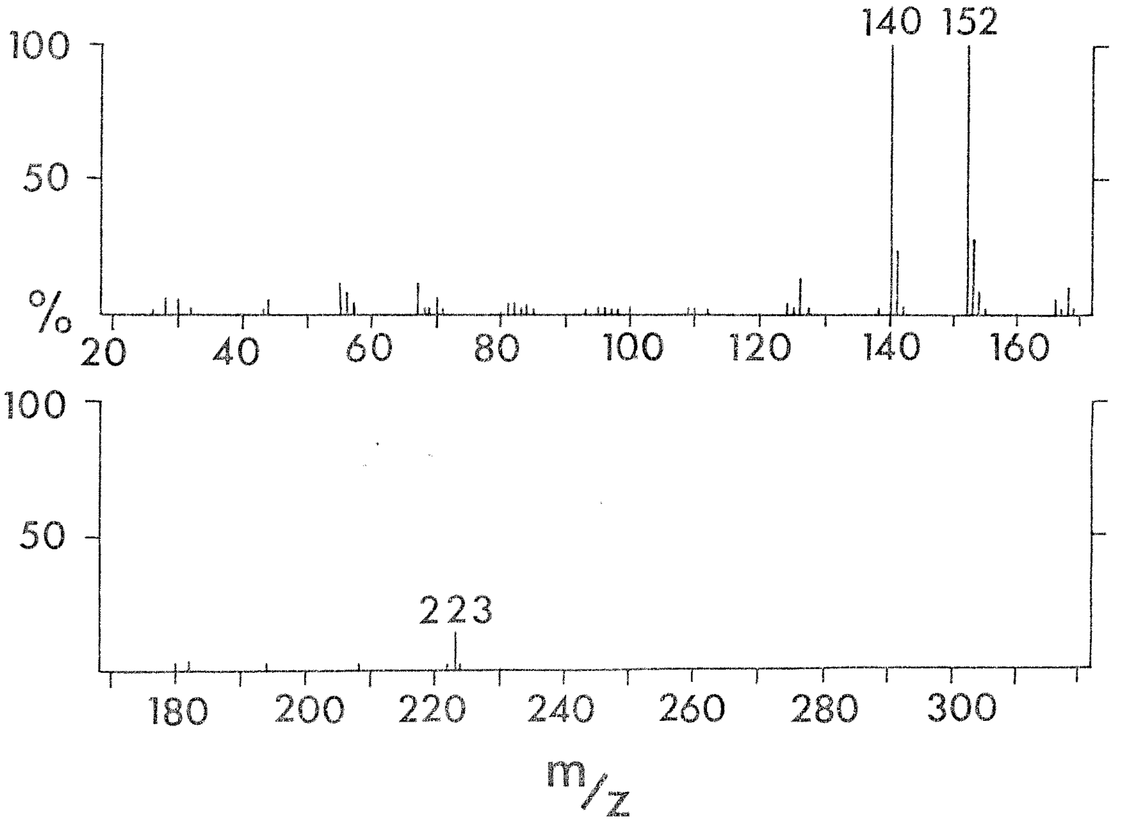
Figure 17. Mass spectrum of GC peak b from fertile queen rinses.

peak a



138
100
50
%
20
40
60
80
100
120
140
160
100
50
180
195
180
200
220
240
260
280
300
m/z

peak b

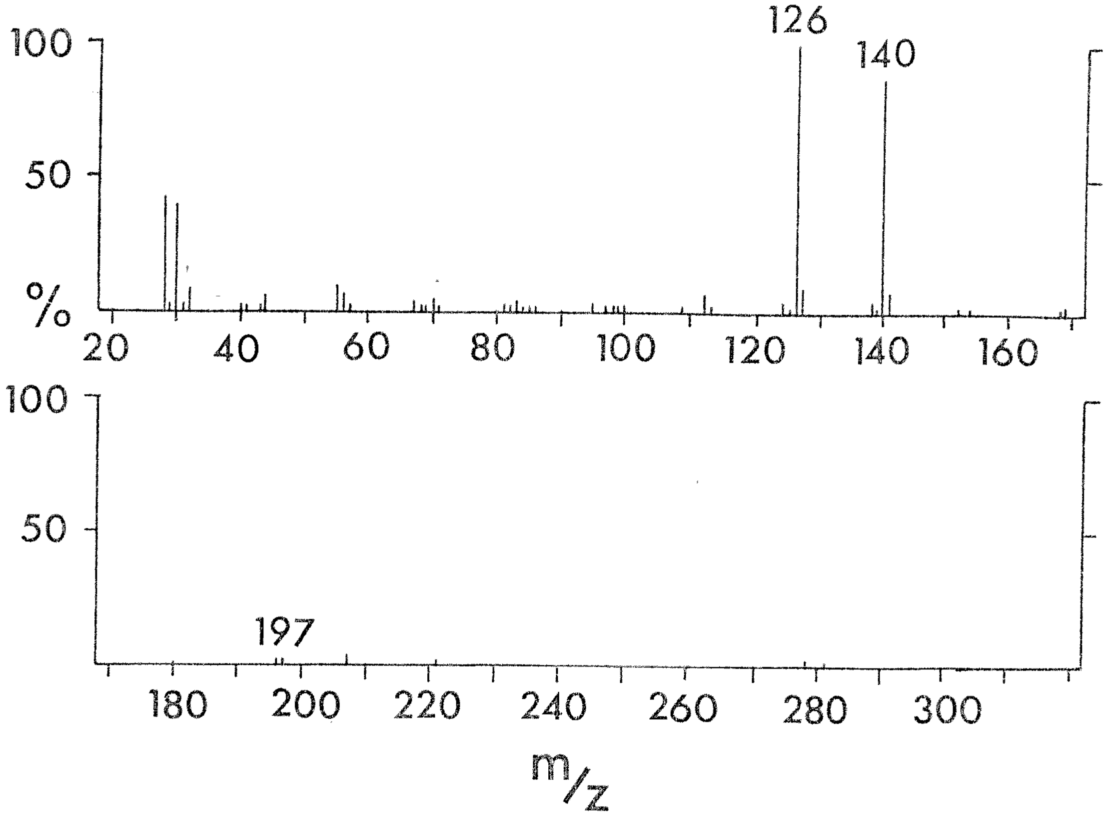


140
152
100
50
%
20
40
60
80
100
120
140
160
100
50
223
180
200
220
240
260
280
300
m/z

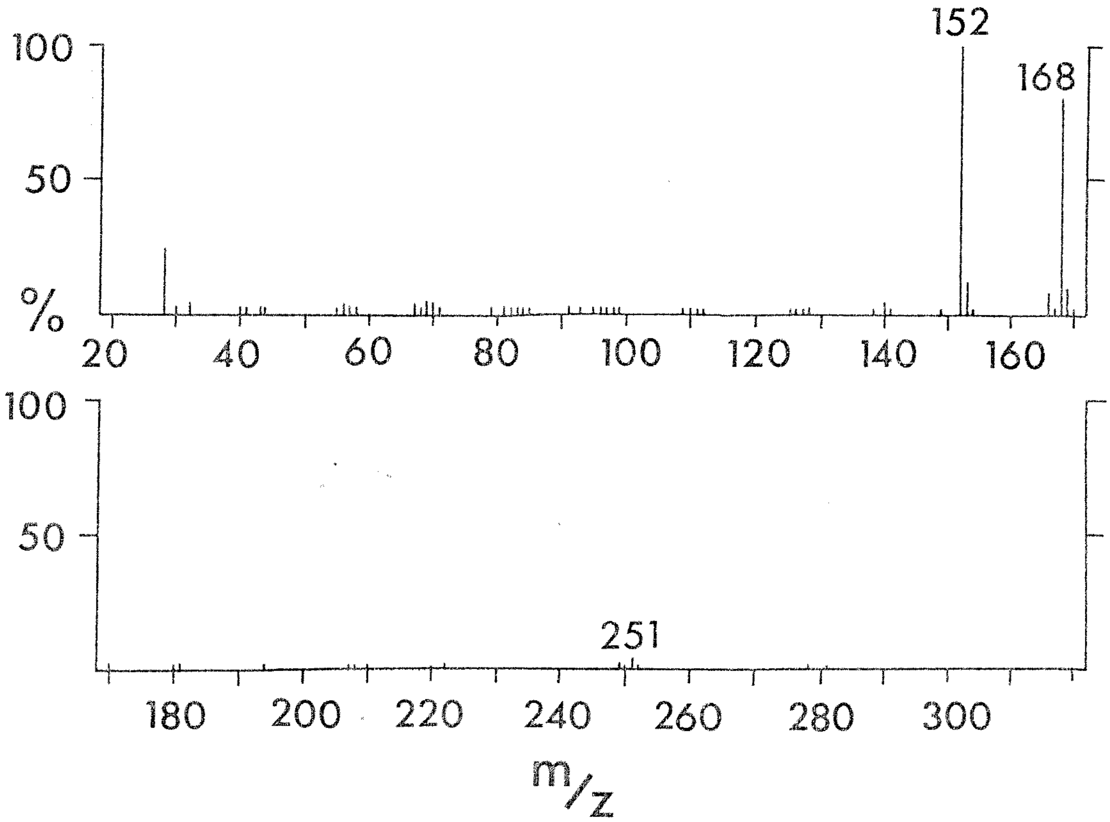
Figure 18. Mass spectrum of GC peak a^1 from fertile queen rinses

Figure 19. Mass spectrum of GC peak c from fertile queen rinses.

peak a^1



peak c



tion and only limited steps were taken to identify the material. However, in this instance, a mass spectrum was obtained and the data suggested a simple hydrocarbon rather than a nitrogen-containing molecule. The most intense signals were at $^{m}/z$ = 205 and $^{m}/z$ 220 (molecular ion?) and the only inference that can be drawn from this spectrum is that the material probably does not belong to the indolizine or pyrrolidine series.

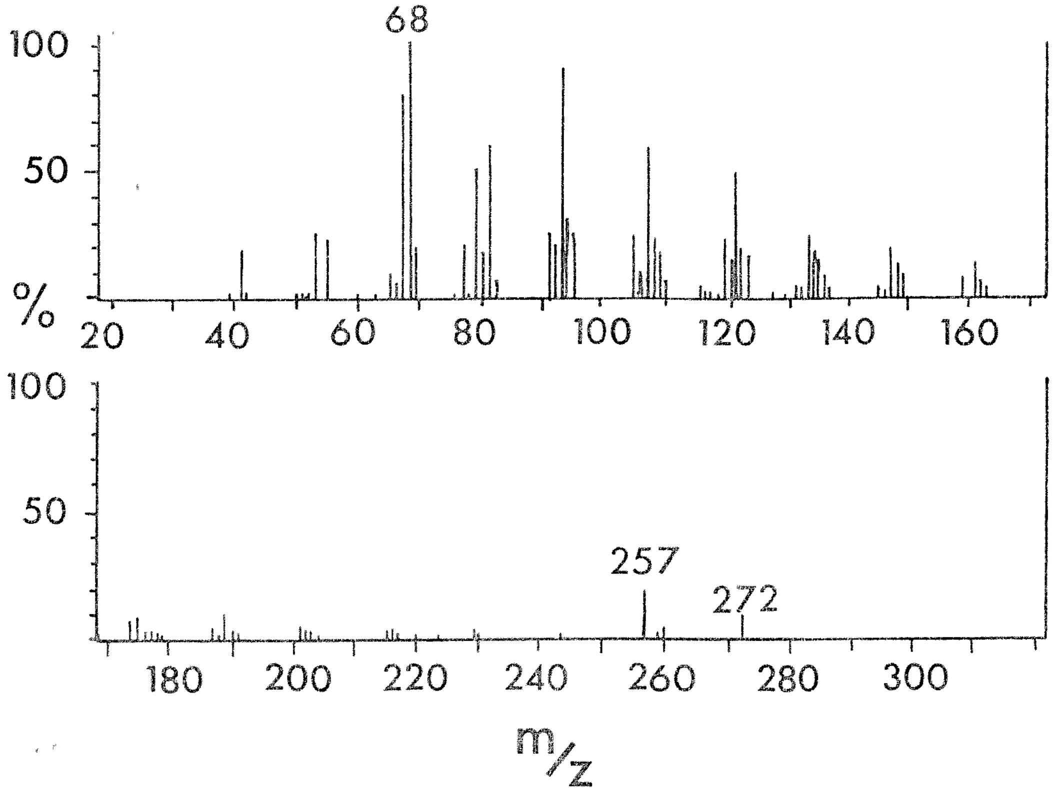
4.2.5 Identification of the structure of peak d from rinses of fertile queens

From the point of view of this study, the most interesting chemical found in the ant rinses was peak d (Figure 13) which was present in fertile queens but absent in virgin queens and workers. Thus, this material was a candidate for the role of 'inhibitory pheromone' previously postulated. The low resolution, electron impact, mass spectrum for this peak kindly provided by Dr. J.B. Siddall (Zoecon Corp. U.S.A.) is presented in Figure 20. The spectrum does not have the characteristic fragment ions associated with the nitrogen-containing pyrrolidines but rather, suggests that the molecule is an unsaturated hydrocarbon with the characteristic 'tufts' indicating losses at intervals of $^{m}/z$ = 14 and 12. The molecular ion appears to be at $^{m}/z$ = 272 indicating a molecular weight of 272 and there is a strong signal at $^{m}/z$ - 257 suggesting the facile loss of a methyl group (M^{+}-15). Another interesting aspect of the mass spectrum of peak d is the rather unusual base peak at $^{m}/z$ = 68. This could be due to isoprene units and suggests that the compound might be a terpenoid. Subsequently, the mass ion at $^{m}/z$ = 272 was confirmed by chemical ionization mass spectrometry (Dr J.B. Siddall). On the basis of the limited data available from the mass spectra (i.e. the probable absence of nitrogen(s) and the possible presence of oxygen(s)) 6 possible empirical formulae were tentatively proposed - $C_{20}H_{32}$, $C_{19}H_{28}O$, $C_{18}H_{24}O_2$, $C_{17}H_{36}O_2$, $C_{18}H_{28}N_2$ and $C_{17}H_{24}N_2O$. Subsequently, a pure sample (600 μg) of peak d was obtained from an ether rinse of 1500 fertile queens by preparative gas chromatography (see methods). This material was subjected to high-resolution mass spectroscopy at the Physico Chemical Measurements Unit, Harwell. The spectrum (Figure 21) was essentially the same as that recorded earlier and the mass recorded at the molecular ion was 272.2513. This was sufficiently close to the calculated mass for $C_{20}H_{32}$ (272.2504) to exclude the presence of nitrogen and oxygen atoms and confirm the empirical formula as $C_{20}H_{32}$. Thus, the molecule has five units of unsaturation (rings and/or double bonds).

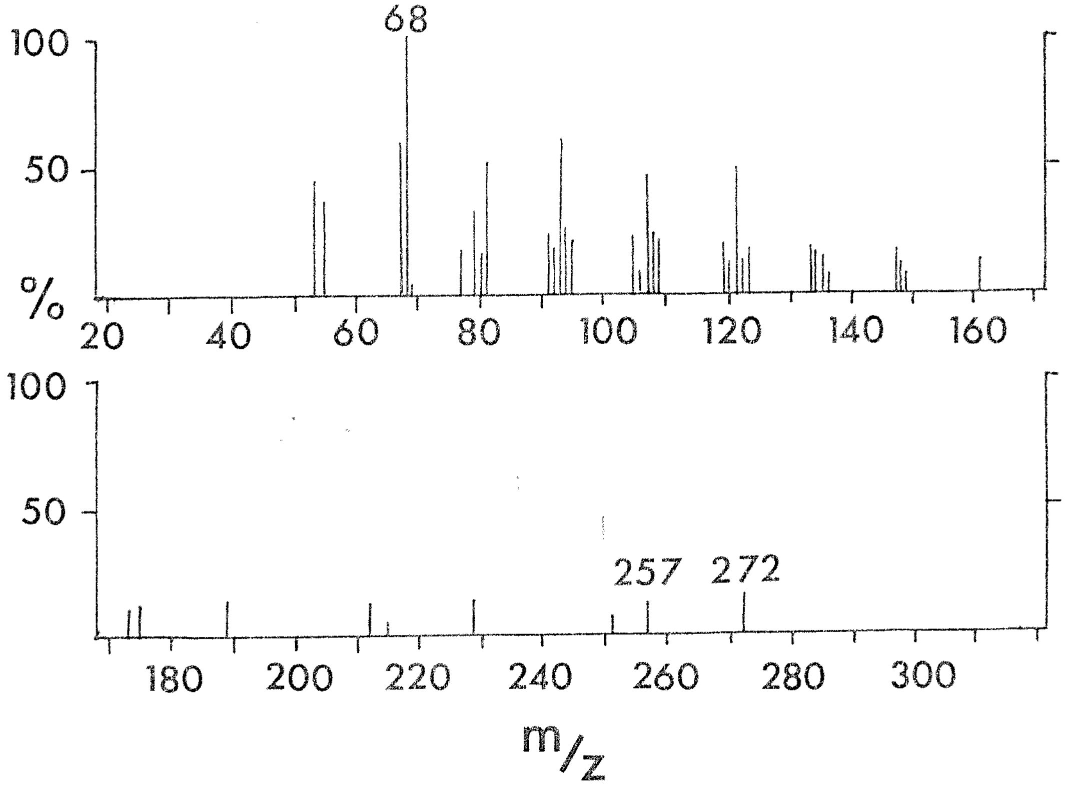
Further information about the chemical structure of peak d was kindly provided by Dr J. Chambers (Slough) and Drs N.F. Janes and J. Pickett (Rothamsted) who ran and interpreted a fourier-transformed nuclear magnetic resonance spectrum on a pure sample of peak d. On the basis of the MS and NMR data it was possible to propose simple structural types that appeared to satisfy the requirements of the analytical results. Some of the possible structures are presented in Figure 22. As indicated in Figure 22, 3 basic structural types are possible: i.e. six-membered ring (A and B), ten-membered ring (C) or fourteen-membered ring (D). Biosynthetically, each structural type could be made by cyclisation from geranylgeranyl pyrophosphate as illustrated in Figure 20. However, formation of B would require the isomerization of the double bonds in the chain. In order to obtain some further indication of the structure of peak d, total hydrogenation of the material (approx 200 μg) was carried out by Dr J. Chambers (MAFF Slough). The rationale behind this procedure was that hydrogenation would saturate only the double bonds present in the molecule which were not associated with the ring(s). Total hydrogenation gave a mixture of four products which had similar GC retention times and similar mass spectra (Table 11). Although the molecular ion of one minor compound (dH_1) was not obvious, all 3 remaining materials gave molecular ions at $^m/z$ = 280(e.g. Figure 24). Since there was no evidence of remaining unsaturated double bonds, this result confirmed the number of double bonds as four, i.e. the molecule was monocyclic. As to the number of carbon atoms in the ring, some indication was obtained from the mass spectra of the hydrogenation products which all gave strong signals at m/z 236 and 237 i.e. M^+-44 or 43. This suggests the loss of 3-carbon units and, since it is likely that such loss would have most easily occurred by loss of the side chain from the ring, it suggests

Figure 20. Mass spectrum (low resolution) of GC peak d from fertile queen rinses.

Figure 21. Mass spectrum (high resolution) of GC peak d from fertile queen rinses.



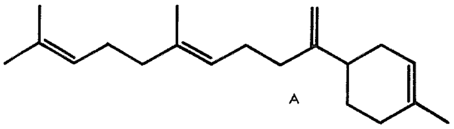
peak d



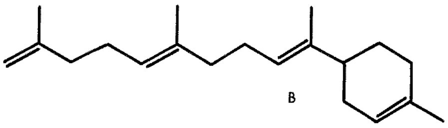
peak d (high res.)

Figure 22. Four basic structural types satisfying the mass spectrometric data obtained from GC peak d from fertile queen rinses.

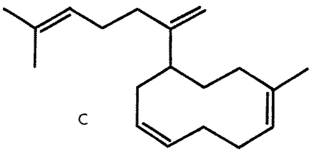
Figure 23. Structures of cembrene and neocembrene.



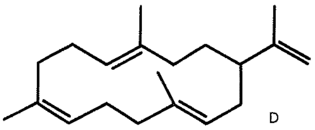
A



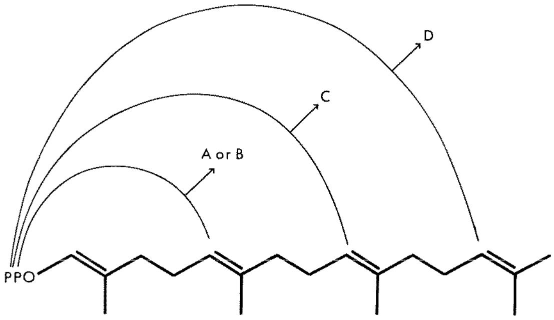
B

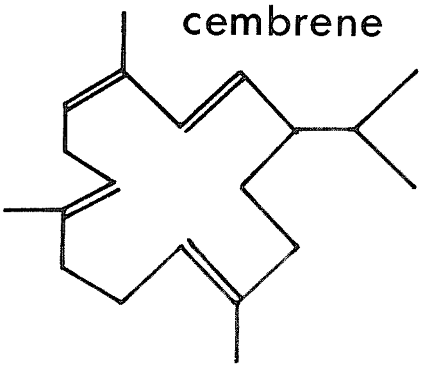


C

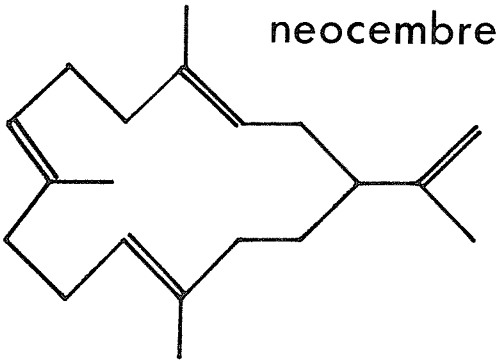


D





cembrene



neocembrene

that the side chain contained 3 carbon units. Further evidence from the NMR data, provided by Dr J. Chambers, strongly suggested that the 3-carbon unit side-chain could only be isopropenyl and, that the remainder of the molecule comprised a fourteen carbon ring (e.g. as in D, Figure 22).

Table 11. Retention times and mass spectral data for four total hydrogenation products of peak d.

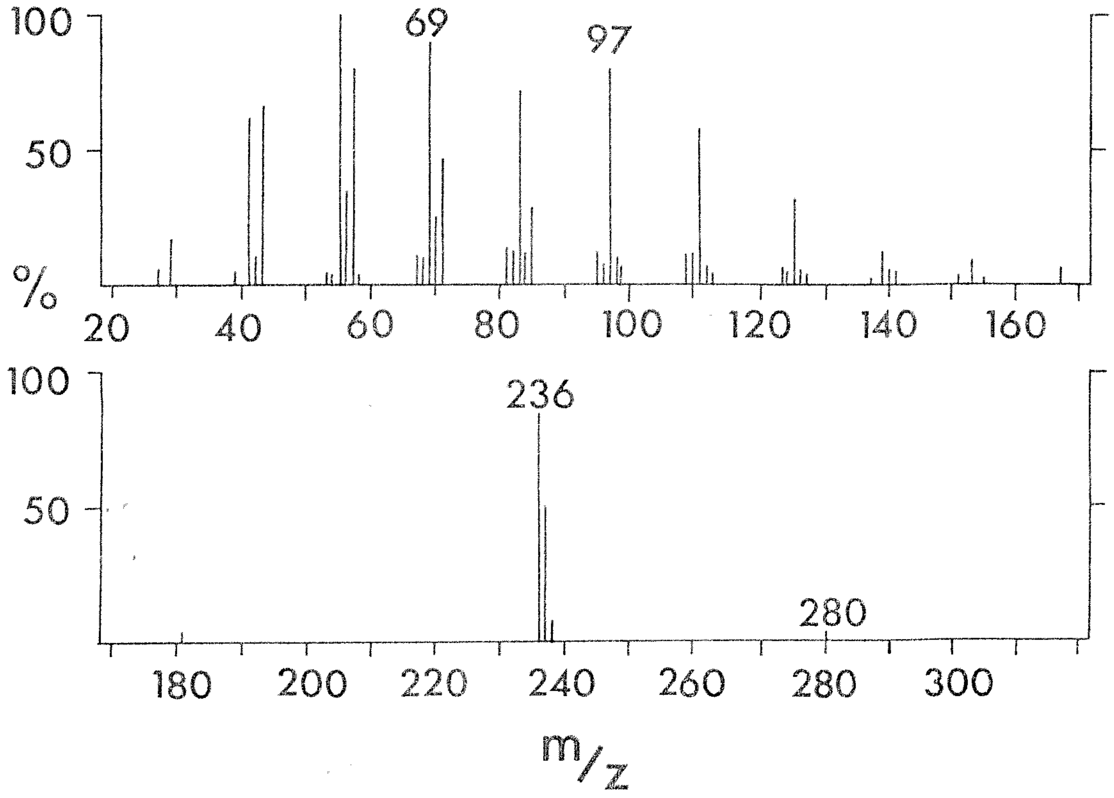
Compound	retention time[a]	$^m/_z$ signals (% response)									
		55	57	69	83	97	111	125	236	237	280(M^+)
dH_1	8.6	100	80	91	67	78	55	30	88	49	0
dH_2	8.8	100	82	92	72	81	58	32	86	51	2
dH_3	8.95	100	83	95	75	86	61	34	89	57	3
dH_4	9.1	100	88	92	71	82	57	32	71	52	2

[a] Isothermal (190°C) capillary GC, 25m OV101

A literature search aimed at identifying compounds with molecular formula $C_{20}H_{32}$ and with other characteristics consistent with the mass spectrometric and NMR data revealed only one compound with appropriate structure. This material, 1-isopropenyl-4,8,12-trimethylcyclotetradeca-3,7,11-triene, (Figure 23) has been independantly identified in natural products from four sources. As a result, it has been given a variety of trivial names. SHMIDT *et al.* (1970) identified this cembrenoid diterpene in the oleoresin from two coniferous trees *(Picea obovata* and *Pinus koraensis)* and called the material neocembrene. The same material was found by PATIL *et al.* (1973) in the gum resin of the tree *Commiphora mukul* but these authors called it cembrene-A. BIRCH *et al* (1972) identified the same material (which they called neocembrene-A) in the trail pheromone of the termite *Nasutitermes exitiosus*. The existence of a trail pheromone in *N. exitiosus* had been suggested by MOORE (1966) who named the (unidentified) active component, nasutitene. Subsequently, the same material was found in another termite,

Figure 24. Mass spectrum of the major hydrogenation product of GC peak d from fertile queen rinses.

hydrogenated d (major peak)



Cubitermes umbratus, by WIEMER *et al.* (1979) who, uncharacteristically, did not give it a trivial name. The stereochemistry of neocembrene A (BIRCH *et al.*, 1972) was determined by KODAMA *et al.* (1975) who showed that, in the natural product, all 3 double bonds have the *trans* configuration. The mass spectrometric data from these authors agreed well with those obtained for peak d from the *Monomorium* queen rinse and with those obtained with a pure sample of synthetic neocembrene (Table 12) which was synthesized and provided by Dr J. Chambers (MAFF, Slough). The combined evidence obtained from analytical data for peak d from *Monomorium* queen rinses, from comparison with published analytical data for certain naturally occurring cembrenoid diterpenes and from data obtained from a sample of synthetic neocembrene, is sufficient to confirm that peak d of the *Monomorium* queen rinse is 1-isopropenyl-4,8,12-trimethyl cyclotetradeca-3,7,11-triene (neocembrene). Furthermore, the data obtained for peak d of the queen rinse, when compared with similar data from known stereoisomers of cembrene A (WIEMER *et al.*, 1979 and KATO *et al.*,, 1980) strongly suggests that peak d (neocembrene) occurs in *M. pharaonis* as the all *trans* isomer.

4.3 Summary of the identification and occurrence of chemicals present in ether rinses of *Monomorium pharaonis* queens

Using a range of standard analytical techniques, it has been possible to assign, tentatively, chemical structures to 5 of the 6 peaks associated with solvent rinses of queen *M. pharaonis* (Figure 25). With the exception of peak d (neocembrene) all compounds identified have been previously recorded as occurring in *M. pharaonis*. Earlier in this chapter the possible functions of the indolizine and pyrrolidine compounds have been discussed but no firm conclusions as to the role(s) of these materials can be reached. During these experiments there was no indication, by GC analysis, of the (unidentified) bicylic hydrocarbon (monomorene) found by RITTER *et al.* (1975) in extracts of virgin queens and which they suggested might act as the sex pheromone previously reported by HOLLDOBLER and WUST, (1973). The fertile queen-specific material responsible for peak d on the gas chromatograms has been identified

Table 12. Comparative mass spectral data for 1-isopropenyl-4,8,12-trimethylcyclotetradeca-3,7,11-triene from different sources and peak d from queen rinses.

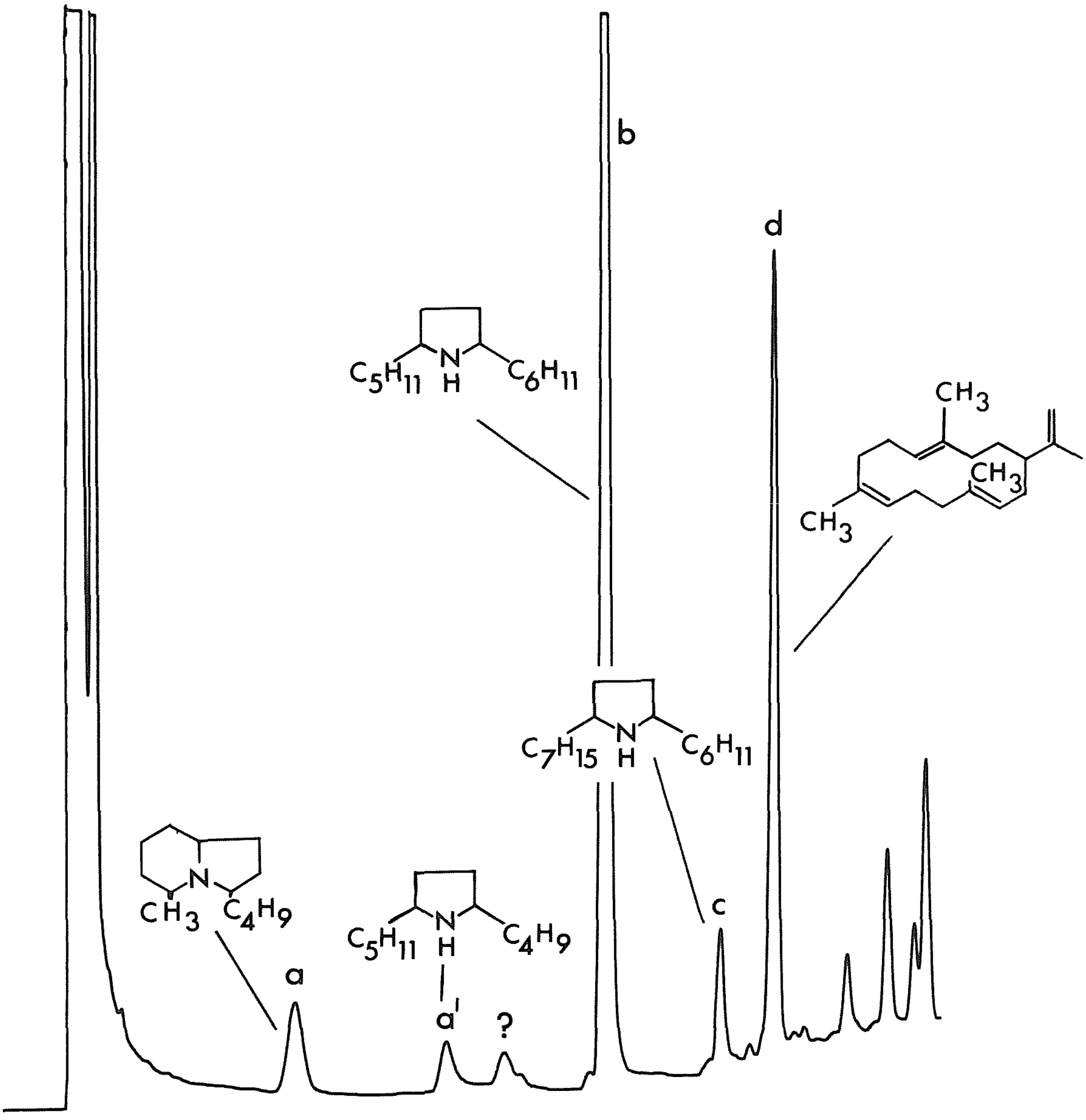
m/z	Compound neocembrene SHMIDT *et al.* (1970)	neocembrene-A BIRCH *et al.* (1972)	peak d (queen rinse)	synthetic neocembrene
272	8	38	15	11
257	-	24	13	11
189	9	15	14	8
161	14	18	13	10
147	18	20	17	13
135	20	26	15	17
133	24	23	19	17
121	45	55	49	44
119	34	23	20	23
107	50	53	46	46
105	32	27	23	17
93)	not reported	73	60	67
81)		64	51	63
79)		25	32	33
69)		27	6	32
68)		100	100	100
55)		33	36	40

[a] Synthesized and provided by Dr J. Chambers (MAFF Slough)

as 1-isopropenyl-4,8,12-trimethylcyclotetradeca-3,7,11-triene (neocembrene). This material has not previously been found in *M. pharaonis*. However, neocembrene does occur naturally in termites and in the resin of some trees. In termites (*Nasutitermes* spp) it apparently functions as a trail pheromone. Its function in other organisms is unknown but it is apparently the active ingredient of the drug Guggulu used in Indian herbal medicine (DASH and KASHYAP, 1980). In this respect, it is reported to be a rejuvenating tonic which can also cure ulcers, leprosy, pimples and haemorrhoids. In addition, it is said to be an effective aphrodisiac.

Because in *M. pharaonis* neocembrene occurs only in fertile queens, it is a prime candidate for the proposed 'inhibitory pheromone' responsible for the suppressing effect of fertile queens on the production of new sexual forms. However, it is not possible to exclude other roles for this caste-specific chemical in the social biology/communication of this species. In the following sections the source, dynamics of production and possible functions of this compound in the pharaoh's ant are investigated.

Figure 25. Chemical structures of peaks present in gas-liquid chromatograms of solvent rinses of fertile queen *Monomorium pharaonis*.


b
d
C5H11 N H C6H11
CH3
CH3
CH3
C7H15 N H C6H11
c
CH3 N C4H9
a
C5H11 N H C4H9
a'
?

CHAPTER 5. SOURCE OF NEOCEMBRENE IN QUEENS OF *MONOMORIUM PHARAONIS*.

5.1. Exocrine glands and semiochemicals in ants.

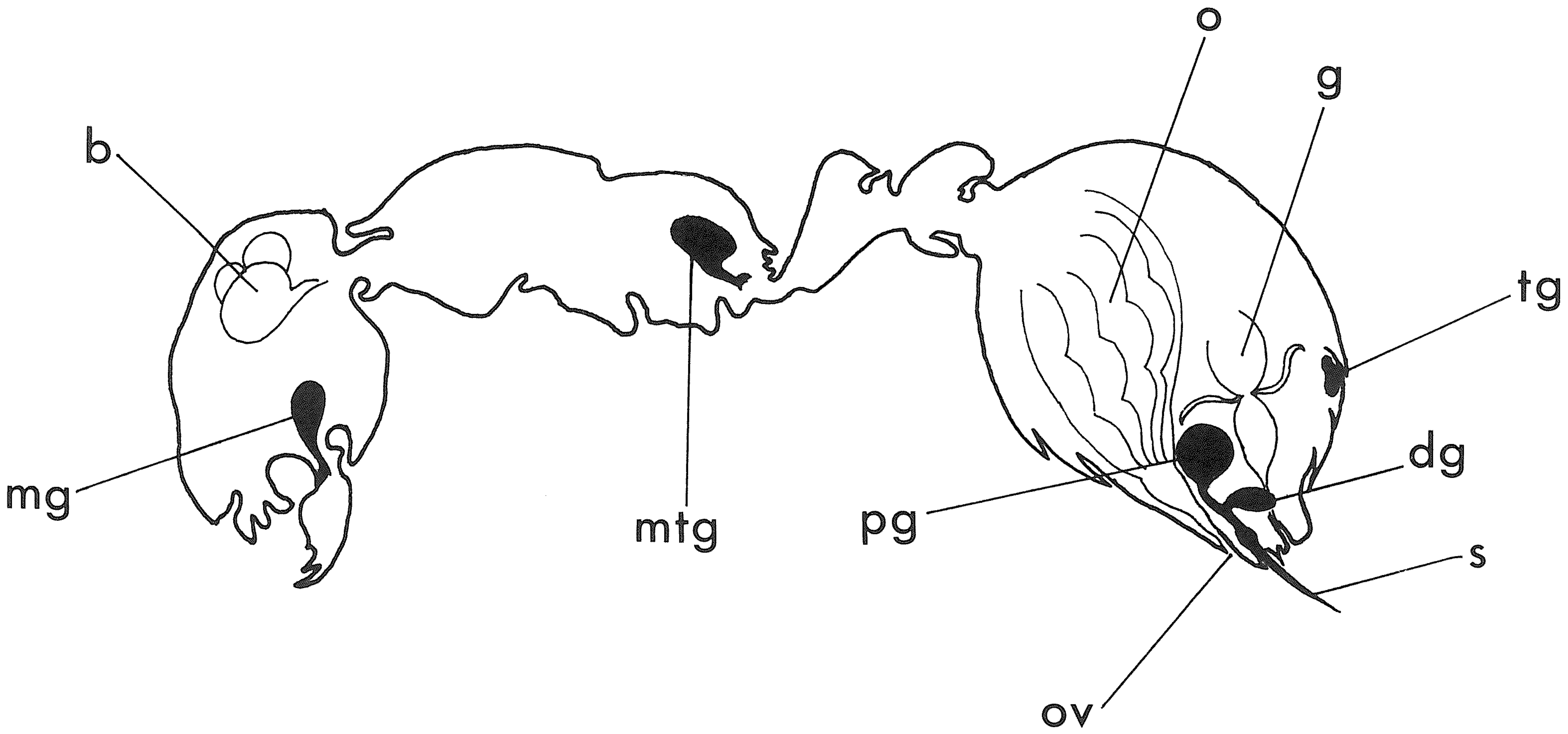
In social insects, perhaps more than in any other group of living organisms, there can be seen the apogee of evolutionary development in the use of chemical signals for communication between individuals. Such chemical signals (semiochemicals) are termed pheromones (KARLSON & LUSCHER, 1959; KARLSON & BUTENANDT, 1959). The variety of behavioural and physiological responses stimulated by pheromones, and the diversity and number of the exocrine organs producing such chemicals in social insects, is an indication of their importance in this group of organisms.

In ants, chemical signals have been shown to play a role in several aspects of social behaviour including alarm behaviour, colony defence, trail following, food marking, colony recognition, brood recognition, queen recognition, sex attraction, identification of dead nestmates and the control of oviposition and ovary development in workers (WILSON, 1971). These aspects of behaviour may be released or modified by a single chemical substance or, in the case of more complex behavioural repertoires, by a mixture of compounds (multicomponent pheromone) as reported by BRADSHAW *et al.* (1975) in the ant *Oecophylla longinoda*. Reviews of the identity and role of pheromones in ants have been published by BLUM, 1973; BLUM and HERMANN, 1978, and PARRY and MORGAN, 1979.

Figure 26 shows the location of the major exocrine glands found in Myrmicine ants. In the head, the mandibular glands have been found to produce a variety of ketones and carbinols (eg. 3-octanol, 3-octanone, 4-methyl-3-heptanone, 3-nonanone etc.) and these, or similar compounds, have been found in a large number of species (BLUM and HERMANN, 1978). These mandibular gland secretions appear to be concerned primarily with the release of alarm behaviour. Interestingly, there is no published information of the constituents of mandibular glands of *Monomorium* or of the related genus, *Solenopsis* . The head of Myrmicine ants also contains the post-pharangeal gland, but there is no evidence that this gland has

Figure 26. Location of the major exocrine glands in Myrmicine ants.

Key: b, brain; mg, mandibular gland; mtg, metathoracic gland; o, ovaries; g, gut; tg, tergal gland; pg, poison gland; dg, Dufour's gland; ov, oviduct; s, sting.


o
g
b
tg
dg
mg
mtg
pg
s
ov

any exocrine function. The thoraces of Myrmicine ants contain the metathoracic glands. There has been much speculation concerning the role of these organs (see WILSON, 1971) but little detailed investigation. In some Myrmicine ants these glands produce acids (e.g. phenylacetic acid, indole-3-acetic acid and β-hydroxy decanoic acid). It has been suggested that these materials function either as selective fungicides or as fungal growth stimulators in the 'fungus gardens' in leaf-cutting ants of the genus *Atta*. However, since the same compounds are also found in *Myrmica rubra*, a species without a 'fungus garden', their precise role in some species remains unclear. The metathoracic glands of *Crematogaster inflata* produce a white, sticky substance which is used in defence (MASCHWITZ, 1974). It has also been suggested that in some species, the products of the metathoracic glands may be involved in colony-specific recognition odours (BROWN, 1968). Because no detailed information was available on the structure and function of the metathoracic glands in *M. pharaonis*, a study of these glands was made.

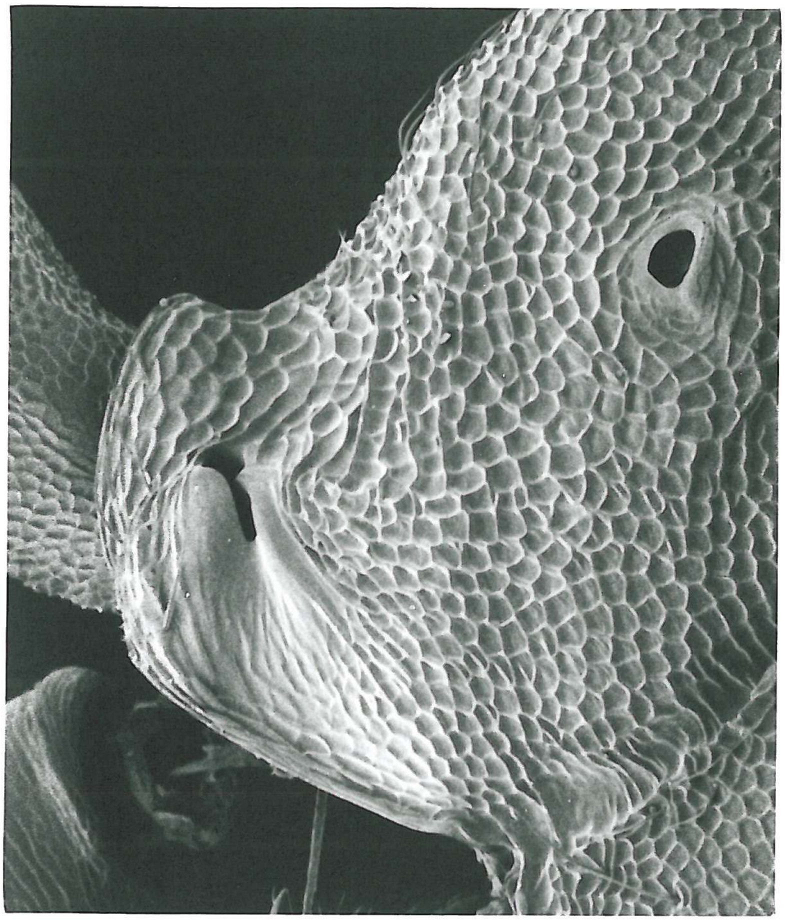
In the majority of Myrmicine species, the most important source of semiochemicals is the gaster. This portion of the body contains the poison gland and the Dufour's gland (Figure 26) as well as a variety of glands associated with the cuticle (HOLLDOBLER and ENGEL, 1978). In *M. pharaonis* workers, the poison gland is the source of the indolizine and pyrollidine substances (RITTER *et al* ., 1981) and the Dufour's gland, at least in workers, is the source of the trail pheromone, faranal (RITTER *et al*, 1977). In the following sections the structure of some of the exocrine glands in queens of *M. pharaonis* is described and an attempt to identify the source of the queen-specific chemical, neocembrene is made.

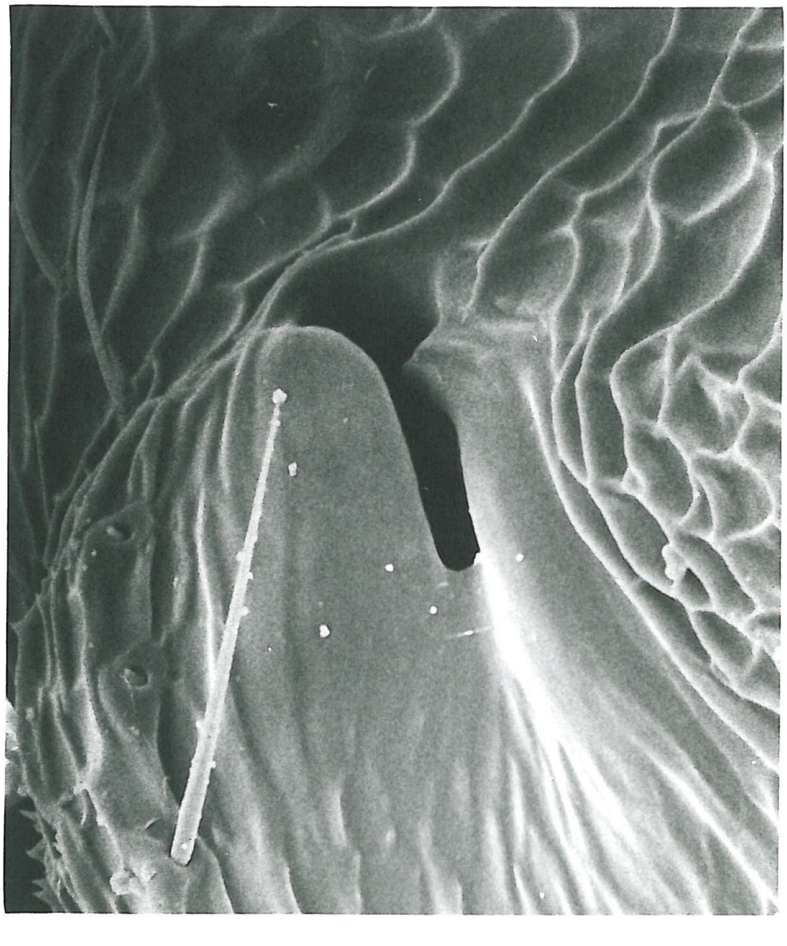
5.2 Structure of the metathoracic glands in queens of *M. pharaonis*

In *M. pharaonis* queens the metathoracic glands open onto the cuticle at the posterior end of the thorax. The opening is a curved slit

Figure 27. Scanning electron micrograph of the posterior thoracic region of queen *M. pharaonis* showing the location of the metathoracic gland opening (X 300).

Figure 28. Close-up (scanning electron micrograph) of metathoracic gland opening of queen *M. pharaonis* (x 700).





(Figures 27 and 28) and there is a partially flattened area of cuticle below the slit which may serve to aid dispersal of the secretion of the gland. Inside the gland opening there is a cuticle-lined cavity or bulla which is surrounded by 3-5 large cells (Figure 29). From these cells, which presumably manufacture and secrete the gland products, fine channels appear to run towards the gland cavity. Thus, the structure of the metathoracic gland in queen *M. pharaonis* is essentially the same as that reported in some other Myrmicine species (BLUM and HERMANN, 1978). This gland is also present in workers of *M. pharaonis,* although no study of the morphology of the gland was made in this caste. The fact that these glands are well developed in this insect, coupled with the large size of the (presumed) secretory cells, suggests that this organ plays an important role in the social biology of this species.

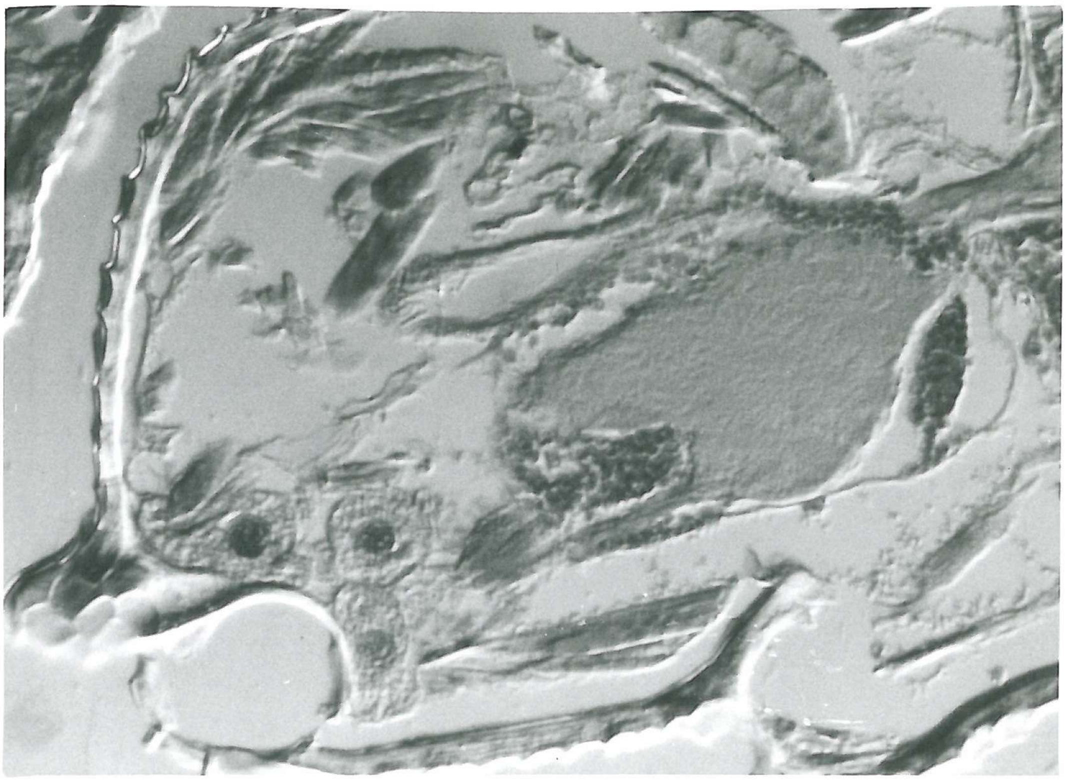
5.3 Structure of the poison gland and Dufour's gland in queens of *M. pharaonis.*

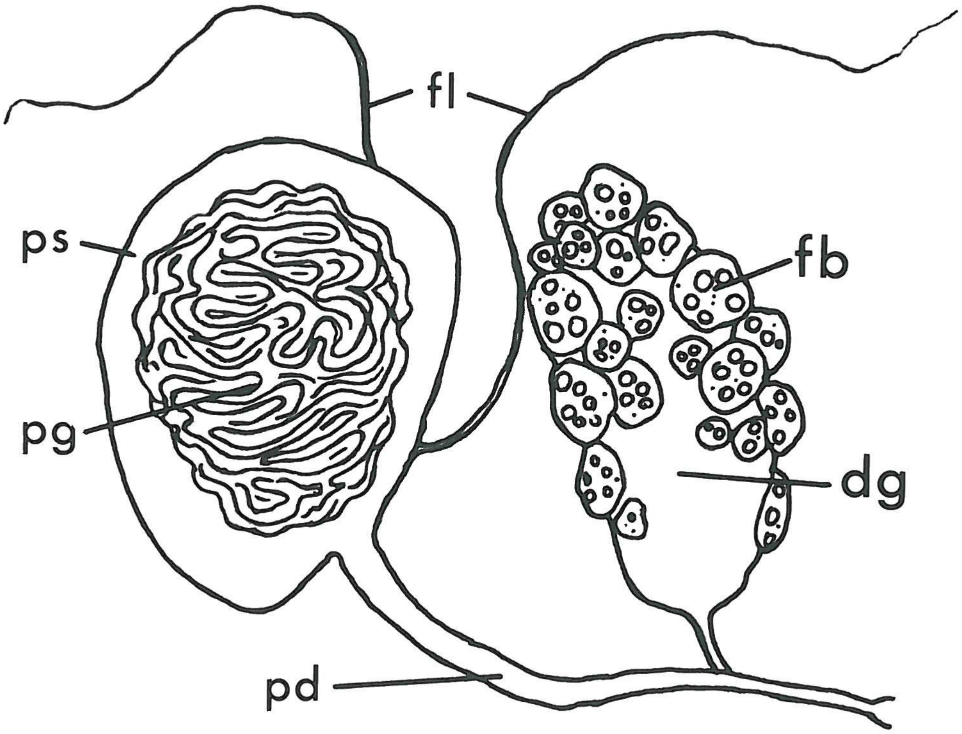
Figure 30 shows the structure of the poison apparatus (poison gland and Dufour's gland) present in queen *M. pharaonis.* The poison gland comprises a large, thin-walled sac enclosing a ball of convoluted tubes from which two fine filaments pass out through the poison sac wall into the body cavity.

The Dufour's gland is a simple tubular gland which connects with the poison duct between the poison sac and the sting (Figure 30). The Dufour's gland is covered by a sheath of fat-body cells which may be involved in the biosynthesis of the gland products. The common duct from both the poison and the Dufour's gland terminates in the sting which can be extended and retracted. There appears to be no gross musculature associated with either gland, or with the poison duct itself. However, it is possible that the extrusion of the sting, by stretching the poison duct, might result in the expulsion of the glandular products at the sting tip. In *M. pharaonis,* it is not known if the products of the poison gland and Dufour's glands can be discharged separately. However, in the related species *Solenopsis saevissima,* CALLAHAN *et al.* (1959) reported that muscles were able to shut-off the poison gland, enabling the

Figure 29. Photomicrograph of L.S. of posterior thoracic region of queen *M. pharaonis* showing large secretory cells surrounding the metathoracic gland cavity (X 250).

Figure 30. Diagram of the structure of the venom glands of *M. pharaonis* queens. ps, poison sac; pg, poison gland; fl, gland filaments; pd, poison duct; dg, Dufour's gland; fb, fat body cells, Scale, 3 cm = 0.1 mm.




fl
fl
ps
fb
pg
dg
pd

Dufour's gland secretion to be discharged separately. In summary, the structure of the poison gland and Dufour's gland in *M. pharaonis* queens does not differ markedly from the same structures reported in other Myrmicine species (BLUM and HERMANN, 1978).

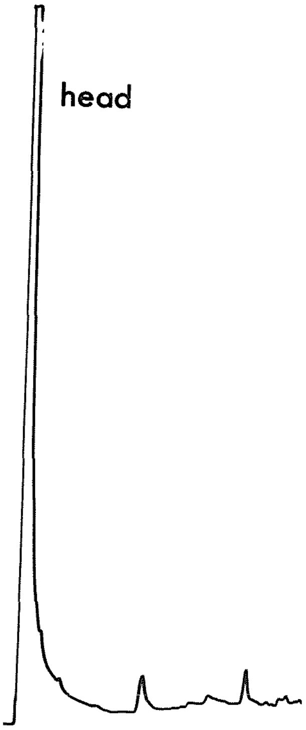
5.4 Location of the source of neocembrene in queens of *M. pharaonis*.

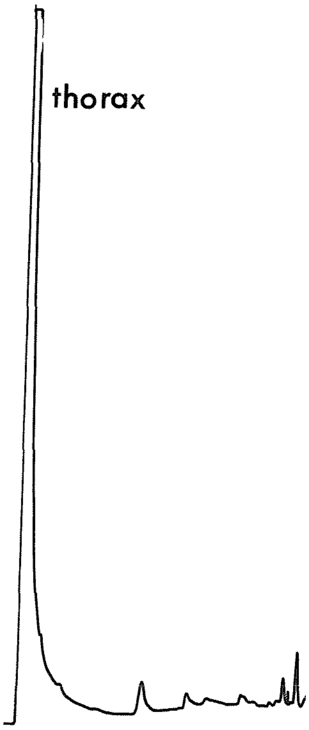
In an attempt to locate the source of neocembrene, 200 fertile queens were cut into the three main body portions (head, thorax and gaster) containing the mandibular glands, metathoracic glands and poison/Dufour's glands respectively. Each lot of body portions was rinsed for 5 min in diethyl ether. The rinses were then reduced to dryness, taken-up in approx 250 µl ether and aliquots analysed by GC. The results are presented in Figure 31 and indicate that only the gastral portion of queens contains significant quantities of the chemicals (including neocembrene) previously identified in rinses of whole queens. This strongly suggested that the source of neocembrene was either the poison gland or the Dufour's gland. However, the possibility that this compound might be produced in a previously unreported gland associated with the cuticle, such as those recently reported in some other species (HOLLDOBLER and ENGEL, 1978) could not be excluded. The fact that the GC peak associated with neocembrene was found only in the rinses of the gaster portion of the body also indicates that this material is not spread over the entire cuticular surface of the insect.

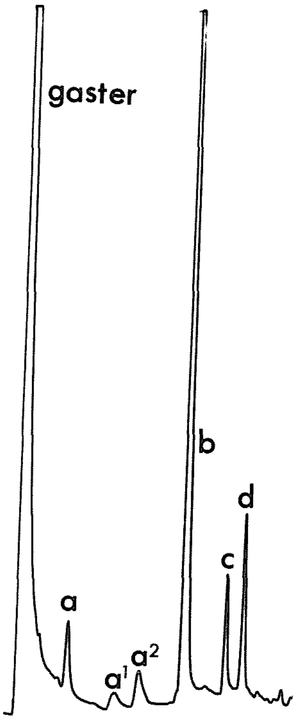
More specific identification of the glandular source of neocembrene in queen *M. pharaonis* was made by careful dissection of the poison and Dufour's glands and separate extraction of these glands in ether, prior to analysis by GC. The results of these analyses are given in Figure 32 and show that neocembrene is associated exclusively with extracts of the Dufour's gland. Further confirmation that the Dufour's gland is the source of neocembrene was obtained by direct injection of dissected glands into the solid-sample system of a mass spectrometer. This analysis was kindly performed by Mr D Lee and Mr J Wilkins at the MAFF Harpenden Laboratory. The data obtained for poison glands and Dufour's glands are presented

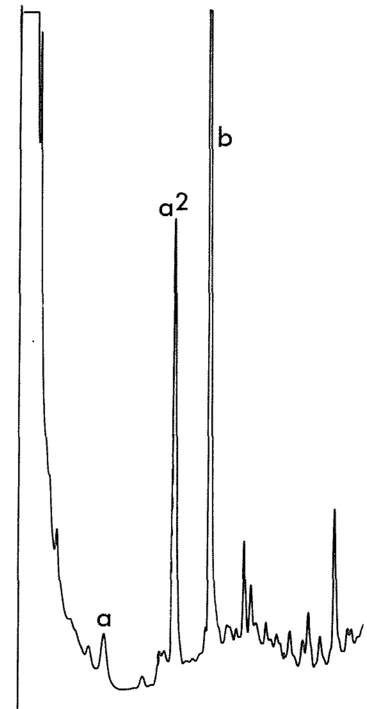
Figure 31. Gas-liquid chromatograms of solvent rinses of major body portions (head, thorax or gaster) of *M. pharaonis* queens. GC Prog. II

Figure 32. Gas-liquid chromatograms of extracts of Dufour's glands and Poison glands from *M. pharaonis* queens. GC Prog. II. One gland equivalent.

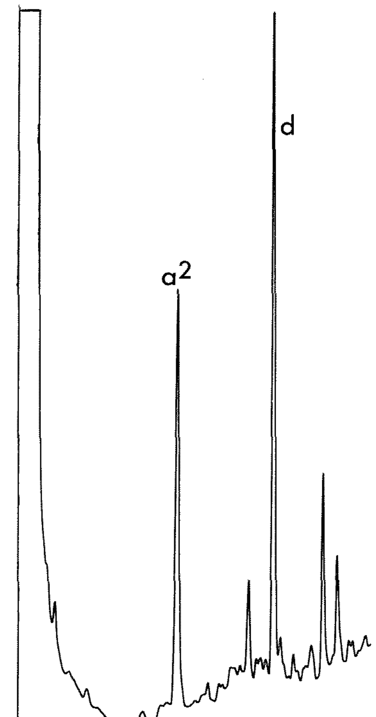








POISON GLAND



DUFOUR'S GLAND

in Table 12, and the major ions of the individual spectra are given in Figures 33 and 34. These results show that the major fragmentation ions associated with neocembrene are present only in the spectrum obtained from Dufour's glands. Thus, it appears that, in *M. pharaonis* queens, the Dufour's gland is the only source of the queen-specific substance, neocembrene.

Table 13. Mass spectral data obtained by direct injection of dissected poison gland or Dufour's gland from queen *M. pharaonis*.

Type of gland	m/z values of major fragment ions	probable inference (chemical type)
Poison gland	168, 152, 140, 97, 85, 71, 57,	indolizine and pyrollidines
Dufour's gland	272, 257, 135, 121, 107, 93, 81, 68	neocembrene

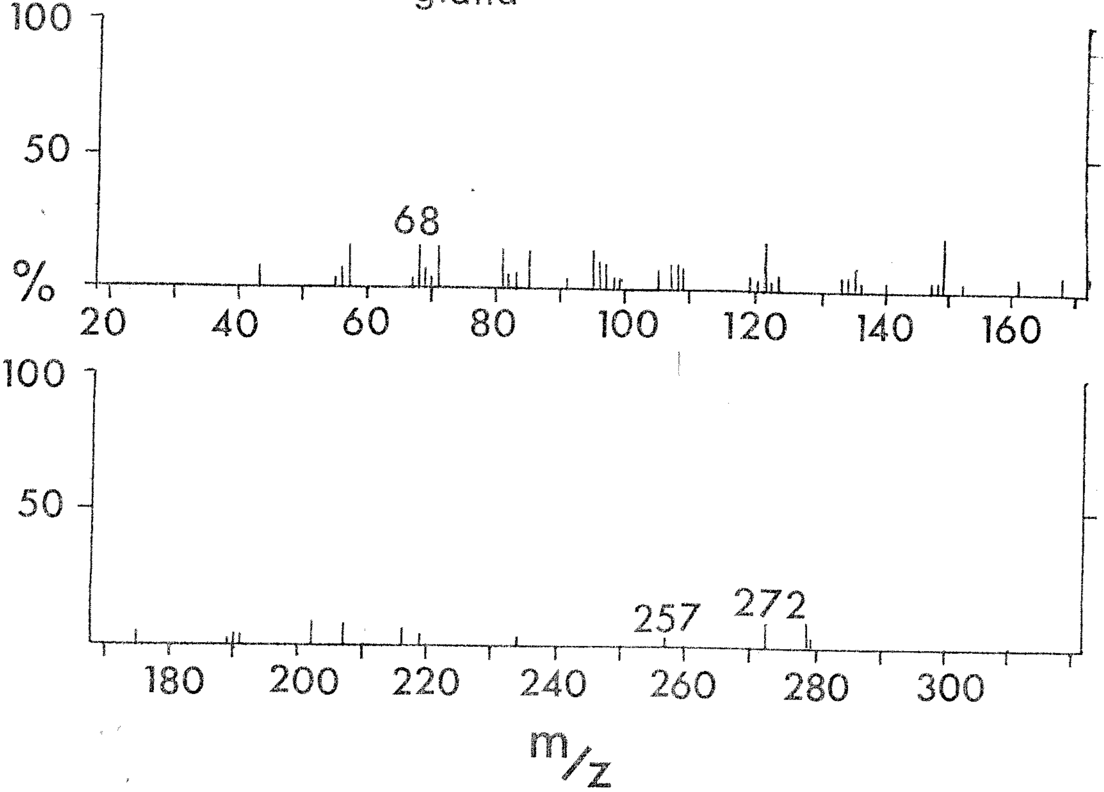
5.5 Dymanics of production of neocembrene during the life-time of queen *M. pharaonis*.

Up to this point in the investigation, the occurrence of neocembrene in fertile queens and its apparent absence in workers and unmated queens was entirely consistent with the possibility that this compound might be responsible for the ability of fertile queens to suppress the development of new sexual forms. However, if this were the case, we might expect to see some quantitative differences in the amount of neocembrene present in young mated queens (0-4 weeks old), fully fertile queens and, perhaps, senile queens. The reason for this is that young queens (and possibly senile queens) are less able to suppress the production of sexuals than are fully fertile queens.

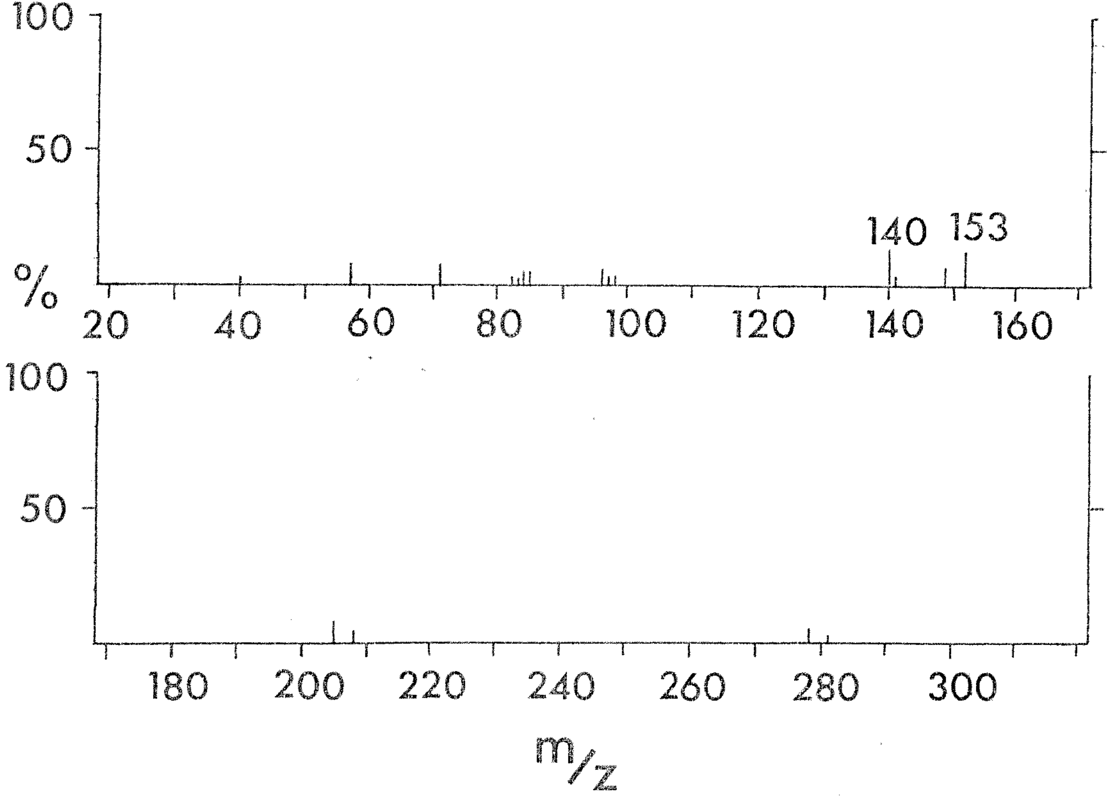
To test this, a survey was made of the quantity of neocembrene produced by queens of different ages. A large laboratory colony containing 2 nests was induced into a phase of sexual production by the removal of all extant queens. Subsequently, when the new batch

Figure 33. Direct-injection mass spectrum of the Dufour's gland of *M. pharaonis* (queen).

Figure 34. Direct-injection mass spectrum of the Poison gland of *M. pharaonis* (queen).



Dufour's gland



Poison gland

of queens emerged as adults, a random sample of ten queens were removed at appropriate intervals from the colony. The removal of these queens was alternated between the two nests to minimise the impact of queen-removal and to prevent such removal resulting in the formation of further sexuals. Sampling of queens continued for 70 weeks, whereupon a new batch of sexuals was produced. Each batch of 10 queens was rinsed for exactly 4 min in 4 ml of dichloromethane and the rinse evaporated to dryness under a stream of nitrogen at 25°C. The residue, contained in a glass ampoule, was sealed under N_2 and stored at -20°C until all samples had been collected. Subsequently, the residues were redissolved in 50 µl diethyl ether and a 5 µl aliquot of each sample was analysed by GC (programme III of methods section). The relative amounts of the compounds monomorine III and neocembrene in each sample were obtained by cutting-out the associated peaks and weighing them. In addition, a standard sample of eicosane (0.1 µg) was injected on to the GC and the weight of that peak also recorded so that the actual amount of neocembrene present in each sample could be estimated.

The results of these experiments are presented in Table 14. The results show several things. First, the amount of monomorine III produced by queens remains relatively constant throughout the brood cycle encompassed by her life-span. However, this is not the case with neocembrene, the levels of which increase by at least 7-fold during the first six weeks. This age-related increase in the amount of neocembrene present in queens is also reflected by a comparison of the quantity of neocembrene present in relation to the quantity of monomorine III (Table 14). Second, it is clear from the results, that once the level of neocembrene has reach a peak in fully fertile queens (by 6 weeks) it does not noticeably decline as the queens age. At first sight this result might seem incompatible with the possibility that the presence of neocembrene inhibits the development of sexual brood since sexuals are produced at the end of the brood cycle when there does not appear to be any reduction in the quantity of neocembrene produced by queens. However, the quantity of neocembrene present in the colony is dependent not only on the quantities produced by extant queens, but also on the numbers

Table 14. Quantitative analysis of relative quantities of Monomorine II and neocembrene present in queens of different ages.

Age of queens (weeks after sexual production)	weight (mg) of GC peaks peak b (monomorine III)	 peak d (neocembrene)	ratio of peak weight (d/b)	weight of neocembrene (ng/queen)
1[a]	38.74	3.52	0.09	78
3[a]	54.22	11.81	0.22	262
6[a]	63.45	25.46	0.40	565
10	49.61	24.42	0.49	542
15	58.52	34.36	0.58	763
20	48.50	31.42	0.64	698
30	29.00	24.69	0.85	548
50	45.68	30.94	0.67	687
60	40.90	24.80	0.60	551
70[b]	43.09	28.67	0.66	637

[a] Winged queens present in nest. [b] New sexual brood present.

present in the colony. Thus, as the senile queens die, the level of neocembrene in the colony as a whole will decrease, even if there is no reduction in the amounts produced by individual queens. Therefore, given the natural mortality of queens, the colony as a whole will be exposed to less neocembrene when queens are young and when they are senile. In this respect, the varying quantity of neocembrene in a colony might still be consistent with its possible role as a pheromone suppressing the development of sexual brood.

CHAPTER 6. INVESTIGATIONS INTO THE BIOLOGICAL ROLE OF NEOCEMBRENE IN THE SOCIAL BIOLOGY OF *M. PHARAONIS*.

In this chapter the results of experiments designed to identify the biological role of neocembrene are discussed. Since the quantity of neocembrene produced by queens is relatively small, these experiments have been made with a sample of synthetic neocembrene (30 mg) generously provided by Dr J Chambers (MAFF, Slough). This material was synthesized by Dr Chambers from a precursor alcohol kindly donated by Dr T Kato (Tohoku University, Japan). Full details of the synthesis of neocembrene will be published elsewhere.

6.1 Effect of virgin queens, topically treated with neocembrene, on the production of sexuals.

Virgin queens are unable to suppress the development of sexual larvae in colonies of *M. pharaonis*. Virgin queens also appear not to produce the compound neocembrene. If neocembrene is the factor responsible for the ability of fertile queens to prevent the development of sexual brood, then it is possible that application of this compound to virgin queens might endow them with the ability to suppress sexual production.

To test this, twelve mini colonies (each containing 60 workers, 50 eggs and 100 mixed brood stages) were set up. Three of these colonies were given one virgin queen. The remaining 9 colonies were divided into 3 groups of 3. The colonies in each group were given either virgin queens treated daily by topical application with 1 μg (approx. 2 queen-equivalents) of synthetic neocembrene, or virgin queens treated daily with 5 μg of total lipids from rinses of fertile queens, or virgin queens treated daily with solvent (hexane) only. Topical treatment was performed using the micro-capillary system developed by HEWLETT and LLOYD (1960). Compounds for topical application were dissolved in hexane and were applied in 0.05μl of solvent. Queens were anaesthetized with CO_2 prior to treatment and any queens which died during the experiment were replaced. The results of these experiments are presented in Table 15.

These results show that, irrespective of treatment, all mini-colonies produced sexual brood. This result suggests that the presence of neocembrene on the body surface of queens is not, in itself, able to endow such queens with the ability to suppress the development of new sexual forms.

Table 15. Effect of topically applied neocembrene and queen-rinse lipids on the ability of virgin queens to suppress the development of sexual brood.

Colony No.	treatment (daily)	sexuals produced (pupae) male	queen	total
1	control	5	2	24
2		4	4	
3		2	7	
4	1 µg synthetic neocembrene	7	1	19
5		4	1	
6		3	3	
7	5 µg total queen lipids	4	1	13
8		1	3	
9		3	1	
10	solvent control (0.05 µl hexane)	2	0	10
11		4	1	
12		2	1	

6.2 Effect of feeding neocembrene to colonies on the production of sexuals.

This experiment was set up to explore the effects of direct introduction of neocembrene into a colony. It was felt that the previous experiment, involving topical application to virgin queens, might have given misleading results if, for example, the behaviour of virgin queens was inappropriate for the normal dissemination of the test compound throughout the colony. Thus, the introduction of neocembrene in food was felt to be a more effective way of ensuring that all colony members had contact with the material. The amount of neocembrene introduced into the colonies was decided upon in a rather arbitrary way and the concentration of synthetic neocembrene in the food (0.5%) was the same as that used previously to evaluate the effects of a synthetic juvenile hormone analogue (EDWARDS, 1975).

Appropriate amounts of dried liver powder were treated with a known quantity of neocembrene dissolved in excess diethyl ether. The mixture was stirred while the ether evaporated and the treated liver powder was then mixed with an equal weight of honey/sponge cake (1:1 w/w) such that the resulting food mixture contained 0.5% by weight of neocembrene. Twelve mini colonies, each containing 50 eggs, 100 mixed brood stages and 50 workers, were set up. Four colonies were given 0.125 g of neocembrene-treated food each week for the duration of the experiment. A further 4 colonies were given food without neocembrene. Of the remaining 4 colonies, two were each given 1 fertile queen and untreated food, and two were given 1 virgin queen and neocembrene treated food. The results of this experiment are given in Table 16. From the results it can be seen that all colonies without fertile queens produced sexual forms. The results of this and the previous experiment indicate that the presence of neocembrene is not the factor responsible for the ability of fertile queens to prevent the development of new sexual forms.

In view of these negative results, the question arises as to the biological role (if any) of the queen-specific compound neocembrene. The caste-specific occurrence of this material, the quantities present in individual queens (approx. 500 ng/queen), and the fact that the material is produced by a specific exocrine gland, all suggest that the compound does have some biological role.

Table 16. Effect of feeding neocembrene (0.5%) on the production of sexuals in colonies of *M. pharaonis*.

Colony No.	Number of queens present	Treatment (weekly)	Sexual pupae produced males	queens	total
1	0	0.125 g food	2	1	3
2	0	containing	5	5	10
3	0	0.5% w/w	4	3	7
4	0	neocembrene	6	4	10
5	0	0.125 g food	6	4	10
6	0	(solvent	12	8	20
7	0	treated only)	8	4	12
8	0		8	1	9
9	1 (virgin)	0.125 g food	6	5	11
10	1 (virgin)	containing 0.5% neocembrene	4	6	10
11	1 (fertile)	0.125 g food	0	0	0
12	1 (fertile)	(solvent treated only)	0	0	0

6.3 Further investigation of the biological activity of neocembrene.

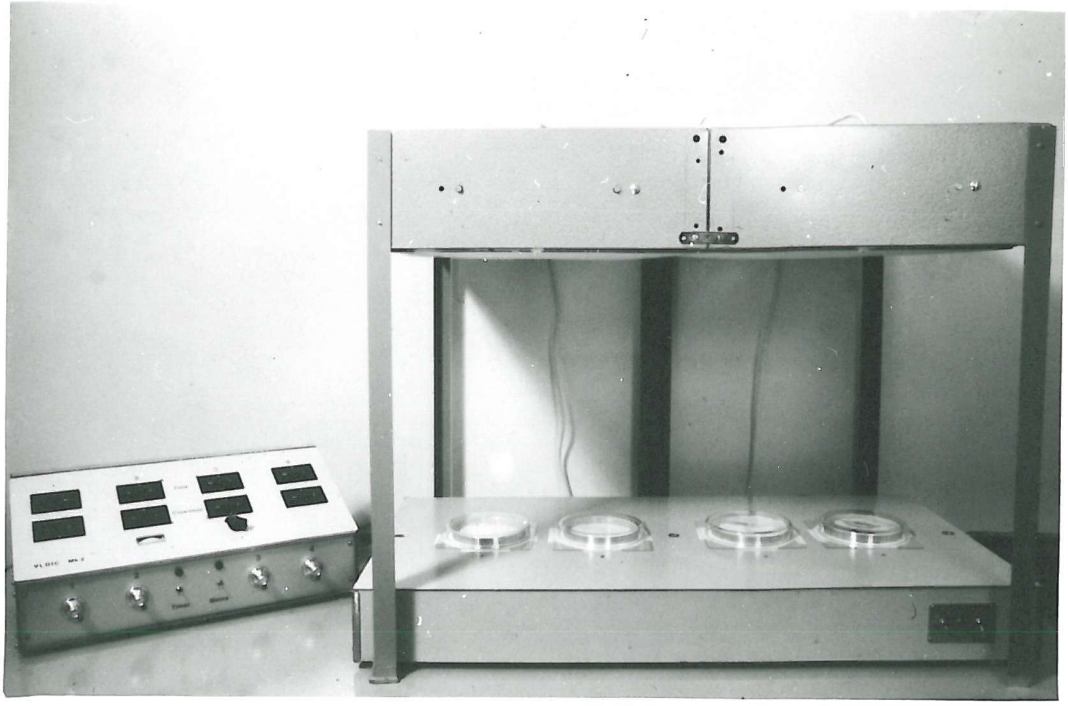
6.3.1 Attractiveness of neocembrene to worker ants.

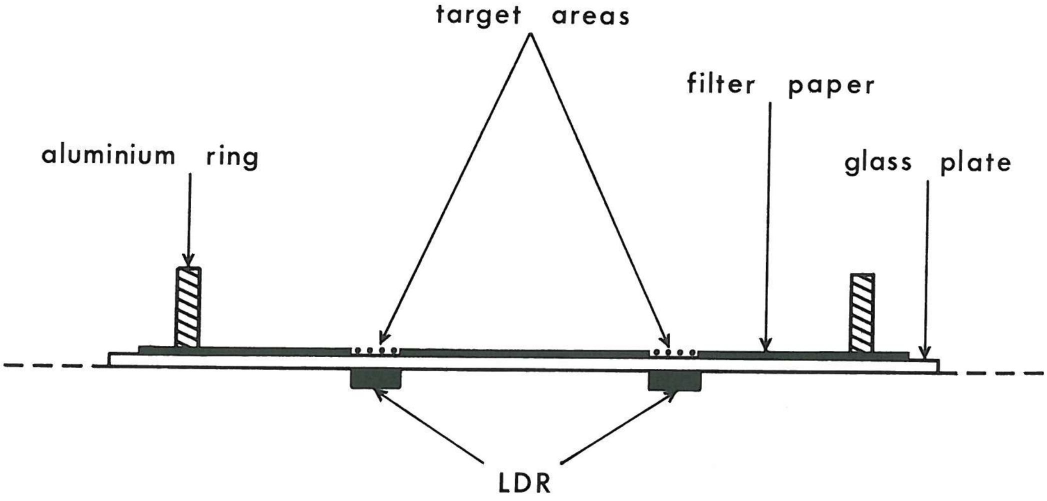
In an attempt to identify the biological role of neocembrene, experiments were conducted to investigate the attractiveness of neocembrene to workers and the ability of neocembrene to induce aggregation in queens.

These experiments were carried out using the 'VLDIC' insect activity detector (Figure 35) developed by PINNIGER and COLLINS (1978)

Figure 35. General view of 'VLDIC' insect activity detector (PINNIGER and COLLINS, 1976).

Figure 36. Section through a test arena from 'VLDIC' insect activity detector showing position of target areas and light-dependent resistors (LDR).


VLDIC Mk 2


target areas
filter paper
aluminium ring
glass plate
LDR

and used by EDWARDS and PINNIGER (1978) to evaluate the biological activity of isomers of monomorine I. This apparatus utilizes light dependent resistors which respond when an insect crosses a target area in a test arena. Using the apparatus it is possible to measure the number of times an insect crosses or enters the target area and the length of time during which insects remain on the area. The test arenas were circles of Whatman No. 1 filter paper on which the insects were contained by PTFE-coated aluminium rings (9.5 cm diameter). Each arena contained two target areas (4 mm^2) located above the light-dependant resistors (Figure 36). In the preliminary bioassay of neocembrene, 1 μl of hexane containing 1 μg of synthetic neocembrene was deposited by glass micro-capillary tube onto one target area, while the other area was treated with 1 μl hexane only. After a period of 10 minutes, during which the solvent evaporated, 25 worker ants were introduced into the test arena. Subsequently, the total numbers of ants crossing each target area and the total time each area was occupied by ants was recorded at 10 min intervals for a total period of 1 hr.

This experiment was repeated four times and the combined results are given in Table 17. These show that the neocembrene-treated target attracted a larger number of workers than did the hexane only treated target and that the amount of time ants spent on the targets was greater when targets were treated with neocembrene. Although, for both parameters (number of 'hits' and time on target) the results of this preliminary experiment were not statistically significant (Student's t-Test) they did suggest that neocembrene is attractive to workers of *M. pharaonis*. However, during this preliminary test, several problems became apparent. First, was the high level of activity of worker ants during the first ten minutes of the experiments. It was thought that this could be associated with the disturbance of ants resulting from their transfer into the test arena. To preclude this possibility, the subsequent tests were conducted after an acclimatization period of 10 min had elapsed following the introduction of workers. Second, it was felt that a true bioassay of attractiveness of a chemical was inappropriate if the attractancy was assayed against nothing (i.e. hexane). The

Table 17. Attractiveness of neocembrene to workers of *M. pharaonis*. (totals from 4 experiments).

Time interval of bioassay (min)	Hexane (control)		Neocembrene (1 μg)	
	No. of hits	time on target	No. of hits	time on target
0 - 10	150	2576	173	2383
10 - 20	70	417	67	578
20 - 30	48	502	71	1031
30 - 40	38	288	64	866
40 - 50	21	113	38	322
50 - 60	25	217	37	403
Totals	352	4113	450	5583

reason for this is that worker ants might be attracted to any material (e.g. a non-repellant, potential food source etc.) that was not positively repellant, and that such a response was not necessarily an indication that the test material had a specific biological role within the species. Thus, a more appropriate test of a 'pheromonal' attractant can only be made by comparison with a similar material without a known biological function. For example, the attractancy of a lepidopteran sex pheromone could be made by assaying an 'inactive' isomer in parallel with the active pheromone. Fortunately, from the point of view of this study, a sample of iso-neocembrene was prepared by Dr J. Chambers (MAFF, Slough) during the synthesis of neocembrene. It was decided therefore to carry out a further assay of neocembrene as an attractant to workers, in conjunction with a similar assay of iso-neocembrene, which is not present in fertile queens. Finally, because in the preliminary bioassay, there was some indication that the attractancy of neocembrene diminished after 30-40 min, the second assay was performed over 30 min. The procedure for the second assay was essentially the same as the first with the exceptions mentioned above. Each assay was replicated 4 times, and the whole experiment was repeated on 3 occasions.

The results are presented in Table 18, and show that neocembrene was more attractive to worker ants than was any other treatment. Overall, neocembrene attracted more than 3 times as many workers as the control (hexane) and these workers remained on the treated target for slightly longer (per unit crossing) than did the corresponding controls. In both cases, the differences between Hexane controls and neocembrene were highly significant (Students t-Test, $P = < 0.001$) By contrast, the iso-neocembrene treated area did not attract noticeably more ants than the untreated (hexane) control area and there was no difference in the times during which ants remained in the two areas. In this experiment, differences between hexane controls and iso-neocembrene were not statistically significant. These results, like those of the previous experiment suggest that neocembrene is actively attractive to worker ants.

6.3.2 Effects of neocembrene on aggregation in queens.

Queens of *M. pharaonis* tend to aggregate together (RITTER *et al.*, 1977a) and this behaviour is particularly noticeable when large numbers of queens are isolated from contact with workers and brood. RITTER *et al.* (1977a) suggested that this aggregation behaviour might be due to a pheromone, and that the indolizine compound, monomorine III, was a likely candidate since the quantity of monomorine III, relative to monomorine I, is much higher in queens than in workers. Because it seemed possible that neocembrene might act as an aggregation pheromone for queens, a test was conducted using the insect activity detector to assay the compound as a queen aggregation factor. The experiment was carried out in a similar way to the first experiments in this section. After treatment of

Table 18. Attractiveness of neocembrene and iso-neocembrene to workers of *M. pharaonis*. (totals from 4 replicates).

Time interval of bioassay (min)	Hexane		Neocembrene		Hexane		Iso-neocembrene	
	No. of hits	Time of target	No. of hits	Time on target	No. of hits	Time on target	No. of hits	Time on target
0 - 10	21	353	85	1308	58	863	61	978
10 - 20	16	265	65	847	44	995	61	1043
20 - 30	15	117	54	937	50	1123	39	697
Total	(52)		(204)		(152)		(161)	
0 - 10	31	229	98	1323	33	595	40	576
10 - 20	14	387	34	1490	34	303	49	963
20 - 30	14	201	44	847	29	729	32	829
Total	(59)		(176)		(96)		(121)	
0 - 10	30	704	79	1485	33	937	31	925
10 - 20	11	74	47	809	43	656	40	783
20 - 30	16	222	53	861	32	673	23	496
Total	(57)		(179)		(108)		(94)	
Grand total	168	2672	559	9907	356	6874	376	7290

one target area with hexane (5 μl) and the other area with 5 μg neocembrene (i.e. approximnately 10 queen-equivalents), 15 fertile queens were introduced into the test arena. However, the queens tended to aggregate at the periphery of the arena and, apart from a short period (about 1 min) after their introduction, no queens were attracted to, or remained on, either target area. For this reason, this assay was rather unsatisfactory and the results do not permit unequivocal conclusions to be drawn as to the role of neocembrene in the aggregation of queens. However, on the basis of these limited results, it would appear as if neocembrene is not a powerful queen aggregation factor.

6.4 General discussion of the biological role of neocembrene.

In this section an attempt has been made to investigate the biological activity of neocembrene in relation to some aspects of the behaviour and biology of pharaoh's ant. No evidence to suggest that this material is directly responsible for the ability of fertile queens to inhibit the development of new sexual forms has been found. It must therefore be concluded that neocembrene has a role other than that previously proposed. Similarly, although on the basis of rather limited experimentation, there is no evidence that the compound is responsible for the aggregating behaviour of isolated queens. However, the above experiments have indicated that neocembrene is attractive to worker ants, and that this attraction is more than just a response of workers to a 'non-repellant' substance. Thus, because neocembrene is present only in the queen caste, and because it is 'recognised' by workers who are attracted to the material, it is possible that this material serves as a queen-recognition substance which enables workers to identify queens from other members of the colony.

The existence of queen recognition pheromones that are attractive to workers has been reported in other Myrmicine ants. For example, extracts of queens of *Pheidole pallidula* (STUMPER, 1956) and *Myrmica rubra* (BRIAN, 1973a) are attractive to workers of the respective species. Queens of the fire ants *Solenopsis invicta* and *S. germinata* also produce substances which are attractive to workers and which are deposited on the substrate (JOUVENAZ *et al.;* 1974). In *S. invicta* this material, which is soluble in inorganic solvents, is produced in the poison gland, although it is chemically distinct from the piperidine alkaloids produced in worker poison glands (VANDER MEER *et al.,* 1980). Because of the close relationship of the genus *Solenopsis* to *Monomorium,* the studies on queen pheromones in the former genus are of particular interest. VANDER MEER *et al.* (1980) reported that the attractive material present in poison glands of queen *S. invicta* was absent in the poison glands of workers and alate (virgin) queens. This situation parallels the occurrrence of neocembrene only in fertile queens of *M. pharaonis.* In *S. invicta,* the source of the queen pheromone appears to be the poison gland, whereas in *M. pharaonis,* the Dufour's gland is the source of neocembrene. In this respect, it may be significant that VANDER MEER *et al.* (1980) report that the Dufour's gland in *S. invicta* queens is atrophied, whereas in *M. pharaonis* this is not the case. Thus, in *S. invicta* the Dufour's gland, which in workers produces the trail pheromone, degenerates in queens which do not lay trails. By contrast, the apparent absence of faranal (the trail pheromone) in the Dufour's gland of queens of *M. pharaonis* suggests that they do not lay trails, but have maintained the gland to synthesize a queen pheromone.

In summary, it appears that in *M. pharaonis,* neocembrene might act as a queen-recognition pheromone, similar in function to those previously reported in other Myrmicine species.

CHAPTER 7. THE BIOLOGY OF QUEENS OF *M.PHARAONIS* IN RELATION TO THEIR ABILITY TO INHIBIT THE PRODUCTION OF SEXUALS.

7.1 Introduction.

It has been shown that virgin queens and fertile queens of *M. pharaonis* differ markedly in their ability to prevent the development of new sexual forms in queenright colonies. Furthermore, the results of previous experiments in this study, and elsewhere (PETERSEN-BRAUN, 1975) suggest that both young fertile queens and senile queens are somewhat deficient in their inhibitory effect on sexual production by comparison with fully fertile (reproductively mature) queens. It is clear that the principal chemical difference demonstrated between virgin and fertile queens (i.e. the presence of neocembrene in the latter) does not appear to be responsible for the difference in the abilities of these two female types to suppress the production of sexuals. The question arises as to whether there are other differences between virgin and fertile queens which might lead to their different effects on the rearing of sexuals.

The only gross morphological difference between virgin and fertile queens is the presence of wings in the former. However, virgin queens do not always remain winged, and the presence of wings in some virgin queens is not responsible for their inability to suppress the development of sexuals since this inability is equally present in artificially de-alated virgin queens (Chapter 3).

Observation of mini-colonies containing workers, brood and either virgin or fertile queens, failed to reveal any obvious differences in the behaviour of, or response to, either queen type by workers. However, virgin queens appeared to spend much less time near the brood than did fertile queens. In view of this observation, a series of tests was set up to investigate further the relative association of each queen type with the developing stages in a nest.

7.2 Association of virgin and fertile queens with the brood in nests of *M. pharaonis*.

The equipment used in these experiments was a video camera (equipped with a low-light-intensity lens system) which was connected to a multi-speed video recorder and monitor. The camera was set on a tripod directly overlooking a white enamelled metal tray (30 x 40 cm) which was illuminated evenly with a dimmer-controlled light system. Four mini-colonies, each containing 50 eggs, 100 mixed brood stages and 100 workers, were placed on the tray such that they were within the field of view of the camera. One fertile queen was introduced into two of the colonies, and one virgin queen was given to each of the two remaining colonies. The colonies were then allowed to settle for 2hr whereupon the camera and recorder were switched on, and the activity within the colonies was recorded for 4hr. This procedure was repeated four times with different colonies and queens. During the recording period, a small clock was also placed in the field of view of the camera. After the recordings were made, the tapes were played-back at 10 x recording speed and the times during which each queen moved away from the brood pile were recorded with a stopwatch. These values were converted to realtime (multiplied by 10) and subtracted from the total time of each recording to give a value for the time each queen spent on the brood pile. The results of this experiment are presented in Table 19 and show that virgin queens spend much less time on the brood pile than do fertile queens. Thus, any interaction between queens and brood-tending workers, or between queens and the brood, would be more pronounced in the case of fertile queens.

7.3 Egg production by virgin and fertile queens.

An obvious physiological difference between virgin and fertile queens is the difference in egg-production between the two forms. This difference is reflected in the different size of the ovaries in virgin and fertile queens, the former being much less developed. That unfertilized queens of *M. pharaonis* lay fewer eggs than corresponding fertile queens has been recorded previously (PEACOCK, 1951 and PETERSEN-BRAUN 1973) as has the fact that, although a few of the eggs laid by virgin females may give rise to males by arrhenotokus parthenogenesis, the majority of such eggs fail to develop into adults (PEACOCK *et al.*, 1954). Moreover, in mini-colonies of the type used in this study, virgin queens seldom laid eggs. Using indirect methods, PEACOCK (1950) estimated that the average egg production of fertile queens was about 1.51 eggs/queen/day (range 0.93 - 2.41) although he acknowledged that this value could be an

Table 19. Comparison of time spent on the 'brood pile' by virgin and fertile queens of *M. pharaonis*.

Queen No.	physiological state	% of time spent on brood pile
1	virgin	27.4
2		21.2
3		2.3
4		20.2
5		39.8
6		22.4
7		25.0
8		25.3
TOTAL (mean ± S.E.)		22.95 ± 3.4
9	fertile	90.0
10		99.75
11		97.25
12		98.72
13		87.25
14		100.00
15		99.0
16		95.5
TOTAL (mean ± S.E.)		95.93 ± 1.5

under-estimate. That this was so, was confirmed by O'TOOLE (1973) who found more than the expected number of mature eggs in the ovaries of queens. In order to obtain further data on the oviposition rate of fertile queens, and any variation in that rate which might occur during the queen's lifetime, the following experiment was conducted. Four mini-colonies, each containing 100 mixed brood stages (no eggs) and 150 workers were set up. Two newly-fertilised queens from a large colony at the end of a phase of sexual production were placed in each mini-colony. To ensure that these queens were fertile, a number of pharate adult queens were confined with workers, brood and 30 males for 2 weeks prior to the start of the experiment. Thus, at the end of this period, the young, fertilised, queens were 0-2 weeks old. After one week, the queens were transferred to 4 new mini-colonies and this procedure was repeated for 37 weeks (i.e. until all queens were dead). The numbers of eggs present in each colony at the end of each weekly period were recorded. This figure represented the total number of eggs laid (excluding any that were eaten by workers) since the incubation period of the eggs is > 7 days (PEACOCK and BAXTER, 1950). In addition, following the transfer of queens, the mini-colonies were retained and the numbers of sexuals produced in each colony (after removal of queens) were recorded.

The results of this experiment are presented in Table 20. The results show that the average egg production of fertile queens (about 20 eggs/day) is much higher than that estimated by PEACOCK (1950a). Furthermore, for the first few weeks of life (upto 4 weeks) the oviposition rate remains below 5 eggs/day. Subsequently the rate rises to reach about 24 eggs per day between 9 and 11 weeks, and this level is maintained for the majority of the queens lifetime. However, approximately 4 weeks prior to death, the oviposition rate appears to decline rapidly to below 10 eggs per day. The times of low oviposition rate apparent in these experiments show a remarkable correlation with the life phases (juvenile, mature and senile) of queens identified by PETERSEN BRAUN (1975) and appear to coincide with the reduced ability of juvenile and senile fertile queens to suppress the development of sexual brood.

Table 20. Egg production and sexual potential of eggs laid during the lifetime of fertile queens of *M. pharaonis*.

Age range of queens (weeks)	Mean oviposition rate (eggs/queen/day) ± SEM.	Sexual eggs laid	
		male	queen
0 - 2	3.35 ± 0.2	-	-
1 - 3	3.53 ± 0.7	-	+
2 - 4	4.47 ± 0.4	-	+
3 - 5	5.08 ± 0.6	+	+
4 - 6	5.35 ± 0.5	+	+
5 - 7	7.58 ± 1.2	-	-
6 - 8	10.95 ± 2.1	-	-
7 - 9	13.47 ± 2.6	-	+
8 - 10	16.62 ± 2.8	-	-
9 - 11	24.58 ± 2.6	-	-
10 - 12	23.54 ± 4.1	-	-
11 - 13	24.99 ± 3.0	-	+
12 - 14	26.38 ± 2.9	-	+
13 - 15	29.41 ± 2.2	-	-
14 - 16	32.60 ± 3.3	+	+
15 - 17	32.71 ± 3.5	+	+
16 - 18	30.22 ± 1.8	-	+
17 - 19	24.87 ± 2.4	-	+
18 - 20	30.15 ± 2.5	-	+

Cont.

Table 20 (cont.)

Age range of queens (weeks)	Mean oviposition rate (eggs/queen/day) ± SEM	Sexual eggs laid male	queen
19 - 21	25.17 ± 3.4	+	+
20 - 22	20.14 ± 1.1	-	+
21 - 23	21.69 ± 2.7	-	-
22 - 24	18.55 ± 1.1	-	-
23 - 25[a]	17.28 ± 1.3	-	+
24 - 26	20.66 ± 1.1	-	+
25 - 27	19.74 ± 1.6	+	+
26 - 28	20.72 ± 1.5	-	+
27 - 29	16.51 ± 1.5	-	-
28 - 30	17.87 ± 1.7	-	+
29 - 31[a]	29.21 ± 1.1	+	+
30 - 32	26.03 ± 0.3	-	-
31 - 33	26.17 ± 1.5	-	-
32 - 34[a]	12.46 ± 1.6	-	-
33 - 35	7.47 ± 0.8	-	-
34 - 36	6.73 ± 1.3	-	-
35 - 37[a]	4.18 ± 1.5	+	+
36 - 38[a]	2.57 ± 1.5	-	+

[a] Indicates period during which queens died.

In the majority of cases, a proportion of eggs laid by queens developed into sexuals irrespective of the age of queens. This fact, coupled with the observation that removal of queens from large laboratory colonies invariably results in the production of new queens and males, irrespective of the time in the brood cycle at which queens are removed, suggests that queens continually lay a proportion of eggs which are capable of giving rise to sexual forms. This is in contrast to the results of a previous study by PETERSEN-BRAUN (1977) which suggested that queens younger than 4 weeks old laid only worker eggs. This was not the case in the present study (Table 20).

7.4 Summary of aspects of behaviour and physiology of queens in relation to their ability to inhibit the production of sexuals.

In the experiments in this section, noticeable differences in the behaviour and biology of fertile and virgin queens have been observed. Virgin queens spend much less time on the brood pile than do fertile queens and this may be associated with the fact that virgin queens lay very few eggs, whereas fertile queens may lay upto 30 eggs per day. Because young queens and senile queens lay fewer eggs than do mature queens, and because the former are less able to suppress the rearing of new sexual forms, it is possible that the rate of oviposition of queens is, in some way, connected with their ability to suppress the production of sexuals. It is also possible that increased egg-production demands an increase in food intake by the laying queens and, in this way, fully fertile queens may compete for food resources at the expense of developing sexual larvae. This mechanism is consistent with both trophogenic caste determination and the existence of 'pro-fertile' food materials as suggested by BUSCHINGER and KLOFT (1973) and PETERSEN-BRAUN (1975). Alternatively, the eggs themselves may act as a signal to workers of the presence of queens, either directly, or as a result of some semiochemical present in or on the eggs. If the latter hypothesis is correct, it should be possible to substitute for the presence of fertile queens in a colony by the introduction of eggs.

CHAPTER 8. THE ROLE OF EGGS IN CASTE REGULATION IN *M. PHARAONIS*.

8.1 The effects of introducing eggs into queenless colonies of *M. pharaonis*.

To test the influence of the presence of eggs on the production of sexual forms in colonies of *M. pharaonis,* the following experiment was performed. Twelve mini-colonies, each containing 50 eggs, 35 workers and 100 mixed brood stages were set up. Three of the colonies were each given one fertile queen, and three more were given no queen. Of the remaining six colonies, three of which were given one virgin queen, all were given 10 eggs per day for a period of 36 days. These eggs were obtained from large colonies containing many mature, fertile queens. During the period when some of the mini-colonies were receiving eggs, the numbers of sexuals produced in all colonies were recorded, and sexuals were removed from the colonies when they reached the pupal stage. After 36 days, the introduction of eggs into the colonies was stopped and the fertile queens were removed from the three colonies in which they were present. Subsequently, the colonies were maintained for a further 35 days and the number of sexuals produced in this second period was recorded.

The results of this experiment are presented in Table 21, and show clearly that the presence of eggs has a dramatic effect on the production of sexuals. No sexuals were produced in the colonies that contained fertile queens, and therefore the eggs which these queens laid. In the colonies which contained either virgin queens or no queens, but which were given 10 eggs per day, only one colony in each case produced sexuals. By contrast, the colonies without queens or eggs, all produced sexuals during the first part of the experiment. Following the removal of fertile queens and the cessation of the introduction of eggs, all colonies (excluding the 'no-queen, no-eggs' colonies) produced large numbers of sexuals during the second period of the experiment. This part of the experiment was considered to be particularly crucial since the only change in the colonies, which hitherto had not produced sexuals, was the removal of fertile queens or the termination of the intro-

Table 21. Effect of the presence of eggs on the production of sexuals in *M. pharaonis* colonies.

Colony No.	Queen type present	Eggs present	Sexuals produced during period of egg introduction male	queen	TOTAL	Sexuals produced after period of egg introduction male	queen	TOTAL
1)	1 fertile	+[a]	0	0	0	0	5	5
2)			0	0	0	1	12	13
3)			0	0	0	0	13	13
4)	1 virgin	+[b]	0	3	3	8	12	20
5)			0	0	0	2	14	16
6)			0	0	0	6	18	24
7)	–	+[b]	1	3	4	2	16	18
8)			0	0	0	5	13	18
9)			0	0	0	4	16	20
10)	–	–	3	11	14)	all brood developed in previous period		
11)			4	2	6)			
12)			1	2	3)			

[a] Eggs laid by fertile queens. [b] Eggs (10/day) artifically introduced.

duction of eggs. This suggests that it was, in fact, the presence of eggs and not some other factor which was inhibiting the development of sexual forms.

Two of the colonies into which eggs were artifically introduced did produce a few sexuals during the period of egg-introduction. This may have been because some of the introduced eggs were damaged during transfer or, perhaps more likely, because the numbers of eggs introduced (10/day) was somewhat below the mean egg-production rate of a fully fertile queen (i.e. approx 24 eggs/day). Notwithstanding this, the majority of colonies into which eggs were artifically introduced did not produce any sexuals during the egg-introduction period, and thus the results indicate that it is the presence of eggs, rather than queens *per se,* which is responsible for the suppression of sexual production.

The complementary experiment to the one described above would be the artificial removal of eggs from colonies containing fertile queens. However, for the following reasons such an experiment would be unlikely to produce meaningful results. First, the removal of eggs would, of course, remove the source of larvae which might develop into sexuals. This would be particularly counter-productive if caste determination were genetic or blastogenic (i.e. sexuals were produced from pre-determined sexual eggs). Second, it seems likely that, unless eggs were removed as soon as they were laid, any signal or stimulus they provide for the colony might still be transmitted. Obviously, the removal of eggs as they are laid by queens is not practically possible.

8.2 Effect of sterilization on the ability of fertile queens to suppress the development of sexual brood.

In previous published experiments (EDWARDS, 1975) it was shown that exposure of colonies of *M. pharaonis* to food containing the insect juvenile hormone analogue, methoprene (isopropyl-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate) induced sterility in queens. This sterility was associated with the atrophy and degeneration of the ovaries (Figure 37) which lacked developing oocytes and trophocytes. Initially, it was thought that this sterility was irreversible, but subsequently, it was shown that with appropriate treatment (i.e. transfer of sterile queens to a thriving colony), some queens recovered their ability to lay eggs. Similar effects of juvenile hormone compounds on the fertility of queens have been reported in the fire ant, *Solenopsis invicta* (TROISI and RIDDIFORD, 1974). Thus, if the presence of eggs (as opposed to the presence of fertile queens) is the factor regulating the development of sexual forms in colonies of *M. pharaonis*, it might be reasonable to expect that sterilized 'fertile' queens would be unable to suppress the development of sexual forms. To test this, 16 mini-colonies each containing 50 eggs, 100 mixed brood stages and 60 workers were set up. Four of the colonies were each given one fertile queen and four were given no queen. The remaining eight colonies were each given a sterilized queen taken from a colony which had been exposed to methoprene-treated food (see EDWARDS, 1975). In addition, three extra mini-colonies, of the same basic composition, were set up and these were each given a sterilized queen captured at a field-trial site at which methoprene baits were being evaluated as a control measure. The appearance of sexual larvae and pupae in all colonies was recorded for a period of 40 days.

The results of this experiment are given in Table 22. In all colonies containing methoprene-sterilized queens, sexual larvae were produced, although in only 5 of these colonies did these larvae survive to the pupal stage. No sexuals were produced in colonies containing fertile queens, but sexuals were produced in all colonies with no queens. In all colonies containing methoprene-sterilized queens, it was noticeable that the queens began to recover their ability to lay eggs between 15 and 29 days after the start of the experiment. This recovery of egg-laying by queens might be the reason why the sexual larvae produced in these colonies sometimes failed to reach the pupal stage. These sexual larvae did not just die of neglect or starvation because, on several occasions, worker ants were seen to remove such larvae from the brood pile and to attack them physically. However, the production of sexual larvae

Table 22. Effect of methoprene-sterilized queens on the production of sexual forms in colonies of *M. pharaonis*.

Colony No.	Queen type in colony	Sexual larvae produced	Sexual pupae produced
1	fertile queen (untreated)	-	-
2		-	-
3		-	-
4		-	-
5	- (no queen)	+	+
6		+	-
7		+	+
8		+	+
9	Sterile queen (from laboratory treated colony)	+	-
10		+	-
11		+	+
12		+	-
13		+	-
14		+	+
15		+	-
16		+	-
17	Sterile queen (from field-treated colony)	+	+
18		+	+
19		+	+

during the period in which sterilized queens were present is further evidence that the presence of eggs is the stimulus which prevents the development of sexual forms. Thus, as the queens recovered their egg-laying ability, the sexual larvae present were unable to complete development to the pupal stage. In this respect, it may be significant that the three colonies that were given queens from the field-trial site, all produced sexual pupae. This is because these queens had longer exposure to methoprene than did the queens from laboratory-treated colonies (10 weeks as opposed to 2 weeks) and therefore recovered their egg-laying capacity more slowly.

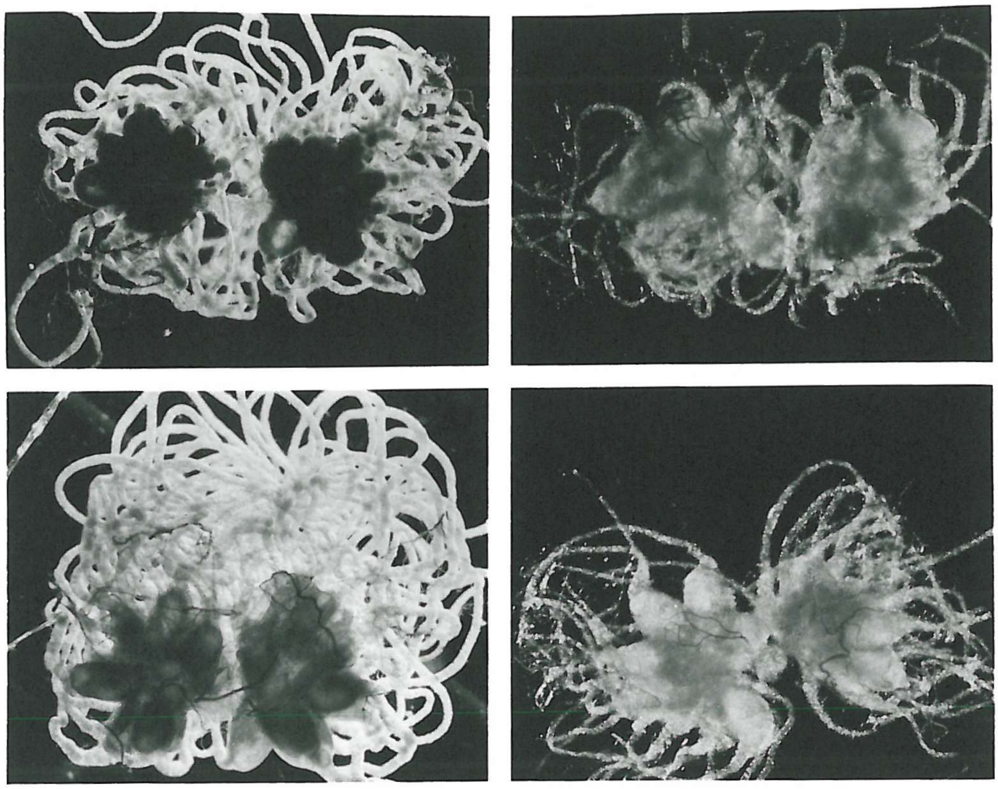
As a follow-up to the previous experiment, groups of 10 ramdomly-selected queens were removed from methoprene-treated and untreated colonies. Subsequently, the queens were rinsed in ether and the rinses analysed by GC (programme II, methods section). Examples of the resulting chromatograms are presented in Figure 38. The results show that all compounds identified in rinses of fertile queens were present in roughly similar quantities in methoprene-treated queens. Thus, the inability of methoprene-sterilized queens to inhibit the appearance of sexual brood, does not appear to be associated with an absence of any particular exocrine product.

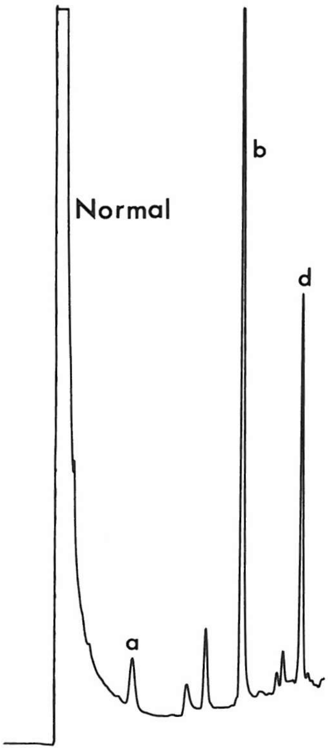
8.3 Eggs as a signal of the presence of fertile queens.

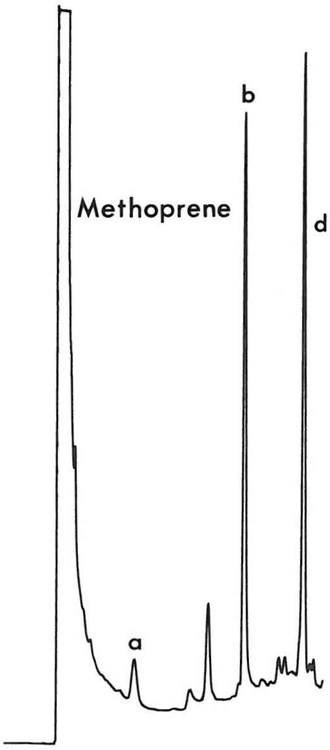
In the previous experiments in this section considerable evidence has been presented to show that the fertile queen exerts her inhibitory influence on the production of sexuals, not by her mere presence, but by virtue of the number of eggs she lays. The oviposition rate of fertile queens varies within her lifetime and, the times of low egg production of juvenile queens and high egg production of mature queens respectively correspond to lesser and greater inhibitory effects on the production of sexual forms. It has also been shown that the presence of fertile queens can, in the majority of cases, be substituted by the presence of a continuous supply of eggs. It follows therefore, that if the presence of eggs is a signal to the colony of the presence of fertile queens, then the colony must be able to detect and recognise the presence of eggs. The recognition of eggs (e.g. by workers) could be either direct (i.e. based on size, shape and 'feel') or as a result of some chemical stimulus on or in the eggs, or as a result of a combi-

Figure 37. Ovaries from normal (left) and methoprene-sterilized (right) queens of *M. pharaonis*.

Figure 38. Comparison of chromtograms of ether rinses of normal (untreated) and methoprene-treated queens.




Normal
a
b
d

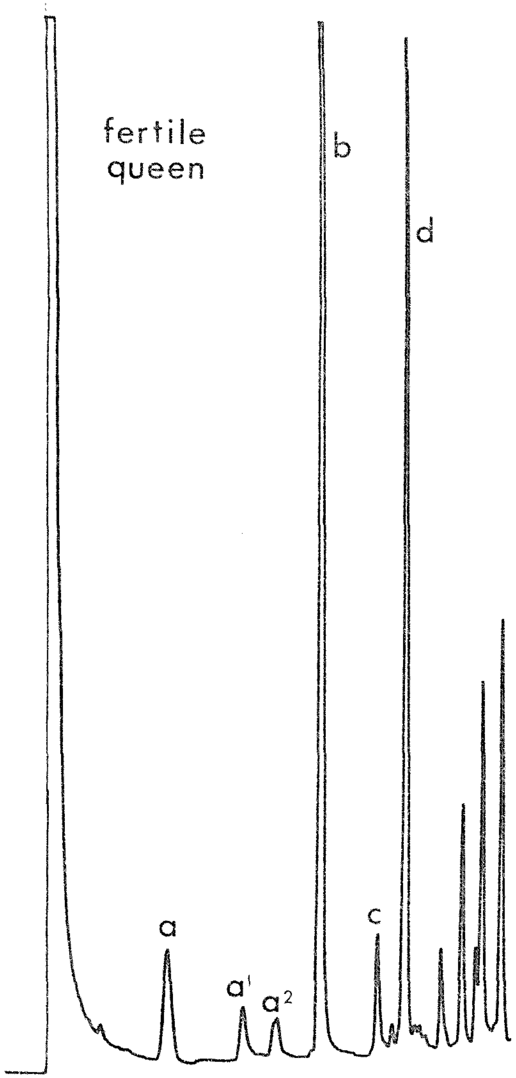

Methoprene
a
b
d

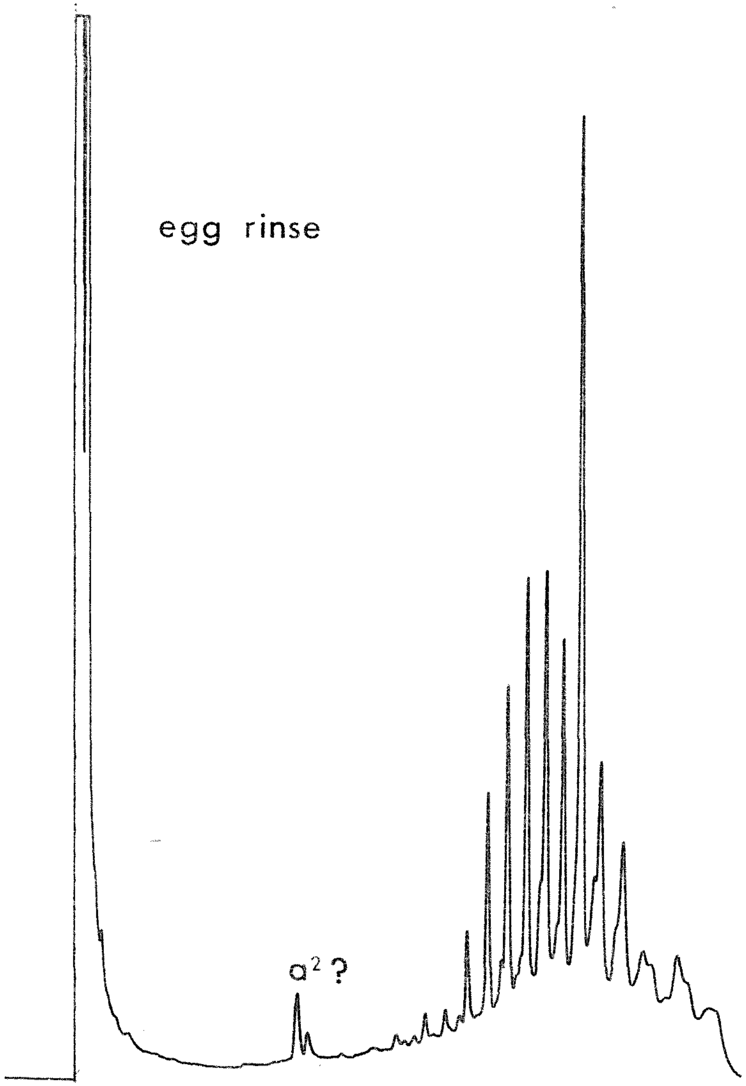
nation of both tactile and chemical stimuli. Direct observation of colonies indicated that workers do spend time on the brood pile touching eggs (and other developing stages) with their antennae and 'licking' them with their mouthparts. Thus, the opportunity for the transmission of a chemical signal from egg to worker certainly exists. A further possibility also requires consideration. Because of the proximity of the sting and the end of the oviduct in queen ants (Figure 26) it is possible that any chemical signal present on the eggs might be put there, by 'topical application' from the sting, as the eggs were laid. Furthermore, the compound neocembrene, previously identified in the Dufour's gland of queens, could be applied to eggs in this way. This possibility necessitates a reconsideration of neocembrene as a candidate chemical signal responsible for the suppression of sexual production in queenright colonies. Indeed, if such a system operated, it would explain why previous attempts to demonstrate the biological activity of neocembrene (by feeding and by topical application to virgins) had failed, because the material was not presented to the colony in an appropriate way (i.e. on the eggs) since in both experiments, a continuous supply of eggs was not available.

To examine these possibilities, 1000 eggs were carefully removed from several large laboratory colonies. These eggs were immediately rinsed in excess diethyl ether for 5 min and the ether then filtered and reduced under N_2 to approximately 250μl before a sample (5μl) was analysed by GC (programme II, methods section). This analysis was conducted alongside an equivalent analysis of a sample of fertile queen rinse and of standard samples of synthetic monomorines I and III, and neocembrene.

A comparison of the analytical traces obtained for the egg rinse and the fertile queen rinse are shown in Figure 39. As can be seen from the traces, the only compound detected in the egg-rinses (apart from some high molecular-weight materials also present in queens) was compound a2 from queen rinses. However, this (unidentified) material is also present in workers, virgin queens and fertile queens and, for this reason, is unlikely to function as an egg-specific semiochemical. None of the other compounds (including neocembrene) previously identified in rinses of fertile queens were detected in the egg rinses. Thus, it would appear that eggs are recognised by their physical characteristics and not by the presence of some lipid-soluble chemical material. That workers are able to recognise and discriminate between eggs and other developmental stages is evident from the fact that brood of the same developmental stage is placed together in different parts of the nest.

Figure 39. Comparison of chromatograms of fertile queen rinse and egg rinse


fertile queen
b
d
a
a^1
a^2
c


egg rinse
a^2 ?

CHAPTER 9. INVESTIGATION OF THE DEVELOPMENT OF SEXUAL BROOD STAGES IN QUEENLESS AND QUEENRIGHT COLONIES OF *M. PHARAONIS*.

9.1. The fate of sexual brood in queenright colonies.

9.1.1. Introduction of sexual brood into queenright colonies.

In previous chapters it has been shown that, throughout her lifetime, a fertile queen lays a certain proportion of eggs which may, given appropriate conditions, develop into new sexual forms.

In the continual presence of fertile queens (signalled by the continuing numbers of eggs they lay) these potential sexual forms do not develop. The question then arises as to what happens to these potential sexuals in a colony containing fully fertile queens? There are several possible answers to this question and they are closely linked with the way in which caste is determined in this species. It is known that males develop only from haploid eggs, and that haploid eggs give rise only to males. Thus, a male egg can only hatch and develop towards adulthood, or die. Therefore, in queenright colonies, the male eggs either do not hatch or do not complete development. If they do not develop, it could be because they are neglected or attacked by workers or queens, or perhaps, because their survival is impeded in some other way (e.g. by a development-inhibiting pheromone).

In the case of the queen, there are more possibilities. If queens are determined in the egg (by genetic or blastogenic factors) and the determination is fixed, then the same limited alternatives that apply to male eggs also apply in the case of queens. If however, there is plasticity in the development of the two female castes (queens and workers) and some or all of the diploid (female) eggs laid are bi-potent, then the non-appearance of queen larvae in queenright colonies might be due to trophogenic factors such as the deficiency of 'pro-fertile' substances as proposed by BUSCHINGER and KLOFT (1973).

To obtain some information on the fate of sexual brood in queenright colonies, several experiments were performed in which sexual larvae, prepupae and pupae were artificially introduced into queenright mini-colonies. In the first series of experiments, a single large sexual larva was introduced into queenright mini-colonies together with a fully-grown worker larva which was taken from the same colony as the sexual larva. In each case, the behaviour of the workers in the foster colony towards the introduced larvae was followed by direct observation with a binocular microscope.

In all cases, introduced worker larvae were accepted by the foster colony and transferred immediately to the brood-pile. Subsequently, workers were observed feeding these introduced worker larvae. By contrast, introduced sexual larvae were immediately attacked by the workers in the foster colony. These workers appeared highly 'excited' when they were presented with sexual larvae, and many more workers gathered around introduced sexual larvae than around introduced worker larvae. Workers attacked sexual larvae by biting them with their mandibles until the cuticle was ruptured, whereupon they appeared to feed upon the internal tissues and liquids of the larva. The whole process, from introduction of sexual larvae to their destruction by cannibalism usually only took 5-10 min. The sequence of photographs illustrating workers attacking an introduced sexual larva (Figures 40-43) was taken within 10 min from the introduction of the larvae. That the attack on sexual larvae was deliberate and not simply a result of the workers inept attempts to move these large larvae from the point of introduction, was evident from the numbers of workers attacking the larvae and from the fact that, in colonies producing sexual forms, workers are adept at moving sexual larvae and other stages without damage. In subsequent experiments, the same cannibalistic behaviour of workers in queenright nests towards introduced sexual pre-pupae, and both male and queen pupae, was observed. However, subjectively, it appeared that attacks on pupae were less sustained and, in some cases, adult males and queens emerged from introduced pupae. When pre-pupae were introduced, there was no difference in the cannibalistic behaviour of workers towards queen

Figure 40. Workers approaching introduced sexual larva (centre) and worker larva (bottom right).

Figure 41. Workers beginning to attack the introduced sexual larva.

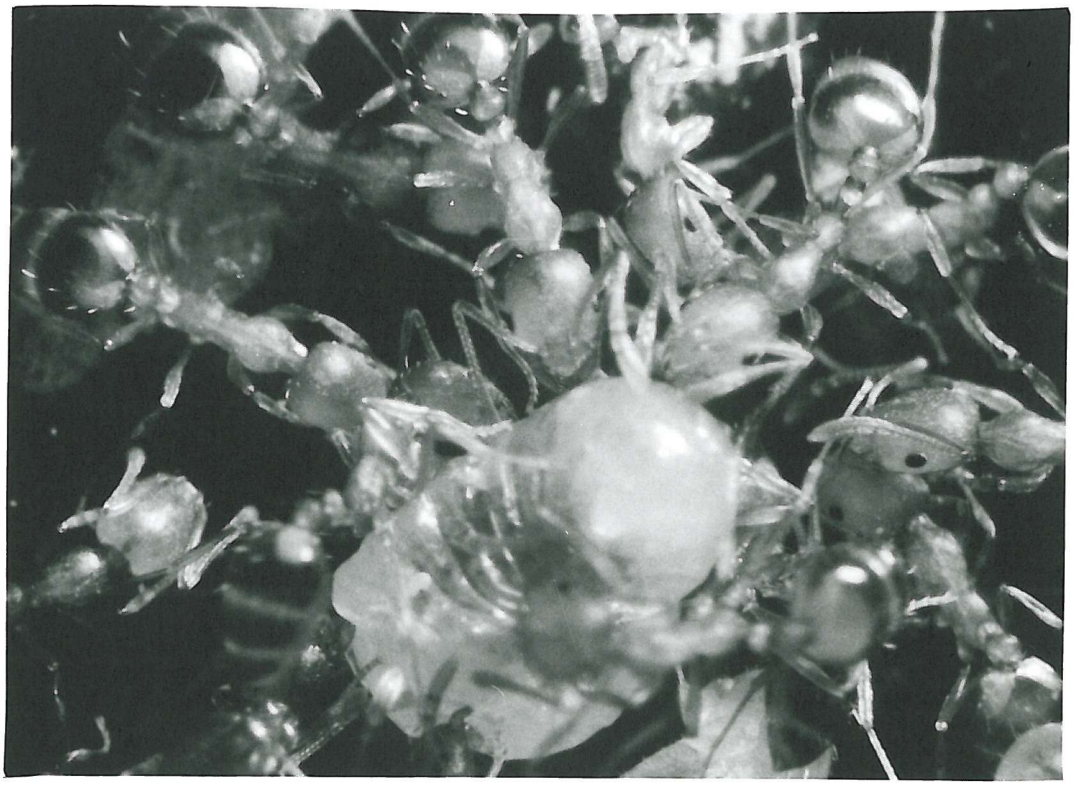
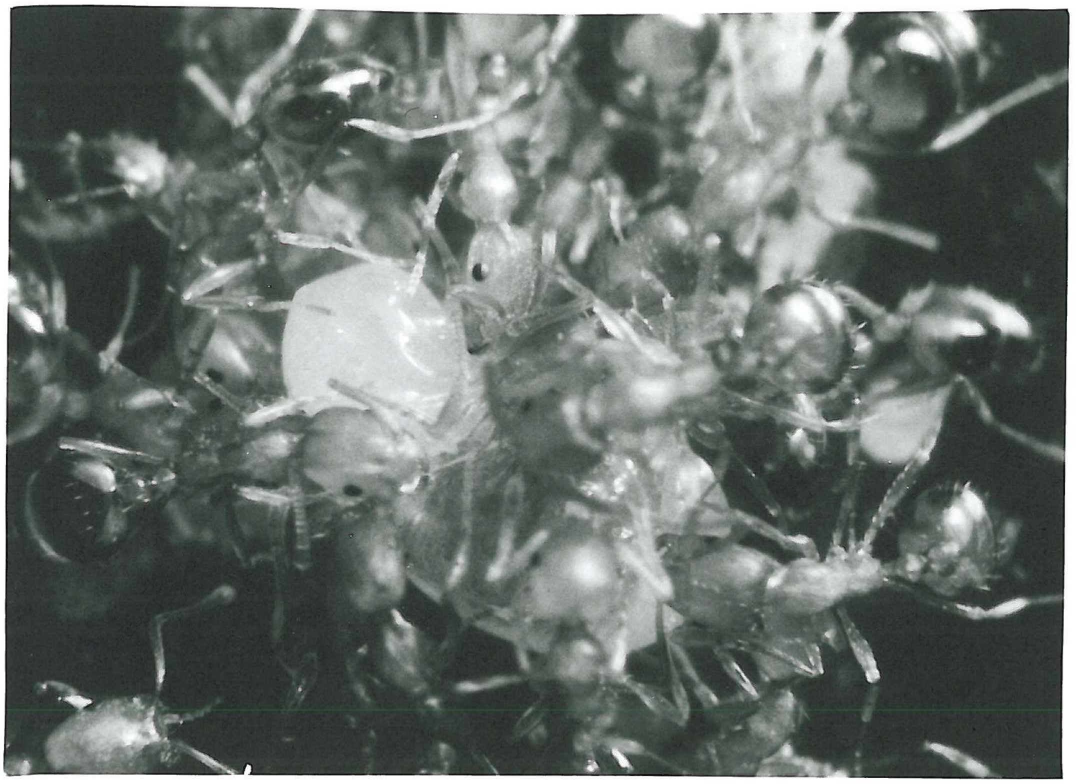


Figure 42. Many 'excited' workers attacking the introduced sexual larva. The worker larva has been removed to the brood pile.

Figure 43. Introduced sexual larva has been killed and is being eaten by workers.



or male pre-pupae, and both sexes were attacked and killed.

In these experiments, the introduced sexual larvae were always about fully-grown and were thus considerably larger than fully-grown worker larvae. For this reason, a further series of similar experiments was performed with smaller sexual and worker larvae. Details are given in Chapter 10 on the features of small sexual larvae which enable them to be recognised even at an early stage in development. The results of this experiment were conclusive, and all 16 sexual larvae (ranging in length from 0.57 mm to 2.80 mm) were killed by workers in the foster colonies. From this experiment it appears that size is not a factor inducing cannibalism since worker larvae, ranging in length from 1.2 mm to 1.87 mm, were accepted in 90% of the experiments. Moreover, those that were killed may have been damaged during introduction to the foster colony.

9.1.2. Introduction of queens into colonies containing sexual brood stages.

In a further experiment, the reverse of the previous experiment was performed (i.e. the introduction of fertile queens into colonies in the act of rearing sexual brood.) Four mini-colonies, each containing 100 mixed brood stages, 50 eggs, 150 workers and 1 virgin queen were set up. After 21 days, when all four colonies contained sexual larvae, two fertile queens were introduced into each colony. The appearance and subsequent disappearance of sexual brood stages was recorded in each colony (Table 23).

The results show that at the time of introduction of the fertile queens (day 21) all colonies contained sexual larvae. Four days after the introduction of fertile queens, the total of 23 sexual larvae had been reduced to 11. Between days 25 and 32 the numbers of sexuals continued to decline, although some were able to complete development to the pupal stage. By day 35 (i.e. 14 days after the introduction of fertile queens) no sexual brood remained in any of the colonies and no further sexuals were produced up to day 44 when the experiment was stopped. Thus, the introduction of fertile queens into colonies producing sexuals leads to the rapid elimination of the developing sexual stages. That the elimination of these sexuals occurred over a period of days, suggests that the influence of the introduced queens took some time to affect all members of the colony. In some cases, the disappearance of the sexual stages was observed to be a result of direct canibalism by workers (Figure 44).

Interestingly, on day 44, it was noticed that the virgin queens, which had been present in the colonies for the duration of the experiment, were all dead. Since these were newly-emerged queens at the start of the experiment, it is unlikely that their deaths were due to 'natural causes' (e.g. old age). Therefore, it is possible that they were also attacked and killed, either by workers or by the introduced fertile queens. That workers or fertile queens might be implicated in the death of the virgin queens is indicated by evidence from two separate sources. First, PETERSEN-BRAUN (1982) has demonstrated that, in *M. pharaonis* , aggression towards queens by workers may occur if the introduced queens are out of synchronisation with the brood cycle. Such would be the case with the virgin queens if the introduced fertile queens had 're-directed' the colony towards a phase of non-sexual production. Second, observations on colonies raising large numbers of sexual stages, particularly those in which the numbers of queens predominated, have revealed that newly-fertilized queens (de-alates) attack, and sometimes kill, unfertilized (alate) queens (Figure 45).

If either workers or fertile queens were directly responsible for the deaths of the virgin queens, then it follows that the attackers must have been able to distinguish between virgin and fertile queens.

Table 23. Effect of introducing fertile queens into colonies of *M.pharaonis* rearing sexual brood stages.

Colony No.	sexual stages present	Days after start of experiment (fertile queens introduced day 21)											
		11	14	16	18	21	23	25	28	30	32	35	44
1	(larvae	0	0	2	5	6	2	2	0	0	0	0	0
	(pre-pupae	0	0	0	0	0	0	0	2	1	0	0	0
	(pupae	0	0	0	0	0	0	0	0	1	1	0	0
2	(larvae	0	0	1	7	6	6	5	4	2	0	0	0
	(pre-pupae	0	0	0	0	0	0	0	1	3	4	0	0
	(pupae	0	0	0	0	0	0	0	0	0	1	0	0
3	(larvae	0	0	3	2	2	1	1	1	0	0	0	0
	(pre-pupae	0	0	0	0	0	0	0	0	1	0	0	0
	(pupae	0	0	0	0	0	0	0	0	0	0	0	0
4	(larvae	0	0	1	6	9	3	3	0	0	0	0	0
	(pre-pupae	0	0	0	0	0	0	0	3	2	0	0	0
	(pupae	0	0	0	0	0	0	0	0	0	2	0	0
Totals	(larvae	0	0	7	20	23	12	11	5	2	0	0	0
	(pre-pupae	0	0	0	0	0	0	0	6	7	4	0	0
	(pupae	0	0	0	0	0	0	0	0	1[a]	4[b]	0	0

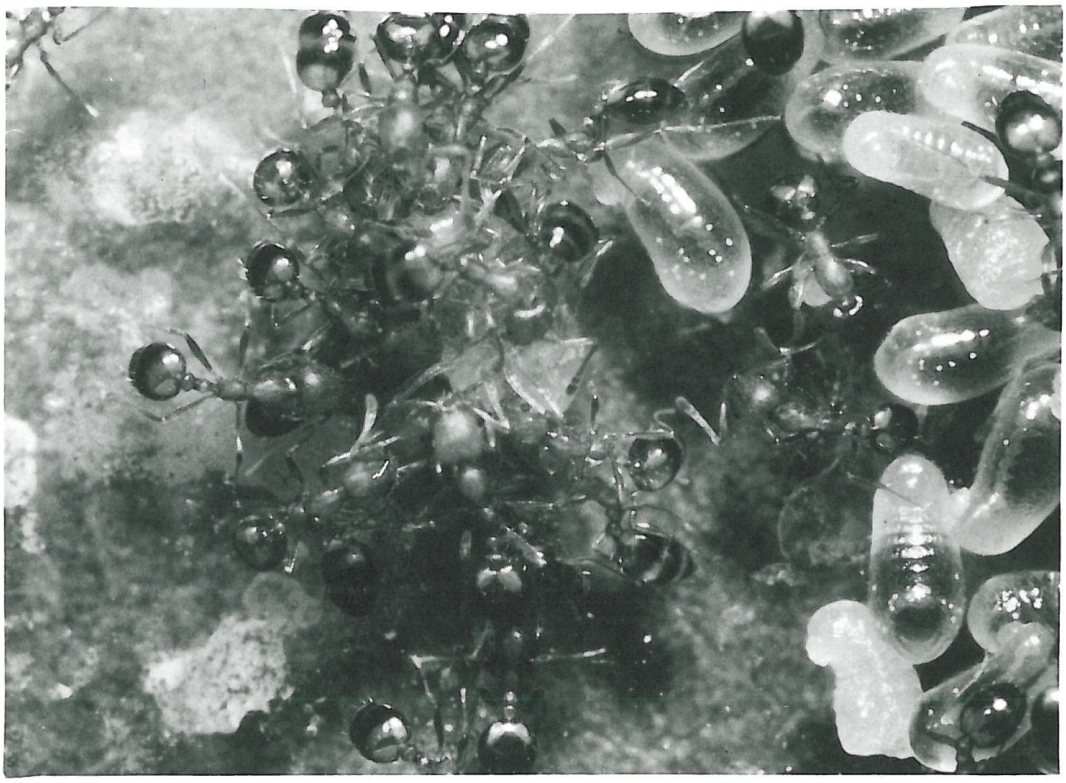
[a] One male pupa [b] One queen pupa and 3 male pupae.

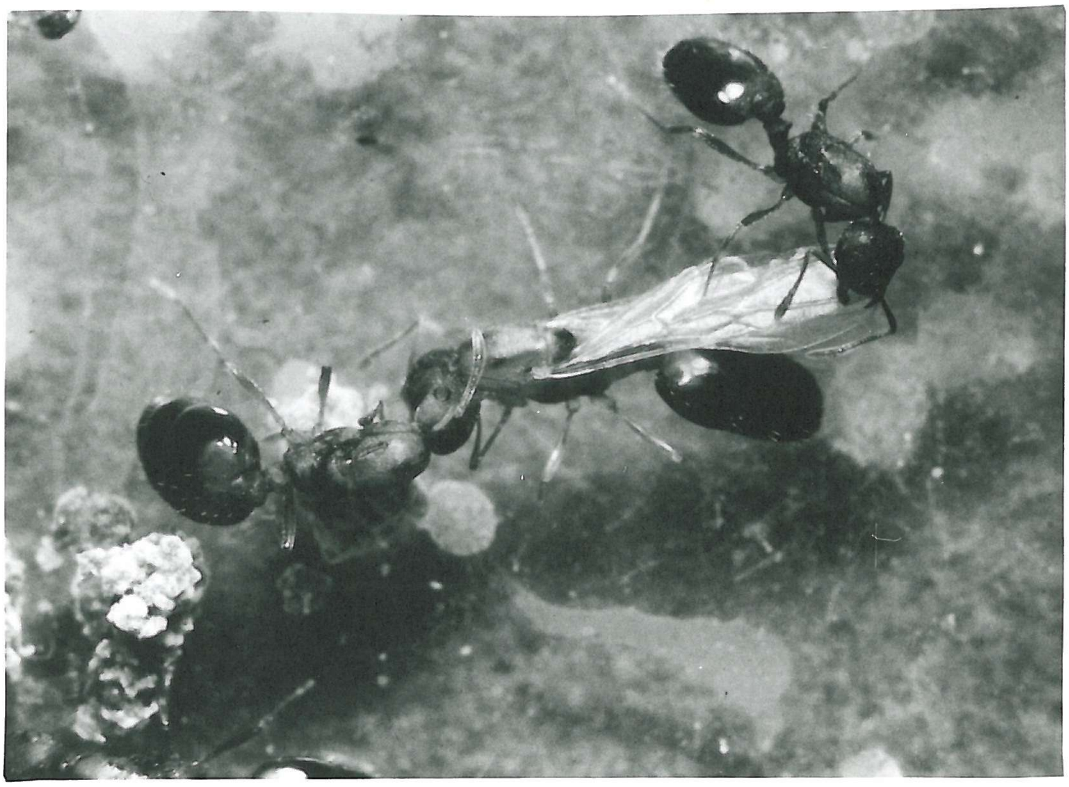
9.1.3. Acceptance of introduced sexual brood by colonies of *M. pharaonis* in relation to the time after removal of fertile queens.

In these experiments, 9 mini-colonies, each containing 100 mixed brood stages, 50 eggs, 70 workers and one fertile queen were set up. These colonies were allowed to "equilibrate" for 4 days, after which time the queens were removed. Into three of the colonies, were immediately introduced 2 sexual pre-pupae and two sexual larvae taken from a large laboratory colony in a phase of sexual production. Similar sexual stages were introduced into the remain-

Figure 44. Workers attacking sexual larva subsequent to the introduction of fertile queens.

Figure 45. Fertilized (wingless) queens attacking a newly-emerged virgin (alate) queen.





ing mini-colonies 4 days later or 7 days later. The survival of these introduced larvae was monitored until they reached the pupal stage.

The results are presented in Table 24, and clearly show that dequeened colonies will not accept introduced sexual pre-pupae or larvae immediately after the removal of queens. Even 4 days after queen removal, the acceptance and rearing of sexual stages is minimal. However, introduced sexuals are accepted and reared when they are introduced into colonies 7 days after the removal of queens. These results indicate that the colony requires between 4 and 7 days (under these experimental conditions) to react to the loss of queens. Furthermore, given that the incubation period of eggs is approximately 7 days, it is possible that the change from rejection to acceptance of introduced sexual brood was linked with the reduction in the number of eggs present in the colonies during the period after the removal of queens.

Table 24. Survival of sexual brood introduced into colonies at various times after the removal of fertile queens.

Colony No.	Time (days) after removal of fertile queens	Number of sexuals introduced		No. reaching pupal stage	% survived
		larvae	pre-pupae		
1	0	2	2	0)	
2	0	2	2	0)	0
3	0	2	2	0)	
4	4	2	2	1)	
5	4	2	2	0)	8.0
6	4	2	2	0)	
7	7	1	2	3)	
8	7	1	2	3)	100
9	7	1	2	3)	

9.2 Developmental periods of worker and sexual brood stages.

Previous studies by PEACOCK and BAXTER (1950) have provided useful data on the developmental periods of workers and sexuals. Their results were obtained from direct observations of *M. pharaonis* colonies fed on raw liver and a mixture of carbohydrates, and maintained at 27°C and 80% r.h. Thus, these data were obtained under conditions which were essentially the same as those used in the present experiments. Preliminary studies at this laboratory (Danby, unpublished) indicate that the values for developmental periods of workers and sexuals are essentially the same as those previously reported by PEACOCK and BAXTER (1950). For reference, the data obtained by PEACOCK and BAXTER (1950) are shown in Table 25. These data show that the average developmental period of sexuals is longer, by 4-5 days, than that of workers, and that this difference is due mainly to a longer larval period. It is noteworthy that considerable variation was apparent in the range of values for worker development. For example, the minimum and maximum values for the larval period of workers are equivalent to a range of -41% to +35% of the mean. Although only mean values were given for sexual brood stages, it is likely that similar

Table 25. Mean developmental periods (days) of worker and sexual brood stages (Taken from PEACOCK and BAXTER (1950))

Stage	Worker (mean)	Worker (range)	Queen (mean)	Male (mean)
egg	7.3	(6.0- 9.0)	7.3	7.3
larvae	17.0	(10.0-23.0)	21.3	21.3
pre-pupa	3.1	(2.0- 4.0)	3.7	3.7
pupa	9.0	(6.5-11.0)	8.9	8.3
Total	36.4	(24.5-47.0)	41.2	40.6

variation exists in the development periods of these insects.

9.3. Observations on the times of appearance of sexual brood stages in colonies of *M. pharaonis*.

Throughout the course of this study, records have been kept of the time of first appearance of sexual pre-pupae and pupae in a large number of mini-colonies. These colonies included those which were set up without queens, those with virgin queens, and those from which fertile queens were removed at a recorded time. Thus, it has been possible to calculate values for the time of first appearance of sexual pre-pupae and pupae in colonies with certain conditions of 'queenlessness'. These data are presented in Table 26.

In the case of colonies (eggs, brood and workers) which were isolated from contact with fertile queens, the first sexual pre-pupae appeared, on average, 25 days after isolation. In those colonies in which virgin queens were present, sexual pre-pupae first appeared, on average, 22 days after the colonies were set up. In colonies without queens and in those with virgin queens, the times of first appearance of queen pupae and male pupae were 30 days and 27 days respectively. Thus on average, male pupae are formed about 3 days earlier than queen pupae. This suggests that the larval stage of male insects is shorter than that of queens. If this is the case, the more rapid development would ensure that adult males were present in the colonies a few days before the adult queens emerged.

In addition to the data obtained on the time of appearance of sexual stages in colonies previously in contact with normal (i.e. mature) fertile queens, there were a few occasions when the appearance of sexuals was recorded in colonies which had previously had contact with either juvenile queens (0-4 weeks old) or senile queens (1-4 weeks before death). These figures are also presented in Table 26 and show that, in such colonies, sexual pre-pupae and pupae appear sooner after the removal of queens than they do in colonies previously in contact with mature fertile queens. Since it is unlikely that the developmental period of sexual stages is

Table 26. Time (days) of first appearance of sexual brood stages in colonies without fertile queens.

Colony type	Time of appearance of sexual stages (mean ± S.E.M)		
	pre-pupae	queen pupae	male pupae
	(n = 99)	(n = 98)	(n = 68)
No queens (mature, fertile)[a]	25.48 ± 0.74	30.15 ± 0.72	27.54 ± 0.76
	(n = 20)	(n = 19)	(n = 21)
Virgin queens	22.20 ± 0.87	30.26 ± 1.72	27.09 ± 0.80
	(n = 3)	(n = 3)	(n = 3)
Juvenile queens[a]	9.0	12.0	13.0
	(n = 7)	(n = 7)	(n = 5)
Senile queens [a]	16.1 ± 2.4	20.0 ± 2.6	21.8 ± 2.5

[a] Type of queen present before de-queening.

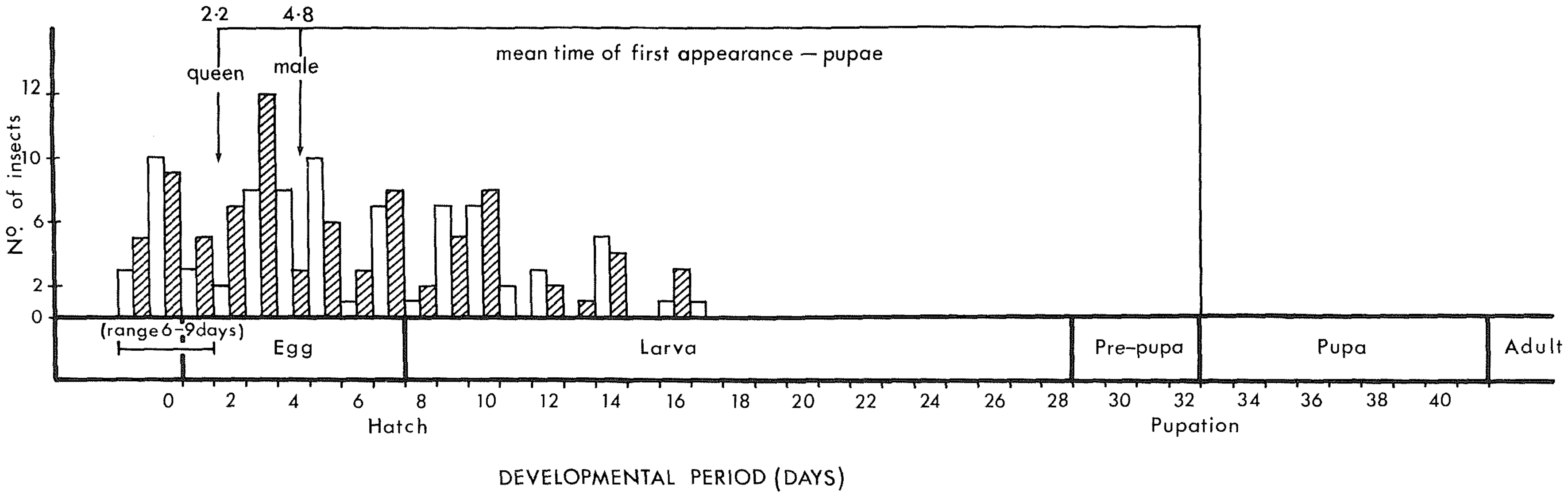
more rapid under such conditions, these results suggest that sexual stages were already developing during the period when the juvenile or senile queens were present. This in turn would indicate that these queens are less able to suppress the development of sexuals than are fully fertile (mature) queens. The fact that sexual pre-pupae first appeared some 3 days earlier in virgin-queen colonies than they did in de-queened colonies (Table 26) is probably not significant since the time of appearance of male and queen pupae in both types of colony was essentially identical. The difference in the times of production of male pupae and queen pupae is not an indication of differences in the pre-pupal period since no distinction was made between male and queen pre-pupae, and the earliest pre-pupae to appear were probably mostly males.

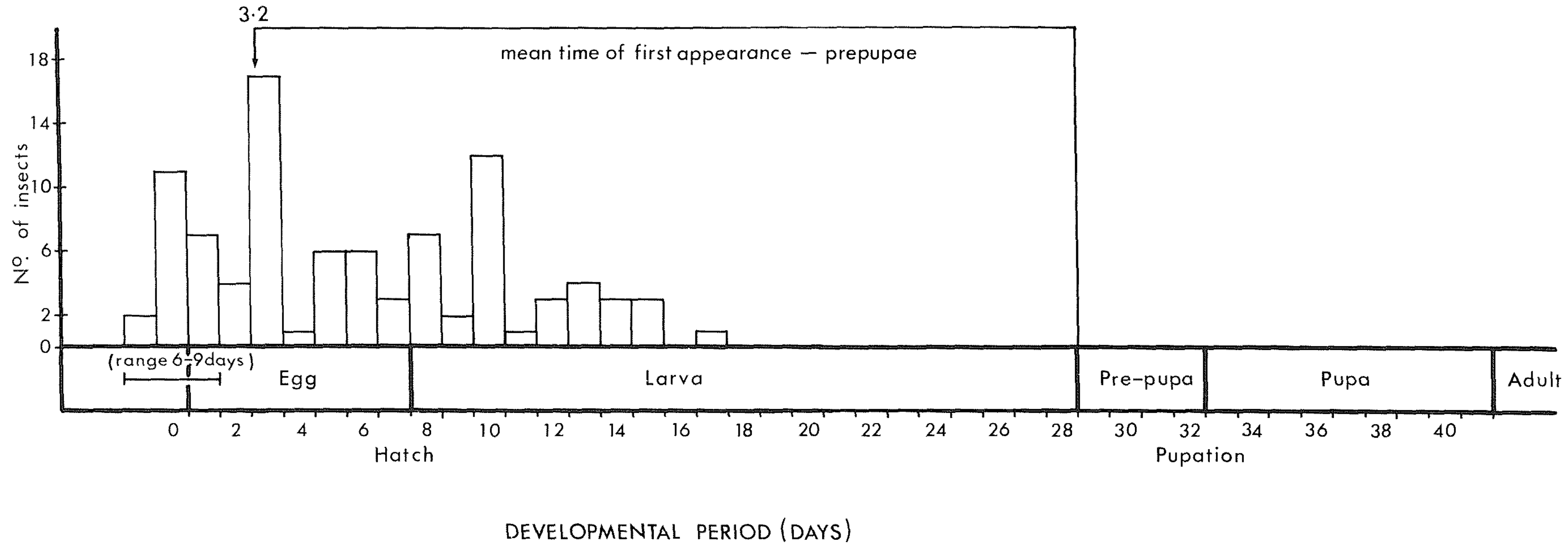
When considering the data presented in Table 26, it is important to bear in mind that the experimental results do not necessarily represent the lengths of the various developmental periods (e.g. egg to pre-pupa) but relate only to the times of first appearance of certain stages in de-queened colonies following the removal of effective contact with fertile queens.

9.4. The time of appearance of sexual brood in queenless colonies in relation to their developmental periods.

Comparison of the time of first appearance of sexual pre-pupae and pupae in colonies without queens (Table 26), with their respective developmental periods (Table 25) provides an estimate of the age of presumptive sexual forms at the time of queen-removal. For example, sexual pre-pupae have a mean developmental period (egg - pre-pupa) of 28.6 days and a mean time of first appearance of 25.4 days. Thus, the average age of these insects at the time of removal of queens was 3.2 days old. Similarly, for sexual pupae, the developmental period is 32.3 days and the time of first appearance of pupae in dequeened colonies was 27.5 days (males) and 32.3 days (queens). Thus the average age of these insects at the time of removal of queens was 4.8 and 2.2 days respectively. From this calculation, we may obtain a total range of expected age of sexuals, at the time of removal of queens, of 2.2 to 4.8 days old. Since the incubation period of the egg is 7.3 days (PEACOCK and BAXTER, 1950) this age-range strongly suggests that these insects were still in the egg stage of development at the time when the colony was isolated from contact with fertile queens. It follows therefore, that in those colonies, the only developmental stage that was present and was able to form sexual pre-pupae and pupae was the egg. Of course, this statement is based on a comparison of mean developmental times with mean time of first appearance of sexual developmental stages, and therefore has to be somewhat modified in the light of natural variation. Thus, because in a number of cases, sexual pre-pupae and pupae first appeared before the mean first appearance time, it follows that these insects will have been older at the time of removal of queens. This is illustrated in Figure 46 which compares the mean time of first appearance of sexual pre-pupae and pupae with the developmental period of sexuals, and shows the distribution of the estimated ages of queen pupae which appeared sooner than the mean developmental period. From Figure 46 it can be seen that, although the

Figure 46. Times of first appearance of male (shaded) and queens (unshaded) pupae (upper histogram) and pre-pupae (lower histogram) in relation to the developmental period of sexuals.


2·2
4·8
queen
male
mean time of first appearance — pupae
No. of insects
0
2
6
10
12
(range 6–9 days)
Egg
Larva
Pre–pupa
Pupa
Adult
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
Hatch
Pupation
DEVELOPMENTAL PERIOD (DAYS)


3·2
mean time of first appearance — prepupae
No. of insects
0
2
6
10
14
18
(range 6–9 days)
Egg
Larva
Pre–pupa
Pupa
Adult
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
Hatch
Pupation
DEVELOPMENTAL PERIOD (DAYS)

majority (68%) of the faster-developing queen pupae were present as eggs at the time of removal of adult queens, some could have been present as young larvae (i.e. upto 9 days old). A similar pattern is evident for pre-pupae and male pupae (Figure 46).

Thus, it appears that in any colony, there is present at all times a proportion of eggs which, upon the removal of fertile queens, may give rise to new sexual forms. In addition, such colonies may contain a (smaller) proportion of young larvae which, in similar circumstances, may also develop into sexual pupae. Furthermore, from the experimental results, it does not appear that queens can develop from larvae aged more than 9 days old, (at the time of queen-removal) because if this were possible, we would expect to see the appearance of queen pupae much sooner after the removal of adult queens than was the case.

A further factor which should be borne in mind is that, the estimated age of presumptive sexual pupae at the time of removal of queens is based on the assumption that the development of the sexuals commences immediately the queens are removed from the colony. It has been demonstrated (Chapter 9) that this may not be the case. Therefore, if the inhibitory effect of the fertile queens persists for a few days after their removal from the colony, this will have resulted in an over estimation of the age of presumptive sexuals present at the time of queen-removal. This may have occurred in those instances where it appeared that sexual larvae, as opposed to eggs, were present at the time of queen-removal. The inference that, in the main, presumptive males are present only as eggs in a queenright colony, merely reflects the fact that the male sex is genetically determined in the egg. The fact that the developmental pattern and times of appearance of queen pupae in queenless colonies closely parallels those of males, strongly suggests that the determination of the female castes occurs in the egg stage. Further evidence in support of this theory is provided by the fact that sexual larvae, particularly at an advanced stage of development, are rarely, if ever, found in colonies containing mature fertile queens.

Because the removal of fertile queens from a colony of *M. pharaonis* typically results in the appearance of sexual forms, and because, queens appear to lay a proportion of eggs which may develop into males and queens, it can be assumed that presumptive sexual stages are always present in a colony. In this section, evidence has been obtained to show that most, if not all these presumptive sexuals are present as eggs. The question then arises as to what happens to these presumptive sexuals in queenright colonies since they do not complete development. In the case of males, where the sex-determination is fixed, these animals may only either develop or die. Since they do not develop, we may assume they die, either by neglect or by direct cannibalism by workers. If the latter is true, then it seems likely that males are killed soon after they hatch from the egg, or perhaps even in the egg stage.

In the case of queens, the situation is potentially more complex and, as discussed in the introduction to this section, is dependent on the way in which the queen caste is determined. If, queen determination is fixed, then, as in males, queens either develop or die because they are neglected or cannibalised by workers. Again, the results of this study suggest that, if this is the case, the destruction of queens occurs at or soon after the egg stage. Alternatively, the determination of caste in females may be plastic and diploid eggs may give rise to workers or queens depending upon the way the larvae are reared (e.g. differences in the quality or quantity of food given to each type of larva). If this is the case, then the results of experiments in this section suggest that the period during which the caste of a female larva is bi-potent is confined to the first few days of larval development.

9.5. Sex-ratio of male and queen pupae.

From approximately 140 mini-colonies which were reared without contact with fertile queens, 100 colonies randomly selected and the incidence of male and queen pupae was recorded. In this sample of 100 colonies, 80 produced both males and queens, 16 produced queen pupae only, and 4 produced male pupae only. These results confirm subjective observations that, in most cases, a colony producing sexuals will produce both males and queens.

To obtain some indication of the sex ratio (males : queens) the total number of queen and male pupae reared in the 100 colonies was recorded. In these colonies the total numbers of male and queen pupae were 328 (39%) and 515 (61%) respectively. The numbers of queens produced was significantly different from the number of males produced (t test, $P = < 0.001$) and the ratio of males to queens was significantly different from 1:1 (Chi^2 test, $P = < 0.001$), the actual ratio being about 2 : 3. This value is reasonably close to the estimates of PEACOCK (1951) who recorded a pupal sex ratio of 1 : 3 (male : queen) and PETERSEN and BUSCHINGER (1971) who reported that there were 3 to 4 times as many queens as males. Although these ratios vary, they all indicate a preponderance of queens over males. This inbalance in adult sexes is reasonable since PETERSEN and BUSHINGER (1971) reported that males mate up to four times.

In 37 colonies reasonable accurate data for the number of eggs present at the time of isolation of the colonies from queens was available. Thus, from the records kept of these colonies, it was possible to calculate the approximate percentage of eggs which gave rise to sexual stages. These values were approximately 8.0% for male eggs and 5.8% for queen eggs, and the populations from which these mean values were derived were not significantly different (t test $P = < 0.001$). However, these estimates may be inaccurate because they do not take into account any mortality which may have occurred during development. PEACOCK (1951) reported that mortality during the development of sexual stages could be as high as 48%. Therefore, it is reasonable to state that the percentage of eggs laid by queens which may give rise to sexuals is probably about 10% for both males and queens.

CHAPTER 10. THE WORKER CASTE - BEHAVIOUR TOWARDS AND RECOGNITION OF BROOD STAGES.

10.1. Age-polyethism in the worker caste.

In a number of ant species it is well established that young, newly-emerged workers tend to remain in the nest whilst older workers tend to leave the nest and scout or forage for food (WILSON, 1971 and contained references). Thus, younger workers are much more concerned with brood-rearing than older workers and, as a result, are likely to have greater or more frequent contact with queens. Because the pigmentation of workers tends to darken with increasing age, colour has frequently been used as a means of estimating the age of workers. Therefore, in order to investigate the possible age-related division of labour (age-polyethism) in workers of *M. pharaonis* the following short study was made. From a sample of approximately 100 workers collected from several places in a large laboratory colony (i.e. nest (brood pile) trail, food dish and periphery) five pigmentation categories (viz. very light, light, medium, dark and very dark) were selected as indicators of age. Subsequently, approximately 200 workers were collected from each of 3 areas of the colony trays. These areas were 1) brood pile in nest, 2) trail to food source and 3) periphery of colony tray. Each worker was then compared with a typical specimen from the 5 colour categories and allocated accordingly.

The results are presented in Table 27, and show a decreasing incidence of lighter workers and a corresponding increasing incidence of darker workers as sampling moved from brood pile, to food trail and to colony boundary. This result strongly suggests that in pharaoh's ant, as in many other species, there is an age-related polyethism in the worker caste. Thus, it appears that, in *M. pharaonis,* younger (lighter) workers tend to remain on the brood pile (where they presumably care for developing stages) whereas older (darker) workers are predominantly responsible for foraging outside the nest. Moreover, the results suggest the possibility that the oldest workers may act as scouts for new food sources foraging further afield and away from established trails.

Table 27. Distribution of pigmentation categories of workers in relation to the part of the colony sampled.

Part of colony sampled		No. of workers in pigmentation class v. light	light	medium	dark	v. dark
Brood pile	total	6.0	60.0	90.0	49.0	0
(n = 205)	%	2.9	29.3	43.9	23.9	0
food trail	total	0	1.0	138.0	69.0	0
(n = 208)	%	0	0.5	66.3	33.2	0
periphery	total	0	0	134.0	70.0	2.0
(n = 206)	%	0	0	65.0	33.9	1.0
totals		6	61	362	188	2
(n = 619)	%	0.9	9.8	58.4	30.3	0.3

10.2 Recognition of brood stages by workers of *M. pharaonis*.

Workers of *M. pharaonis* can recognise brood stages. If, for example, eggs, larvae, pre-pupae or pupae are placed outside the nest, the workers will immediately retrieve them and return them to the brood pile. This occurs even if the brood stages are from another nest. As a preliminary to the next section, it was decided to carry out some simple investigations into the way in which workers recognise brood. For these experiments, worker pre-pupae were the stage used because a) they are not fed by workers and thus are not likely to exhibit any behavioural responses (e.g. during trophallaxys), and b) because they have no mouth or anus through which gut contents could contaminate solvent rinses of cuticular components. The experiments were performed using mini-colonies containing 2 fertile queens, 200 workers and approximately 200 mixed brood stages (including eggs). The mini-colonies were allowed to form a discrete brood pile, whereupon a 1 cm diameter circle was marked on the bottom of the crystalizing dish at a point diametrically opposite the brood pile. Subsequently, 10 pre-pupae (treated or untreated) or 10 surrogate pre-pupae were placed in the marked circle. The number of pre-pupae remaining in the circle was recorded after 30 min. In addition to normal (control) pre-pupae, some pre-pupae were rinsed for 15 min in a range of solvents (water, methanol or ether) and others were killed by exposing them to ethyl acetate vapour for 15 min. In other cases, surrogate pre-pupae were made by cutting small pieces of pith to the size of a pre-pupa. Some of these pith 'pre-pupae' were used untreated, while others were treated with acetone or with an acetone solution containing cuticular materials rinsed from normal pre-pupae. In the latter case, pith 'pre-pupae' were treated with 0.5μl acetone containing twice the estimated pre-pupal rinse equivalent.

The results of these experiments are given in Table 28. These show that only in the case of normal pre-pupae were all the introduced insects removed by workers. The fact that rinsing pre-pupae in solvents, particularly organic solvents, appeared to reduce the ability of workers to recognise and remove them, might indicate the existence of a brood-recognition pheromone which is soluble in organic solvents. However, since killing pre-pupae prior to their introduction also resulted in a partial loss of ability on the part of workers to recognise and remove these stages, it is possible that the solvent-rinsed pre-pupae died as a result of rinsing, and that this was the reason that they were not recognised. The possibility that recognition of pre-pupae by workers is not entirely a result of some chemical signal is further indicated by the results of the experiments with surrogate (pith) pre-pupae, which were treated with a solution of lipids rinsed from the cuticle of normal pre-pupae. None of these 'pre-pupae' were recognised or removed (Table 28). Obviously, it is not possible on the basis of these limited experiments, to make unequivocal statements about the way in which workers of *M. pharaonis* recognise brood stages. Nevertheless, the results suggest that brood recognition is not chemically mediated and that more complex factors (e.g. 'touch' and 'feel') may be involved. Moreover, there is other (circumstantial) evidence which suggests that brood recognition involves, to some extent, the topography and/or size of brood stages. This is indicated because, within the nest, workers segregate brood of different developmental stages into discrete areas of the brood pile.

Table 28. Effect of various treatments on the ability of workers to recognise worker pre-pupae and surrogate pre-pupae.

Treatment and type of pre-pupa	Number of pre-pupa introduced	Numbers of pre-pupae not removed by workers	% not removed
Control (Normal pre-pupae)	40	0	0
Water-rinsed (15 min)	20	4	20
Methanol-rinsed (15 min)	20	18	90
Ether-rinsed (15 min)	20	19	95
Dead pre-pupae (15 min ethyl acetate)	20	9	45
Surrogate Control (untreated pith)	20	20	100
Surrogate (Acetone treated)	20	20	100
Surrogate (pre-pupal rinse)[a]	20	20	100

[a] Treated with $0.5\mu l$ acetone containing 2 pre-pupal extract equivalents.

10.3 Differences between worker and sexual brood stages.

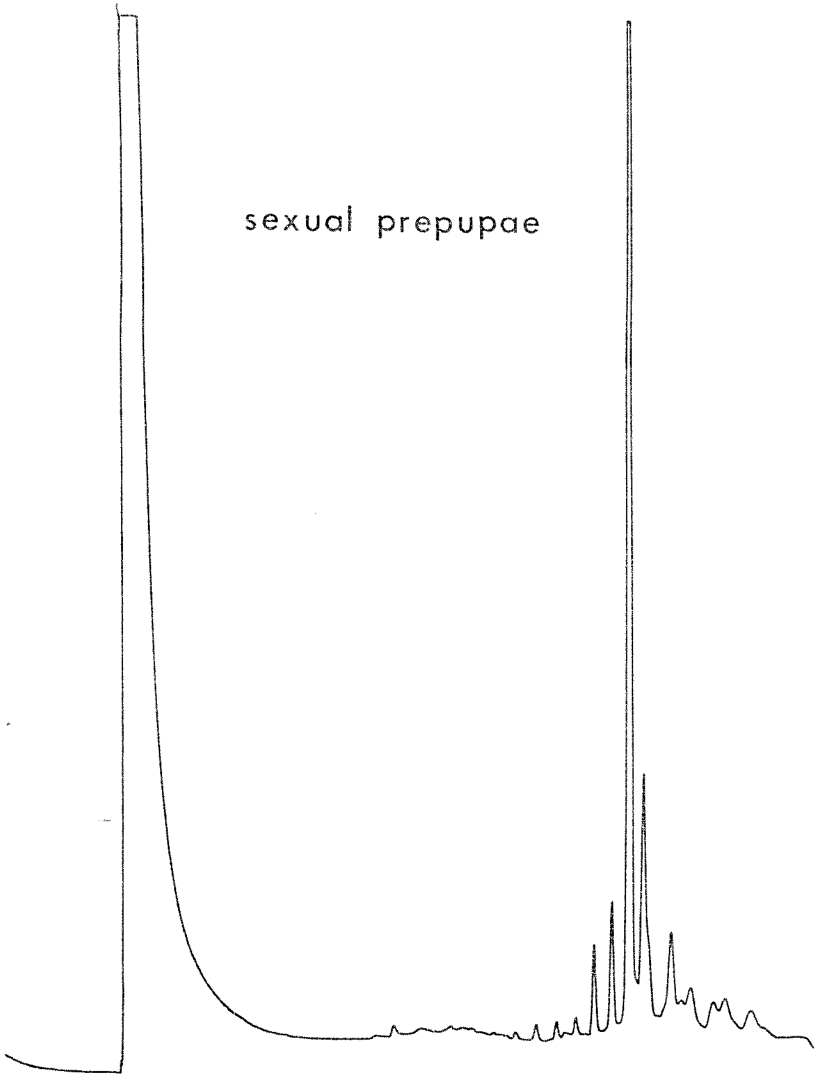
Whatever the mechanisms by which workers recognise brood as such, it is clear that they can differentiate between worker and sexual brood stages. This is evident from the way in which they attack sexual larvae when these are introduced into queenright colonies. There are a variety of possible ways in which workers may differentiate between sexual and non-sexual brood stages. For example, sexual brood may produce (or lack) a specific recognition substance, or there may be physical or 'behavioural' characteristics which workers can recognise.

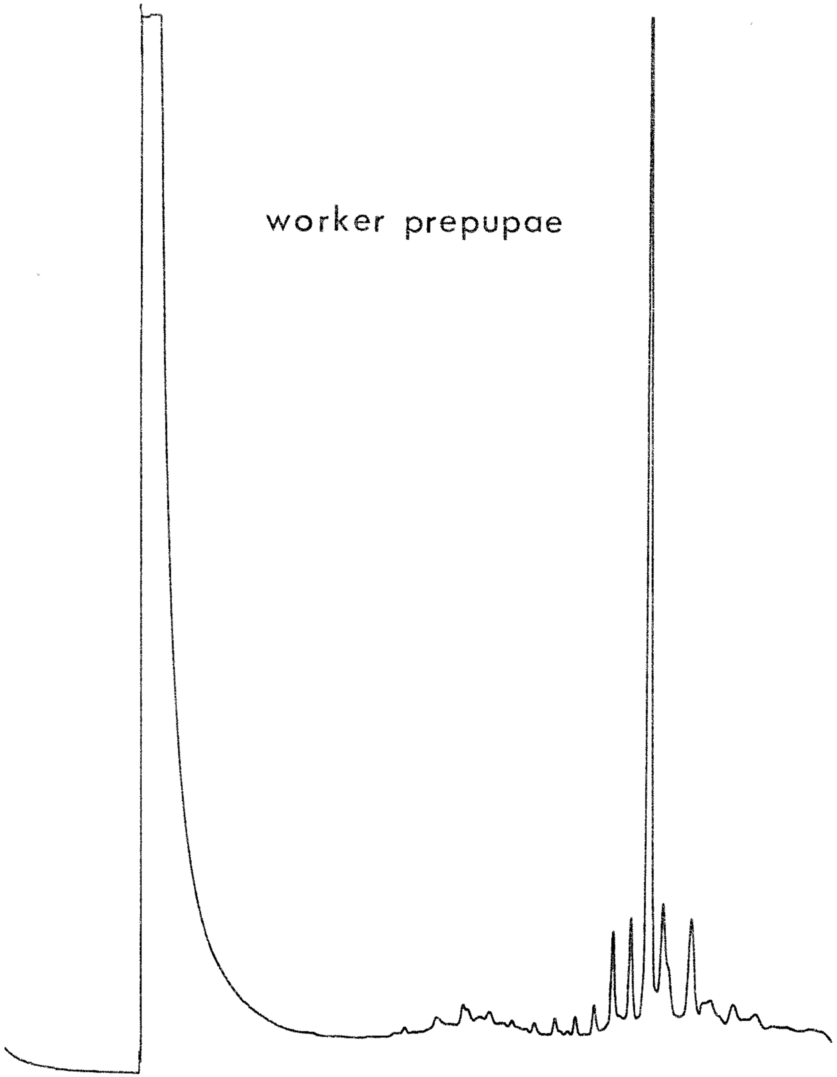
It seems unlikely that behaviour is a key to recognition of sexual brood since pre-pupae and pupae are recognised and neither have any obvious 'behaviour'. Although fully developed sexual larvae are much larger than corresponding worker larvae, size alone can not be important in recognition of sexuals since even small sexual larvae are recognised and attacked when they are introduced into queenright colonies. Therefore, with the probable elimination of size and behaviour, only chemical signals and/or physical characteristics remain as the most likely alternatives.

10.3.1. Chemical differences between worker and sexual pre-pupae.

In order to investigate any chemical differences between sexual and nonsexual brood, pre-pupae were rinsed in organic solvent (diethyl ether) and the rinses analysed by GC. Pre-pupae were chosen because they do not feed and because, as mentioned above, the gut contents of the larva are voided as the pre-pupa is formed. Therefore solvent rinses are unlikely to be contaminated with materials from the digestive tract. Because sexual pre-pupae weigh about twice as much as worker pre-pupae, rinses were made with 200 worker pre-pupae and 100 sexual pre-pupae. These insects were rinsed for 5 min in excess diethyl ether which was then filtered and reduced to dryness, under a stream of nitrogen, at 25°C. Subsequently, the residues were taken up in 200μl diethyl ether, and an aliquot (6μl) analysed by GC (programme II, materials sections). The resulting chromatograms are shown in Figure 47. From these it can be seen that

Figure 47. Gas-liquid chromatograms of ether rinses of sexual and worker pre-pupae.


sexual prepupae


worker prepupae

there are no major differences, either quantitative or qualitative, between solvent rinses of sexual and worker pre-pupae. This result would suggest that there are no lipid-soluble cuticular components which are specific to sexual or to worker pre-pupae. However, these results do not preclude the possibility that specific polar (water soluble) materials are present in or on such larvae.

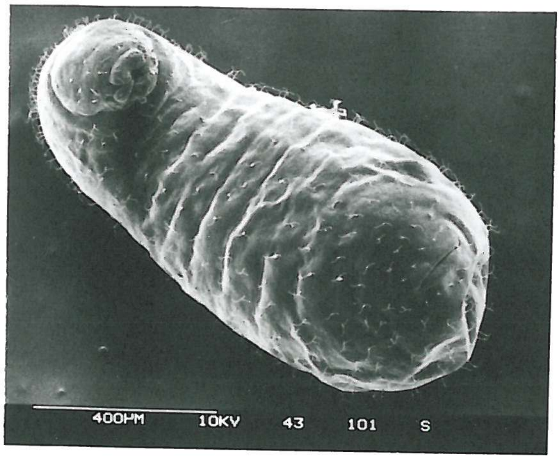
10.3.2. Morphological differences between sexual and worker larvae.

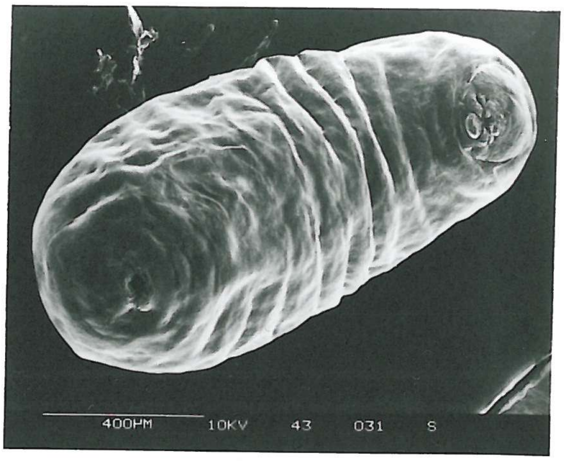
It has been reported previously (HALL and SMITH, 1953) that there are differences in shape between sexual and worker larvae, and differences in the colour of the gut-contents in these two types of larvae (PEACOCK and BAXTER, 1950). Both statements are true - sexual larvae tend to be more 'pear-shaped' or 'rounded' than worker larvae, and this is so in the case of both young and older sexual larvae. Moreover, as the larvae develop, the gut contents of sexual larvae appear lighter than those of worker larvae, although it is not clear if this difference is due to differences in nutrition, or to the greater rotundity and size of the body of sexual larvae.

As part of this study, a detailed investigation (using scanning electron microscopy) was made of the cuticular surfaces of sexual and worker larvae. Initially, these studies were made with a view to identifying possible exocrine gland openings on the body surface of larvae (none were found). However, it soon became clear that there were obvious differences in the pilosity of these larval types which were not obvious on casual visual observation. In essence, worker larvae are hairy and sexual larvae are hairless or almost so. These differences are illustrated in the scanning electron micrographs in Figure 48. These early electron micrographs were taken of well-developed larvae, but subsequently small sexual and worker larvae were used. In the smaller larvae the differences were similar (Figure 49) and workers were covered with many rows of large, bifurcated, hooked setae, whilst sexual larvae were very sparsely endowed with short curved setae (Figures 50 and 51).

Figure 48. Scanning electron micrographs of large sexual (right) and large worker (left) larvae.

Figure 49. Scanning electron micrographs of small sexual (right) and small worker (left) larvae.


400µM 10KV 43 101 S


400µM 10KV 43 031 S


200µM 7.5KV 71 006 S

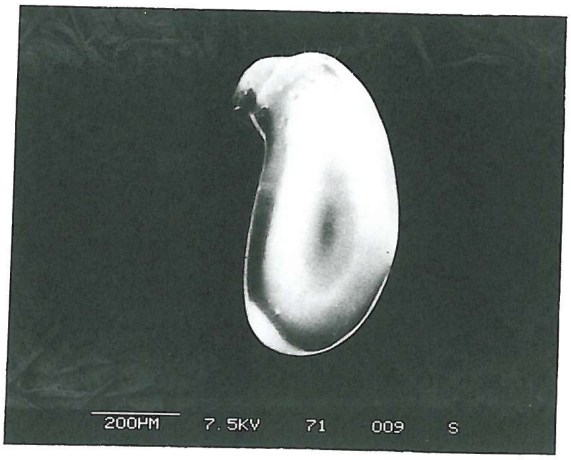
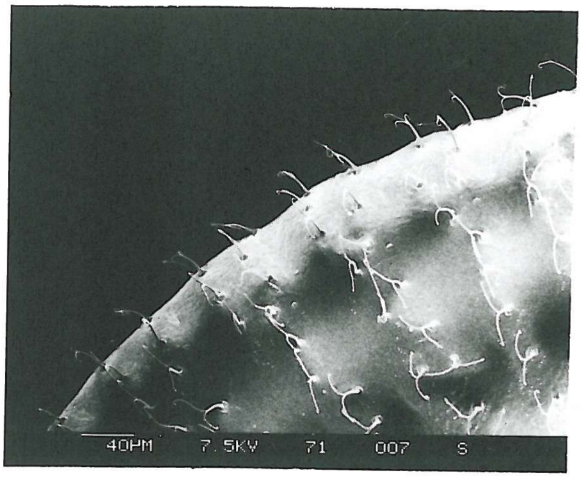
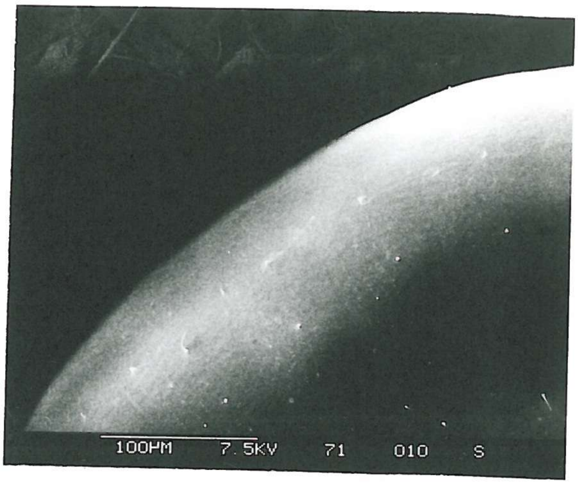
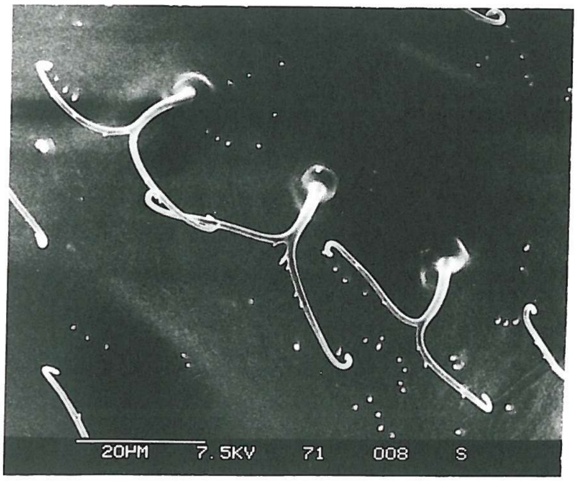

200µM 7.5KV 71 009 S

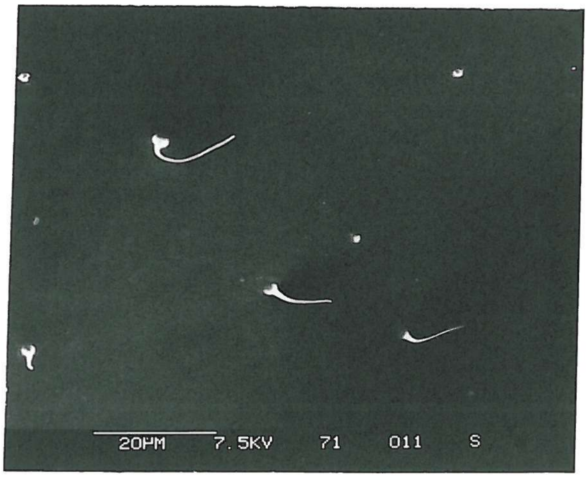
Figure 50. Scanning electron micrograph of part of the dorsel cuticle of sexual (right) and worker (left) larvae.

Figure 51. Scanning electron micrographs showing detail of hair structure on sexual (right) and worker (left) larvae.


40µm 7.5KV 71 007 S


100µm 7.5KV 71 010 S


20µm 7.5KV 71 008 S


20µm 7.5KV 71 011 S

The presence of many hooked hairs in worker larvae may serve to hold the larvae together in bunches (for ease of transportation by workers), but the absence of these hairs in sexuals may well enable workers to differentiate between the two types of larva. In the pre-pupal stage sexuals are hairless and workers hairy, and of course, sexual pre-pupae are much larger than worker pre-pupae. Similar differences in pilosity between worker and sexual larvae have been reported by SCHMIDT (1974) in *Formica polyctena*, and by WHEELER and WHEELER (1979) in a variety of (unnamed) species.

10.3.3. Protein content of worker and sexual larvae.

A simple experiment was performed to examine any gross differences in larval protein content. Since the gut of larvae occupies a relatively large proportion of the body, it was felt that any large differences in total protein content might indicate that extra protein was being fed to one type of larva. The experiment was performed by homogenizing a known weight of sexual or worker larvae (11.5 and 9.3 mg respectively) in 3 x 100μl TRIS/HCl buffer solution, pH 7.4; from which 10μl aliquots were removed and analysed by spectrophotometry after reaction with a standard coumassie blue reagent solution. The reagent and spectrophotometer were calibrated for 0 to 50μg total protein with standards prepared with Bovine serum albumen. The results obtained from this experiment revealed no apparent gross differences in the total protein content of sexual and worker larvae (20.31 and 24.54μg/mg body weight, respectively). This suggests that sexual larvae are not selectively fed a high-protein diet by workers.

CHAPTER 11. DISCUSSION, SUMMARY AND CONCLUSIONS.

11.1 Introduction.

Because the significance of many of the experimental results obtained in this study has been discussed previously in the appropriate chapters, this final chapter attempts only to bring together the main conclusions of the work, and to relate them to caste determination and regulation in other ants.

For reasons given earlier (section 1.2) the mechanisms of caste regulation and caste determination in *M. pharaonis* will be discussed separately. Because virtually all previous studies on caste in ants have not rigorously distinguished between caste regulation and caste determination, there is not sufficient information to permit a general discussion of caste regulation (in the restricted sense). For this reason no separate discussion of caste regulation in other ants is possible. However, the little relevant information available (e.g. that on *Myrmica rubra*) is included in the section covering caste regulation in *M. pharaonis* .

In addition to the discussions of caste regulation and determination, this chapter also includes some general observations on the social organisation and life-style of *M. pharaonis* .

11.2 Caste regulation in *Monomorium pharaonis*.

If queens are present in a colony of *M. pharaonis* they generally inhibit the production of sexuals. If however queens are absent, the colony responds by rearing new queens (and males) from the brood. In this way the colony regulates the development and appearance of the two female castes (workers and queens). The present study has sought to identify the way in which this caste regulation occurs in *M. pharaonis* .

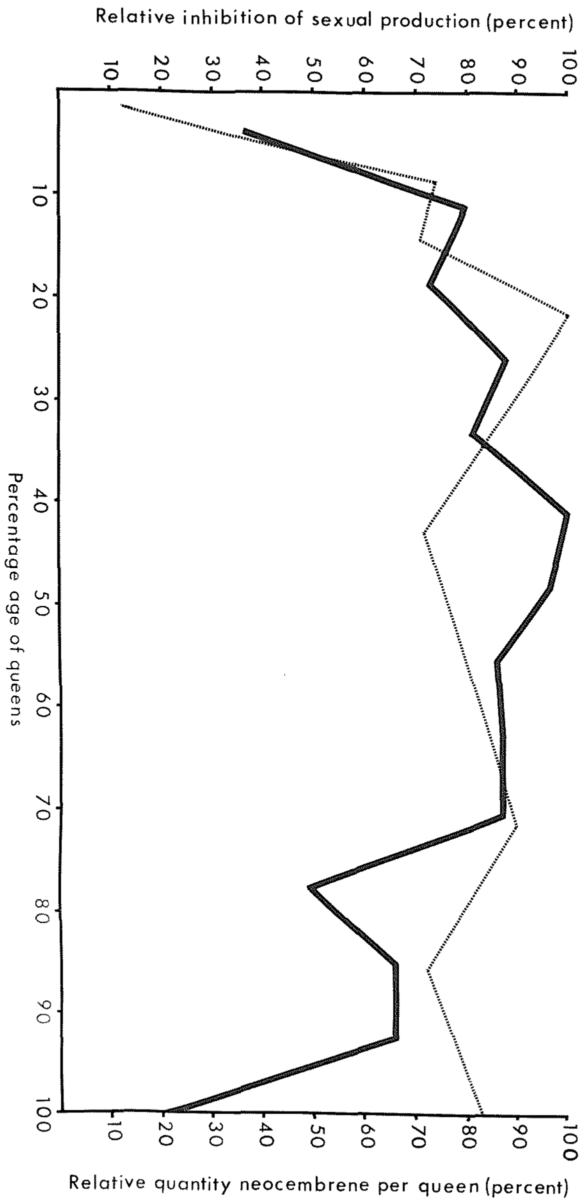
Although extant queens are usually able to inhibit the production of new queens, their ability to do so is closely associated with their physiological state. Thus, live fertile queens can inhibit sexual production, whereas neither live virgin queens (section 3.3) nor dead fertile queens (section 3.5) have this ability. Moreover, mature, fertile queens are more effective inhibitors of sexual production than newly fertilized (juvenile) queens (section 3.3). In addition, some evidence was obtained to suggest that senile queens (0-4 weeks prior to death) were also less able to inhibit the production of sexual forms than fully fertile (mature) queens (section 3.3). This indication is in agreement with the findings of PETERSEN-BRAUN (1975). Finally, the inhibitory action of fertile queens requires that there be physical contact between the queens and workers, (section 3.2) and that the queens be present in the colony for about 40% of the time (section 3.2). In retrospect, the parameters associated with the queen's ability to prevent the development of sexuals can be linked to the fecundity of queens. Thus, the relative fecundity of queens is closely linked to their ability to inhibit the production of new sexual forms (Figure 52).

However, the results of the queen-rinse experiments (section 3.6) indicated that the inhibitory effect of fertile queens might have been due to the presence of a queen inhibition pheromone analagous to the queen substance, 9-oxodec-trans-2-enoic acid, produced by queens in honeybee colonies (BUTLER *et al.*, 1962; BUTLER, 1961). The same conclusion was reached by BERNDT (1977) on the basis of similar experiments. However, in the present experiments, the mortality of rinsed queens was high and their oviposition rate appeared to be reduced (section 3.6). Thus, it is possible that the loss of inhibitory activity in rinsed queens was due to reduced fecundity (resulting from a sub-lethal toxic effect of the solvent) rather than to the removal of an inhibitory pheromone.

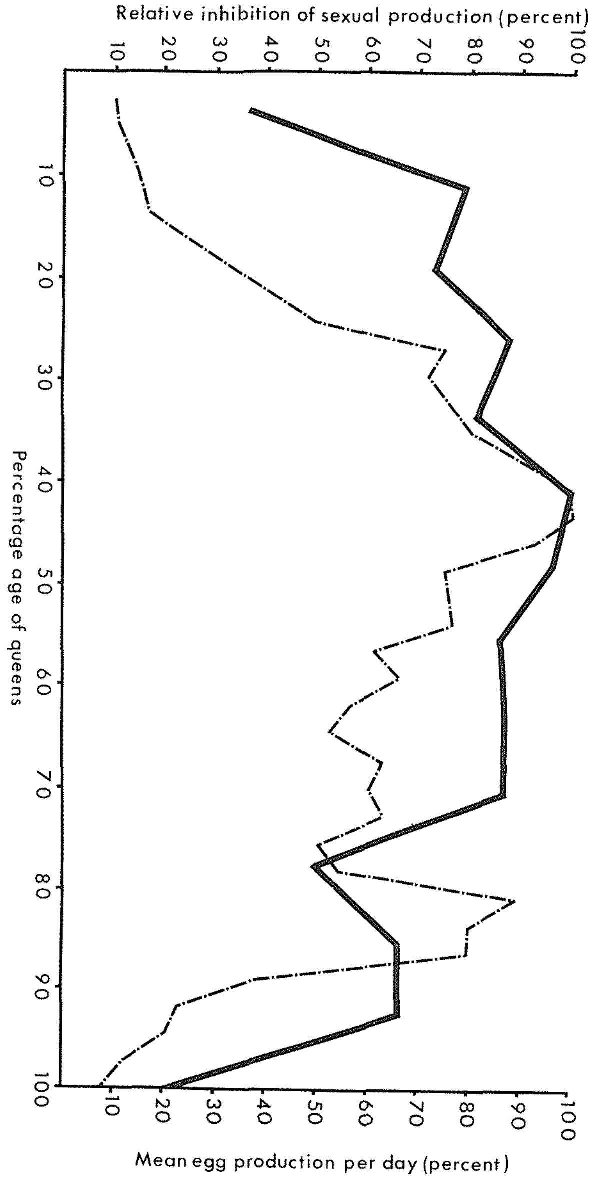
That it is the presence of eggs, rather than the presence of queens *per se* which inhibits the appearance of sexuals in queen-right colonies is confirmed by the results of the experiments with sterilized queens (section 8.2). However, the 'egg-signal' is not merely an indicator of the physical presence of queens in a nest.

Figure 52 Relationship between mean egg production/day of queens throughout their lifetime (dotted line) and their relative ability to inhibit the production of sexuals (solid line).

Figure 53 Relationship between quantity of neocembrene present in queens throughout their lifetime (dotted line) and their relaive ability to inhibit the production of sexuals (solid line).



Relative inhibition of sexual production (percent)
10
20
30
40
50
60
70
80
90
100
Percentage age of queens
10
20
30
40
50
60
70
80
90
100
Relative quantity neocembrene per queen (percent)



Relative inhibition of sexual production (percent)
10
20
30
40
50
60
70
80
90
100
Percentage age of queens
10
20
30
40
50
60
70
80
90
100
Mean egg production per day (percent)

This is because it conveys information about both the number and the fertility of the queens present in the colony. It appears that workers are able to detect changes in the numbers of eggs present in the colony, perhaps by monitoring the number of egg-contacts per unit time. Such a system would transmit to the workers information regarding the number of queens (eggs) in the nest and allow response to changes in the ratio of eggs (and therefore queens) to workers in the colony. Thus, an excessively large number of workers and a small number of queens will result in fewer egg/worker contacts, and thus to the rearing of more queens and males to redress the balance. This might explain why even the removal of a proportion of queens from a colony can lead to the production of new sexuals (PEACOCK *et al.,* 1955a).

In addition, should the number of queens remain constant, but their fertility become reduced (e.g. as they become senile),the workers will be 'informed' about the declining reproductive potential of the colony by the decline in the numbers of eggs. In relation to the social biology of *M. pharaonis,* such a system is biologically efficient, because while extant queens are maximally fecund, there is little requirement to produce more fertile females. However, the system also permits a pre-emptive response to the loss of old queens through natural mortality. Thus, as the fecundity of old queens declines through the senile phase, the colony responds by rearing new sexuals before the nest is entirely denuded of queens. This ensures continuity of maximum reproductive capacity at the end of each sexual cycle. Moreover, optimal production of new sexuals is assured because neither extant senile queens, nor newly-emerged fertile queens will impede the development and maturation of the sexual brood. Thus, the number of sexuals produced at the start of each sexual cycle will be limited only by the resources available to the colony (i.e. number of nurse workers, availability of food, etc.)

The mechanism of caste regulation in *M. pharaonis,* is ideally suited to the mode of colony dispersal and dissemination in this species. Sociotomy, or budding, involves the emigration of a portion of the established colony to a new nest site. Obviously, the numbers of workers, brood stages and queens that constitute these budding groups will vary depending on circumstances. In general, budding will occur as the colony outgrows or exhausts the resources in its home range. However, the ability to rapidly disseminate the contents of a nest would be an important attribute in emergencies (e.g. attack by predators, food shortage, flooding etc.) In such emergencies the composition of emigrating groups will be rather haphazard. However, the absence of queens in the budding group will be signalled by the absence of a continuing supply of eggs. In such circumstances workers will rear new sexuals from the existing brood. In this respect it may be significant that it takes workers between 4 and 7 days to respond to the loss of queens (section 9.1.3) but because the incubation period of the eggs is more than 7 days (PEACOCK and BAXTER, 1950), the availability of brood with potential to form sexuals is ensured. If however, fertile queens accompany the budding group, then their eggs will prevent the rearing of new sexual forms so that the colony will grow optimally by maximising the production of workers.

For such a system to operate, it is essential that in each budding group there is always the potential to rear new queens and males should the need arise. Thus, there must always be present in a colony, brood which has the potential to give rise to the adult sexual forms. In *M. pharaonis* this appears to be the case since fertile queens continually lay a proportion of eggs which are capable of developing into both queens and males (section 7.3).

The use of eggs as a signal of the presence of fertile queens necessitates that this signal be recognised by workers. Because there does not appear to be any chemical basis underlying the recognition of eggs by workers (section 8.3) it seems likely that workers recognise eggs by shape, size and texture. This also appears to be the case in relation to the recognition of, and discrimination between, other brood stages (see below).

It is difficult to see why *M. pharaonis* has evolved a system which utilizes the presence of eggs as a signal to workers of the presence of queens, rather than a system in which the presence of queens is signalled directly. Moreover, it is strange that, given that queens produce a caste-specific chemical (neocembrene), this material is not utilized as a signal of the presence of queens. Perhaps eggs, being more numerous than queens, are a more effective way of signalling reproductive potential to large numbers of workers, than is physical contact with the relatively few queens present in the nest.

Having recognised the presence of fertile queens in a nest, the workers prevent the development of new sexual forms by cannibalising sexual brood stages (section 9.1). Thus, not only can workers distinguish between different developmental stages of the worker caste, they can also recognise and distinguish between workers and sexual brood stages (section 9.1). The mechanisms of brood-recognition in ants have been studied in relatively few species. In *Myrmica rubra,* larvae are recognised by a chemical signal which is distributed evenly over the cuticle (BRIAN, 1975). In this species, neither the presence of hairs nor the proctodeal and stomodeal exudations of larvae serve to identify them to workers (BRIAN, 1975). This latter observation is relevant because larvae of *M. pharaonis* also produce gastric secretions which are consumed by workers (WUST, 1973). These secretions contain mostly amino-acids and may be the major source of these materials for workers whose raw food intake is mainly carbohydrate (BUSCHINGER and KLOFT, 1973). In *Solenopsis invicta,* workers identify their brood because the latter produce a contact pheromone (WALSH and TSCHINKEL, 1974). BIGLEY and VINSON (1975) characterised this brood pheromone as triolein, although there appears to be some doubt as to whether this material is the true brood pheromone or a contaminant from food material (M.S. BLUM, pers. comm.) In *M. pharaonis* there is no evidence that developing stages produce a brood recognition pheromone (sections 10.2 and 10.3), although this possibility cannot be excluded. However, it seems more likely in view of the workers ability to distinguish between different developmental stages, that topographical cues are more important than chemical cues for the recognition of brood. Workers of *Myrmica rubra* are also able to distinguish between different developmental stages, and BRIAN (1975) has suggested that each developmental stage may possess specific chemical recognition cues. This seems unlikely. If the recognition of brood by workers of *M. pharaonis* is accomplished on the basis of shape, size and 'feel', then these criteria might also allow discrimination between worker and sexual brood. For, not only do sexual larvae have a different shape than worker larvae (HALL and SMITH, 1953), but the former are almost hairless whereas the latter are covered in rows of bifurcate hairs (section 10.3.2). This situation is not unique among ants since out of 35 species in which both sexual and worker larvae were examined, 14 had caste-related differences in body shape and 4 had caste-specific differences in the abundance of body hairs (WHEELER and WHEELER, 1979). Moreover, SCHMIDT (1974) reported marked differences in the pilosity of worker and sexual larvae in *Formica polyctena*. Thus, there is considerable evidence that there are marked morphological differences between worker and sexual larvae in a number of ant species. Furthermore, it seems reasonable to suppose that in some of these species, these differences may enable workers to distinguish between the two types of brood.

In *M. pharaonis*, the paucity of hairs in sexual larvae may further help workers to locate such larvae and to separate them from the, more numerous, worker brood. This might occur because the hooked hairs present on worker larvae probably serve to hold these larvae together in bunches. Thus, any larva without such hairs would have a tendency to remain separate from the surrounding bunches of worker larvae.

Once sexual brood has been identified by *M. pharaonis* workers in a queenright colony, it is destroyed by cannibalism (section 9.1). This mechanism of caste regulation has parallels in other social Hymenoptera. For example, in the honeybee, *Apis mellifera*, diploid drone larvae are normally removed from the colony by cannibalism (WOYKE 1963). Furthermore, these diploid drone larvae are recognised by workers because they produce a specific chemical signal (WOYKE 1967). In the ant *Myrmica rubra,* some large, queen-biased larvae in queenright colonies are attacked and bitten by workers (BRIAN, 1973; 1974a). Again, these larvae appear to be recognised because they produce a chemical signal (BRIAN, 1970; 1973). The worker attack on these large larvae does not usually result in their destruction, but leads to their premature metamorphosis into workers (BRIAN, 1973). It is interesting in view of their widely different life styles, that both *Myrmica rubra* and *Monomorium pharaonis* utilise worker attack on developing sexual larvae as a mechanism of caste regulation. In *M. pharaonis,* the attack is more violent, and usually results in the death of the victim (section 9.1). These differences in the severity and result of the behaviour of workers towards sexual larvae appear to reflect the different mechanisms of caste determination in the two species. Thus, in *Myrmica* where caste is plastic throughout the major part of larval development and where determination occurs late in larval life, female larvae can be diverted towards the most appropriate caste even at the latest stage of larval development. In *M. pharaonis* however, where caste determination appears to be fixed at a very early stage in development, queen-determined larvae can not be 're-determined' to become workers and must therefore be destroyed. In this context it is also clear why workers attack sexual larvae of *M. pharaonis* much earlier in their development than is the case in *Myrmica.* However, in neither species is the process wasteful. In *Myrmica,* the investment of colony resources in the rearing of large larvae, which are plastic with respect to caste, is efficient because such larvae can be channelled into queens or workers as necessary. In *M. pharaonis,* the system operates differently, but the investment in producing sexual brood is not lost, but recycled whenever these stages are cannibalised.

11.3 Caste determination in ants - further considerations and the possible role of the endocrine system.

In the introduction (section 1.2) we have considered the main theories concerning the mechanism of caste determination. In essence, it appears that caste determination can occur at an early stage of development, in which case it may be determined genetically (i.e. as a result of the specific composition of the genes) or blastogenically (i.e. determined by embryonic factors like quantity of yolk in the egg). In such cases, the caste of individuals is fixed and may not be altered by environmental factors. It has been proposed that this method of caste determination be termed 'monomorphogenic', because eggs laid by queens are programmed to give rise to only one phenotypic form. Conversely, caste determination can be deferred until the larval stage of development. In such cases, plastic (bi-potent) larvae may be diverted towards either queen or worker development as a result of environmental factors (e.g. nutrition). For this reason, this type of caste determination could be termed 'polymorphogenic'. It is also possible that combinations of these mechanisms may exist in some species.

So far, when considering caste determination, discussion of the development of sub-castes (e.g. soldiers) has been avoided. Notwithstanding the absence of sub-castes in *Monomorium pharaonis,* recent progress in the understanding of the development of sub-castes in other Myrmicine species merits discussion, since as a result, some light may be thrown on the mechanisms of caste differentiation in general. Whether or not this is so will largely depend upon the 'status' of the soldier sub-caste in relation to the two main female castes (queens and workers).

WILSON (1971) states that three basic female castes are found in the ants: the worker, the soldier and the queen; and that males constitute an additional 'caste' only in the loosest sense. A true caste must exist within the same sex and must be stable during one or more instars (WILSON, 1971). Moreover, slight continuous variations in colour or size do not provide the bases for regarding such individuals as belonging to separate castes. It is interesting that WILSON (1971) regards soldiers as a distinct caste, and thus, by implication, that this caste is equivalent in status to queens and workers. However, a comparison of soldiers, workers and queens would suggest that this may not be the case.

In many species, including *M. pharaonis* (HALL and SMITH, 1951) the worker caste is monomorphic, although the mean size of workers may vary depending upon the time in the brood cycle at which they are produced (PETERSEN-BRAUN, 1975). In other species there exists a continuous size variation from very small workers to very large ones. Often they are no gross morphological differences between workers throughout the size-range. Conversely, in some species with a continuous variation in size range of workers, some large workers (soldiers) may be morphologically distinct from smaller workers. The latter are sometimes referred to as 'minor workers'. Finally, in some species, there is complete dimorphism within the worker caste, and two distinct size classes of workers exist. Again, in many cases the larger workers (soldiers) may exhibit major morphological differences from the minor workers. Typically, soldiers have disproportionally large heads and mandibles - features commensurate with their role as defenders of the colony. As a rule, the differences in morphology between small workers and soldiers are a result of disproportionate (allometric) growth of certain body parts (e.g. head and jaws). In some species these differences may extend to the relative size and utility of exocrine organs (LAW *et al.*, 1965). However, even the most morphologically distinct soldiers are fundamentally 'workers' because, unlike queens, soldiers do not possess any caste-specific organs which are absent in minor workers. Moreover, the growth patterns which lead to the formation of soliders are fundamentally the same as those which give a continuous size variation in workers. The only difference being that, in the case of soldiers, increased growth of body parts is limited to specific body areas. Therefore, it seems clear that the differences between workers and soldiers are fewer and less fundamental than the differences between workers and queens. Moreover, whereas allometric growth might easily lead to the development of unusually large heads or exocrine glands in some individuals, such growth alone would be insufficient to account for the development of caste-specific organs like wings and ocelli. For these reasons it is probably best to regard soldiers and minor workers as sub-castes of the worker caste.

Interestingly, considerable progress has been made in the understanding of the development and regulation of the soldier sub-caste in the Myrmicine genus *Pheidole*. In several species within this genus, the ratio of the number of soldiers to minor workers in a colony is self-regulating and appears to be controlled by a feedback mechanism (PASSERA, 1982). Furthermore, the results of several investigations indicate that the juvenile hormone plays an important role in the development of the soldier sub-caste. For example, in *Pheidole bicarinata* treatment of the colony with juvenile hormone increases the numbers of soldiers (WHEELER and NIJHOUT, 1981). A similar phenomenon was observed by EDWARDS *et al.* (1981) in *P. megacephala*. ONO (1982) found in *P. fervida* that the size of the corpora allata in last instar larvae was greatest in queens, intermediate in soldiers and smallest in minor workers. However, these differences probably merely reflected the different body size of each type of larva. This author also demonstrated that topical application of juvenile hormone to last instar larvae led to an increase in the proportion of larvae developing into soldiers (ONO, 1982).

Interestingly, some authors have reported that the juvenile hormone is implicated in caste determination *sensu stricto*. For example, feeding colonies of *Pheidole pallidula* with juvenile hormone-impregnated *Tenebrio* larvae leads to the development of queens (PASSERA and SUZZONI, 1978). However, the same authors reported that topical application of juvenile hormone to queens of this species, induced them to lay a greater proportion of queen-biased eggs (PASSERA and SUZZONI, 1978a). Thus the hormone-induced appearance of queens may not be a result of direct action of juvenile hormone on larval development.

In other Myrmicine genera there is also some evidence that juvenile hormone may be involved in caste determination. In *Myrmica rubra,* treatment of large larvae with juvenile hormone promotes their development into queens (BRIAN, 1974). However, because of the complexity of caste-regulating factors in this species, it is difficult to assess the implications of this result. Treatment of colonies of *Solenopsis invicta* with juvenile hormones produces a number of results: first, morphological deformities and death of developmental stages (CUPP and O'NEAL, 1973), effects similar to those found in *M. pharaonis* (EDWARDS, 1975). Second, feeding juvenile hormone to colonies of *S. invicta* reduces the egg production of queens and induces the appearance of queen pupae 20-30 days after treatment (VINSON and ROBEAU, 1974) and males (TROISI and RIDDIFORD, 1974). Similarly, in *M. pharaonis,* feeding juvenile hormone analogues does occasionally result in the appearance of sexual brood stages (EDWARDS, unpublished results). Moreover, in such treated colonies there is a marked reduction in oviposition by queens which eventually become sterile (EDWARDS, 1975). In both *Solenopsis* and *Monomorium* it is tempting to conclude that the appearance of sexual stages in juvenile hormone-treated colonies is a direct result of the action of the hormone on larvae - analagous to the effect of the hormone on the development of soldiers in *Pheidole.* However in *M. pharaonis,* a reduction in egg-production by queens will automatically lead to the rearing of sexual brood which would otherwise be destroyed. Thus, the appearance of sexual brood in *M. pharaonis* colonies treated with juvenile hormones is likely to result not from a direct effect on developing larvae, but because of the sterilizing effect on queens. The same phenomenon may be responsible for the appearance of sexuals in colonies of other species (including *Pheidole* spp) exposed to juvenile hormone. So, whereas there is considerable evidence that the differentiation of the soldier sub-caste in Myrmicine ants is directly controlled by juvenile hormone, the evidence that caste determination *per se* is similarly controlled is much less substantial.

However, since the juvenile hormone and the moulting hormone are intimately involved in the control of development, moulting and metamorphosis in all insects, it is possible that the endocrine system may act as a regulating system for caste in social insects. M.V. Brian and others have suggested that the worker caste represents a neotenic form of the adult female insect. That is, that workers can be regarded as dwarf, prematurely metamorphosed, individuals with suppressed reproductive capacity. Such precociously-formed sterile 'adultoids' can be produced in some non-social insects e.g. Hemiptera (BOWERS, 1976) and Lepidoptera (KUWANO and ETO, 1983) by treatment with anti-juvenile hormones. It is possible that the *corpora allata* of worker-determined larvae stop producing juvenile hormone relatively early in larval development, leading to a 'premature' moult to a small, underdeveloped adult. By contrast, the same glands in queen-determined larvae may persist with juvenile hormone production until later in larval development, allowing full development of female organs before metamorphosis. Such a system might explain why, in *M. pharaonis*, queen-determined larvae attain a greater size and take longer to develop than worker larvae. Moreover it is possible that, in some species, the queen-determined larvae have an extra larval instar, during which they attain full developmental maturity. Thus, in species where caste determination is genetic, the endocrine system would be pre-programmed to run for shorter or longer depending on caste. In species where caste determination is not genetically controlled, the activity of the endocrine organs could be influenced by external factors such as food, temperature and photoperiod. Certainly, in non-social insects there is ample evidence that these environmental factors can influence the activity of the endocrine system.

11.4 Caste determination in *Monormorium pharaonis*.

Several previous authors have suggested that caste in *M. pharaonis* is determined by trophogenic factors (HALL and SMITH, 1953; BUSCHINGER and KLOFT, 1973; PETERSEN-BRAUN, 1977). Therefore, in discussing the implications of the results obtained in the present study, it is appropriate to review the evidence for trophogenic determination of caste in *M. pharaonis*.

The first suggestion that caste in pharaoh's ant was determined by nutritional factors resulted from the observations that there were differences in the colour of the gut contents between sexual and worker larvae (PEACOCK and BAXTER, 1950). However, more detailed observation revealed that this difference was due to the fact that the darkening of the gut contents occurred some 5 to 6 days earlier in worker than in sexual larvae (HALL and SMITH, 1953). These authors concluded that this phenomenon was indicative of different feeding regimes for the two types of larvae and therefore, that caste was trophogenically determined. However, it is equally possible that the different timing of the darkening of the larval gut contents, simply reflects the difference in length of the developmental period of worker and sexual larvae. (PEACOCK and BAXTER, 1950). Thus, because darkening of the larval gut contents occurs at the time of formation of the meconial sac, i.e. at the end of larval development, it appears to occur sooner in worker larvae because they have a shorter larval period. HALL and SMITH (1953) were unable to distinguish any differences in the colour of the gut contents between male and queen larvae. This is interesting, since there is no doubt that in *M. pharaonis,* males are genetically determined.

Further evidence for the trophogenic nature of caste determination in *M. pharaonis* was claimed by BUSCHINGER and KLOFT (1973). Using radiolabelled food materials these authors demonstrated that adult queens were fed less glandular food for a short period immediately after fertilisation, and again later in life, when the new sexual brood was being produced. During the intervening period, when queens were maximally effective at inhibiting the development of new sexuals, they were fed, almost exclusively, with glandular food. These authors suggested that this glandular food was essential for the development of larvae into queens. Thus, only when queens were consuming little glandular food, could this material be diverted to larvae allowing these to develop into queens. However, these results can also be interpreted in another way. This is because the consumption of food by queens is likely to be closely associated with egg-production. Thus, the consumption of glandular food by queens is low when they are juvenile and senile because egg production is low at these times (section 7.3). Conversely, high intake of glandular food occurs during the period of optimum egg production. It is true that, since the rate of egg-production has a direct bearing on the regulation of sexual production in *M. pharaonis*, feeding differences associated with egg-production will be correlated with caste regulation, but this does not mean that they are caste-determining. Similarly, the association between food consumption and egg-production in queens may be reflected by the relative state of expansion of the thoracic crop (PETERSEN-BRAUN and BUSCHINGER 1975) the changes in which have also been cited as evidence for trophogenic caste-determination in *M. pharaonis*. However, expansion of the capacity of the digestive system in fully fertile queens becomes necessary for reasons unconnected with caste determination. First, the increased size of the ovaries reduces the space available for the gastric crop in fertile queens. Second, this reduction in feeding capacity must be compensated for (e.g. by dilation of the thoracic crop) if optimal egg-production is to be maintained.

In *M. pharaonis*, ERPENBECK (1981) has demonstrated that both adult queens and all larval stages receive more protein (relative to carbohydrate) than adult workers, although he does not record whether sexual larvae receive more protein than worker larvae. He also showed that colonies of *M. pharaonis* consume more protein during the periods when new sexual brood is being produced than during periods when only workers are reared. From these observations ERPENBECK (1981) concluded that it was the quantity, and not the quality, of food consumed by larvae which determined their caste. Again however, there is no direct evidence to support this conclusion and it is equally reasonable to suppose that, at a time when the colony is producing a significant proportion of large, fully developed, sexually mature individuals, its overall requirements for protein will increase. Once more, this raises the question of whether sexual larvae develop because they are fed more or 'better' food, or are such larvae fed differently because they are sexuals? These questions assume that sexual larvae are indeed fed differently compared to worker larvae. In *M. pharaonis*, there is only indirect evidence that this is the case. Moreover, chemical analysis of amino-acids and sugars in the gut contents of larvae revealed no differences between sexuals and workers (BRIAN, 1957) neither is there any detectable difference in total protein (mg/body weight) between these types of larvae (section 10.3.3). Thus, in retrospect, the evidence that caste determination in *M. pharaonis* is trophogenic and results from either quantitative or qualitative differences in larval nutrition, is hardly convincing.

PETERSEN-BRAUN, (1977) suggested that, in addition to trophogenic factors, caste determination in *M. pharaonis* was, at least in part, blastogenic (i.e. a function of some influence on the developing embryo). This conclusion was reached because she was unable to rear sexuals from eggs laid by juvenile queens (i.e. those less than four weeks old). Such queens laid eggs that were relatively large by comparison with eggs laid by fully fertile queens. Because these larger eggs developed exclusively into workers PETERSEN-BRAUN, (1977) concluded that they were blastogenically determined as worker eggs. However, the size of eggs may be associated more with their rate of production than with their potential developmental fate (CHERIX and FLETCHER 1982) and the present study has shown that sexuals can develop from eggs laid by juvenile (0-4 week old) queens (section 7.3). PETERSEN-BRAUN (1977) also reported the first batch of workers, produced from what she believed to be blastogenically-determined eggs, were distinctly smaller than workers produced later in the brood cycle. A similar phenomenon (small first-brood workers) occurs in other species, especially those in which single fertilised queens initiate new colonies in claustral nests (WILSON, 1971).

In the absence of any direct evidence that caste determination in *M. pharaonis* is either trophogenic or blastogenic we must consider the possibility that caste determination, in this species, is genetic. This possibility would mean that queens lay two (genetically distinct) types of diploid egg, and that subsequently, the larvae hatching from these eggs follow separate developmental paths leading to either queens or workers. Moreover, in a pure system of this sort, no plasticity would exist between the two developmental types and no amount of special feeding would alter the developmental pattern. That is not to say that relative under-nourishment will not reduce overall size, but it will not change a queen-determined larva into a worker or *vice versa*. It is strange that, with the exception of some species of bee (KERR, 1950) the idea that caste could be genetically controlled in social insects has received relatively little support (WILSON, 1971). This is even more surpising because it is generally accepted that sex in social insects is genetically determined (i.e. a result of haplodiploidy) and, as discussed below, there are marked similarities between the development of males and queens especially in ants like *M. pharaonis*.

Thus, in pharaoh's ant, males are haploid and are thus genetically determined. Moreover, whatever the mechanism of determination of the female castes in this species, it is clear that it occurs at an early stage in the insect's development. The evidence presented in this study strongly suggests that determination in *M. pharaonis* occurs either in the egg or during the first few days of larval life (sections 9.3 and 9.4). If determination occurs in the egg, it could be a result of genetic factors or of blastogenic factors (e.g. quantity of yolk). If determination occurs in the larval stage it could be a result of some non-genetic factor such as nutrition. However, as discussed above, feeding differences are unlikely to be responsible for caste determination in this species. Moreover, such an explanation is even less tenable in the case of very young larvae. The reason for this is that newly-hatched larvae do not appear to receive food from workers, but seem to derive most of their nourishment by feeding on unhatched eggs in their vicinity. Interestingly, this phenomenon has also been reported in *Myrmica rubra* (WEIR, 1959).

It is possible that caste determination in *M. pharaonis* is blastogenic and that, for example, queen eggs contain more yolk than worker eggs. However, despite the fact that blastogenesis is repeatedly cited as a mechanism of caste determination in the social Hymenoptera, there is little conclusive evidence that this mechanism actually occurs in any species. In *Formica polyctena,* eggs which give rise to workers and queens (winter eggs) have more RNA and a larger polar plasm than summer eggs which give rise only to workers (BIER, 1953; 1954). However, since winter eggs give rise to both queens and workers, final determination occurring during larval life (GOSSWALD and BIER, 1957), the differences recorded in the egg can not be responsible for caste determination *per se*.

In some stingless bees (*Melipona* spp) caste is genetically determined and queens are heterozygous for 2 or 3 pairs of genes; homozygosis for any one of which makes the individual develop into a worker (KERR, 1950). This mechanism illustrates just one of the many potential ways in which the composition or regulation of the genetic material present in the germ cells might determine the caste of an individual.

In *Monomorium pharaonis* there is some evidence that caste determination is controlled on a genetic basis. First, there is a marked similarity in both the pattern of development and the times of appearance of queens and (genetically determined) males (sections 9.3 and 9.4). In addition, there are considerable differences in both overall shape and pilosity between sexual and worker larvae. Moreover, these morphological differences are present from the earliest stages of larval development (section 10.3). Such differences, which are undoubtedly genetically controlled in males, are more likely to be similarly determined in queens, since it is unlikely that such differences could result simply from differences in nutrition.

Second, the appearance of adult sexuals in a queenright colony is prevented by the cannibalistic behaviour of workers towards developing sexual larvae. In the case of male-determined (haploid) brood, this process must be continuous, since male eggs are laid throughout the queens lifetime and males only appear at the times of sexual production. It is not unreasonable to suppose that this system also operates in the case of queens. Thus, a species utilizing this simple and efficient method for producing and regulating one sexual form (males) is perhaps unlikely to evolve another mechanism for regulating the other sexual form (queens). Moreover, a single mechanism would be advantageous since it is essential that the production of the two sexual forms should be synchronised.

Finally, further evidence that caste in *M. pharaonis* is genetically determined can be obtained from consideration of certain naturally occuring, morphological abnormalities. HALL and SMITH (1954) reported the sporadic appearance in *M. pharaonis* colonies of a small number of individuals which were intermediate between males and workers or between males and queens. Such individuals are termed ergatandromorphs and gynandromorphs respectively, and appear to be mosaics of male and female tissue. Since the sex of a pharaoh's ant is determined genetically, by haplodiploidy, these abnormalties most likely result from intermixing of sex-determining genetic material in the germ cells. In addition to male/female intermediates, HALL and SMITH (1953) found a small number of queen/worker intermediates (ergatogynes) in *M. pharaonis* colonies. Some of these specimens were basically worker-like in appearance but had, in addition, ocelli and/or other queen structures e.g. vestigial wings and thoracic sutures. Interestingly, the ergatandromorphs were also worker-like with additional ocelli and thoracic sutures (HALL and SMITH, 1954). It is perhaps significant that the types and patterns of abnormality seen in male-worker intermediates (ergatandromorphs) are similar to those seen in queen-worker intermediates (ergatogynes). This would suggest that both types of abormality occur as a result of the intermixing of genetic material. Moreover, in the case of ergatogynes, it seems unlikely that such abnormal morphology, encompassing the development of caste-specific organs, would result from qualitative or quantitative changes in larval nutrition. Thus, whereas it is reasonable that sub-optimal nutrition might produce smaller than average females, and that extra nutrition might produce unusually large females, it seems unlikely that these factors would lead to either the absence or presence of certain organs.

In conclusion, the evidence obtained from the present study does not allow an unequivocal interpretation of the mechanism of caste determination in *M. pharaonis*. However, it is clear that earlier suggestions that the process was trophogenic, and that either qualitative or quantitative nutritional factors determined caste, were probably inaccurate. Caste determination in *M. pharaonis* appears to occur in the egg. In this sense, determination may be either genetic or blastogenic. For a variety of reasons, discussed above, it seems most likely that some genetic pre-determination within the egg is responsible for the development of females into either queens or workers. Such a genetic control might be exerted through the programming of the activity of the endocrine system during larval development.

11.5 Some aspects of the social biology of *Momomorium pharaonis*.

Although the main aim of the present study was to examine the mechanisms of caste regulation and determination in *M. pharaonis*, the investigation has provided some additional information pertinent to the understanding of the social organisation of the species.

Pharaoh's ant has evolved a number of advanced social traits (WILSON, 1971). For example, it has abandoned a nuptial flight; it does not have territorial boundaries between colonies; it uses sociotomy as a method of dispersal and dissemination; and it has evolved, probably secondarily, polygyny. Because *M. pharaonis* has embraced these unusual aspects of social behaviour, which are not the normal pattern found in the majority of ant species, it may be regarded as a rather atypical ant. Moreover, the species appears to have adopted a rather unique system for the regulation and determination of the female castes.

Colonies of *M. pharaonis* are polygynous and usually contain several, sometimes several hundred, fertile queens. The polygynous condition is primitive in eusocial insects (WILSON, 1971) and where it occurs in more advanced genera, it is probably, secondarily evolved. The same condition may be evolving in related genera like *Myrmica* and *Solenopsis*. For example, in polygynous colonies of *Myrmica rubra*, the queens in the nest tend to avoid contact with each other, (EVESHAM, 1982) a situation which might represent the early stages in the evolution of a polygynous and polydomic society from a truly mongynous ancestor. Moreover, the discovery of a few apparently polygynous colonies in the normally monogynous species, *Solenopsis invicta* (FLETCHER *et al.*, 1980) may signal the same process occuring in fire ants.

In *M. pharaonis*, where polygyny is advanced (i.e. there are large numbers of non-aggressive queens in a nest) the condition must be particularly advantageous in relation to the life-style of the species. HOLLDOBLER and WILSON (1977) have suggested that the evolution of polygyny has pre-disposed such species to live in habitats which are isolated and irregularly distributed, but which will support substantial populations when colonized. Such conditions are, of course, exactly those present in the man-made 'tropical' environments found in domestic institutions in non-tropical regions. HOLLDOBLER and WILSON (1977) have remarked that polygyny is often associated with polydomy, and that propagules of such species are rapidly able to colonize specialised or transient habitats and encounter a potential "bonanza" in such environments. Thus, one of the advantages of polygyny is that it allows optimal colony expansion in times of unlimited resources. In monogynous species, where the queen has a finite, if large, egg-production capacity, this capacity will be the main limiting factor on colony growth. In polygynous species, the egg-laying capacity of individual queens is not limiting, because more queens can be produced if necessary. This is, of course, assuming that there are always queen-potential eggs present in the colony. Moreover, although the egg-laying capacity of each individual queen in polygynous nests may be less than that of individual queens in monogynous colonies, as is the case in *Solenopsis invicta* (FLETCHER *et al.*, 1980) the potential egg-production of the polygynous nest will always be much greater.

HOLLDOBLER and WILSON (1977) have also suggested that polygyny is an adaptation which permits the evolution of sociotomy as a method of colony dissemination. However, in *M. pharaonis*, where the viability of the budding group is not dependent on the presence of an accompanying queen, it seems that the most important mechanism ensuring successful sociotomy is the continuous presence of queen and male eggs in the colony. Other traits which have predisposed *M. pharaonis* to its particular life-style are the absence of aggression between nests, and the ability to nest in a variety of holes and crevices. These factors are both important for the evolution of a polydomic society, and have, no doubt, been important in the adoption of sociotomy as a means of dispersal. Furthermore, all these factors have equipped the species with the ability to respond quickly to changing environments. However, the use of sociotomy as the only mechanism of colony dispersal, and the abandonment of the nuptial flight has severely limited the opportunity for mixing genetic material between polydomic societies. Indeed, where colonies are geographically isolated, continuous inbreeding between successive generations of brothers and sisters, might lead to the rapid evolution of distinct subspecies. Such inbreeding could even be detrimental in the longer term. Alternatively, the acceptance of queens by all colonies could, in fact, be a result of inbreeding which has reduced intra-specific variation to such a degree that there are no longer effective mechanisms for recognising queens from other nests as 'foreign'.

Another interesting aspect of the social biology of *M. pharaonis* is that new males and queens can be produced at any time, given that the ratio of queens to workers permits their development and survival. This is an adaptation permitting sociotomy, but it is also a move away from the more usual 'seasonal' production of sexuals found in many ant species. The production of a new batch of sexuals, as an annual event, is probably more important in temperate (as opposed to tropical) regions, and most temperate species seem to have an annual brood cycle. PETERSEN-BRAUN (1975) has suggested that the endogenous brood cycle in *M. pharaonis* is 3-4 months, based on her estimate of the longevity of queens. However, the present study has revealed that the longevity of queens is nearer 12 months, (section 3.3) indicating that this species has one brood cycle per year. This might represent the vestige of an annual or 'seasonal' cycle of sexual production.

In many ant species, it appears that caste determination is not fixed at an early stage of development. In *M. pharaonis* caste determination is probably fixed in the egg. The question, in what ways would a species benefit from the early and rigid determination of caste, then arises. In ants where a nuptial flight, coupled with claustral nest foundation, is the main mode of colony dissemination, it may be important that all female eggs are plastic with regard to caste. Single fertilised queens in claustral nests can then ensure maximum production of workers from eggs laid at the founding stage of the colony. In such cases it would be inefficient to lay queen-determined eggs at a time when their survival would not increase the growth of the colony and would place severe demands on the food supply of the claustral nest at the expense of developing workers. Even if such queen-determined eggs or larvae were destroyed and recycled, their production would still detract from the maximum production of the worker caste, so important at this critical stage of colony foundation. Thus, queens in claustral nests must lay either eggs that are all worker-determined, or eggs that are plastic with regard to caste. However, where colony dispersal occurs by sociotomy, as in *M. pharaonis,* the continued presence of both queen and male eggs in the brood may be advantageous, if not essential. Thus, in the absence of a plastic caste-determining system, queens must continually produce a proportion of male and queen eggs alongside the majority of eggs which give rise to workers. Indeed, such a system may be a prerequisite for the evolution of the polydomic ant society and the ability to found nests by sociotomy.

It is likely that *Monomorium pharaonis* evolved from ancestral forms which lived by lestobiosis (entering the nest galleries of other ant species and stealing their brood). This life-style is common within the tribe Solenopsidini, and *M. pharaonis* retains the chemical defence system (diakyl pyrrolidines) utilised by several species which have retained the lestobiotic habit e.g. *Solenopsis fugax,* BLUM, *et al.* (1980); *Solenopsis molesta,* JONES, *et al.* (1979) and *Monomorium minumum,* JONES, *et al.* (1982). In a species with such a life-style, it would be important for workers to be able to distinguish between the brood of other species (prey) and its own developing stages. Thus, it might seem surprising that *M. pharaonis* does not appear to utilise a simple chemical signal (brood pheromone) as a means of recognising its brood. Instead, it seems that the workers are able to distinguish brood stages on the basis of their shape, size and 'feel'. If workers of *M. pharaonis* are able to use these criteria to distinguish between their own brood stages and those of other species, this suggests that the sense of 'feel' is well developed. Further evidence that this is so has come from the discovery that workers appear to recognise the presence of fertile queens by the presence of the eggs laid by such queens. In this sense, the species utilizes one of its own developmental stages (eggs) as a signal between two adult stages (workers and queens). This method of communicating the presence of queens, though unusual, is probably not unique to *M. pharaonis* and may be more widely used in the social Hymenoptera. For example, in *Myrmica rubra* (BRIAN and EVESHAM, 1982), the presence of eggs laid by queens changes the type of egg laid by workers from reproductive (fertile, male) eggs to trophic (food) eggs. In the ant *Plagiolepis grassei,* a social parasite of *Plagiolepis pygmaea,* workers of the former species, despite a marked gynecoidy (queen-like morphology) are unable to inhibit the egg laying of host workers, though their own queen is highly inhibitory (PASSERA, 1977). This lack of inhibition is thought to be due to the inability of *P. grassei* workers to lay eggs (PASSERA 1977). Moreover, as discussed above, juvenile hormone-induced sterility in queens of some species may lead to the production of new sexuals as a result of the removal of the 'egg signal' of the presence of queens. Thus, there is some evidence that eggs are important 'messages' in several species. Interestingly, BRIAN and EVESHAM (1982) record that, in *Myrmica rubra,* contact between workers and newly laid eggs, especially eggs as they are being laid, is the most important factor ensuring transmission of the signal. These authors also suggest that the workers respond to a combination of contact and chemical signals present on the eggs (BRIAN and EVESHAM, 1982). Perhaps the same system operates in *M. pharaonis,* and newly-laid eggs are more important as a signal of the presence of queens than older eggs. Although there is no evidence that randomly-selected (mixed-age) eggs of *M. pharaonis* contain a chemical signal (e.g. neocembrene), it is possible that such a chemical signal exists on newly-laid eggs but is removed (licked off) by workers attending the laying queen.

Queens of *M. pharaonis* produce a caste-specific chemical (neocembrene) in their Dufour's gland, which is absent in virgin queens (section 4.1) and less abundant in newly-fertilised queens than in mature fertile queens (section 5.5). The absence of neocembrene in virgin queens may simply reflect the fact that they are very young queens, and may not be associated with any physiological differences between fertile and virgin queens. The presence and relative quantity of neocembrene in queens does not appear to be associated with their ability to inhibit the production of new sexual forms (Figure 53). However, the material is highly attractive to worker ants, and it has been proposed that the chemical serves as a queen-recognition pheromone (section 6.3). Thus, the material is utilized to distinguish queens from other colony members. Moreover, because it is absent in young alate queens which are reproductively inactive, it may serve to distinguish between egg-laying queens and non-productive queens. Why then, is it important in a species which uses the presence and relative number of eggs as indicators of the presence and fecundity of queens, to have an 'additional' queen-recognition signal? The answer to this question may revolve around the nature of the information carried by each signal. The presence and number of eggs is a signal of the overall growth potential of the colony (i.e. the number and fertility of queens present in the colony). Thus, when many eggs are being produced, there is no need to increase the number of egg-producers (queens). However, the egg-signal is not an indicator of the special social status of individual queens, despite the fact that it is essential that such important individuals should be recognised and given special treatment (feeding, grooming and protection) by workers. It is for this reason that workers must be able to recognise individual queens as well as being aware of the general reproductive potential of the colony.

11.6 Summary and conclusions - caste regulation and determination in *Monomorium pharaonis* (L.)

11.6.1 Summary

i) The inhibition of the production of sexual forms by fertile queens occurs only when there is physical contact between these and workers (section 3.2).

ii) Virgin queens are unable to inhibit the production of sexual forms (section 3.3) even if their wings are artificially removed (section 3.4).

iii) The ability of fertile queens to inhibit the production of sexuals is age-related. Thus, young (juvenile) queens and, probably, old (senile) queens are less effective inhibitors of sexual production than mature queens (section 3.3).

iv) Dead fertile queens (section 3.5) and fertile queens rinsed in organic solvents (section 3.6) lose their ability to inhibit the production of sexual forms.

v) Solvent rinses of fertile queens contain a number of chemicals, one of which is absent in young alate (virgin) queens and in workers (section 4.1).

vi) Some of the chemicals in rinses of fertile queens have been identified as monomorines - compounds previously identified by other researchers (section 4.2).

vii) The fertile queen-specific compound has been identified as neocembrene : 1-isopropenyl-4,8,12-trimethylcyclotetradeca-3,7,11-triene (section 4.2).

viii) The structure of some of the exocrine glands (metathoracic glands section 5.2 and poison/Dufour's glands section 5.3) of queens has been investigated.

ix) Neocembrene is produced in the Dufour's gland of queens (section 5.4).

x) Newly-fertilised queens produce less neocembrene than older queens. Senile queens do not appear to have reduced levels of neocembrene (section 5.5).

xi) Neocembrene, when topically applied to virgin queens (section 6.1) or when fed to queenless colonies (section 6.2) does not prevent the production of sexual forms.

xii) Neocembrene is attractive to worker ants, but does not appear to be responsible for the aggregation behaviour of queens (section 6.3).

xiii) It is proposed that neocembrene may serve as the queen-recognition pheromone in *M. pharaonis* (section 6.4).

xiv) Fertile queens spend more time on or near the 'brood pile' in the nest than do virgin queens (section 7.2).

xv) Fertile queens show age-related fecundity : young queens and very old queens lay fewer eggs than mature queens (section 7.3).

xvi) The average egg production of mature fertile queens has been shown to be much greater than previously reported (section 7.3).

xvii) Throughout their lifetime, queens lay a proportion of eggs which may, in appropriate circumstances, give rise to sexual forms (section 7.3).

xviii) The inhibitory effect of fertile queens on the production of sexuals can be substituted by the artificial introduction of eggs into queenless colonies (section 8.1).

xix) Queens sterilised by exposure to an insect juvenile hormone analogue lose their ability to inhibit the production of sexuals (section 8.2).

xx) Eggs laid by queens appear to act as a signal to workers of the presence of queens in a colony and of their relative fecundity (section 8.3).

xxi) Sexual larvae introduced into queenright colonies are rapidly attacked and destroyed by workers (section 9.1.1).

xxii) Introduction of fertile queens into colonies rearing sexuals induces workers to destroy the extant sexual brood (section 9.1.2).

xxiii) In queenright colonies, the only brood stages capable of forming sexuals are eggs and very young larvae (section 9.3). This suggests that the caste of females is determined at an early stage in development, probably in the egg.

xxiv) Workers of *M. pharaonis* exhibit age-polyethism (section 10.1).

xxv) Workers appear to recognise brood by shape, size and 'feel' rather than by any specific chemical signal (section 10.2).

xxvi) There are no detectable differences in the cuticular lipids of sexual and worker pre-pupae (section 10.3.1).

xxvii) Worker larvae are covered with many bifurcate hairs whereas sexual larvae are essentially hairless (section 10.3.2).

xxviii) Workers may use these differences in pilosity to distinguish between sexual and worker larvae (section 10.3.2).

xxix) There appears to be no gross quantitative differences in the total protein content of worker and sexual larvae (section 10.3.3).

11.6.2 Conclusions : Caste regulation and determination in *Monomorium pharaonis*.

In the light of the available evidence, it is possible to propose a mechanism for the regulation and determination of the two female castes (queens and workers) in the ant *Monomorium pharaonis*.

In this species, the number of fertile queens in a colony, in relation to the number of workers and the quantity of brood, is self-regulating. That is, in the presence of an adequate number of queens, the production of new queens (and males) will be inhibited. It is not simply the presence of extant queens which prevents the development of new queens. Although individual fertile queens are recognised by workers because they produce a caste-specific chemical (neocembrene), their inhibitory effect on the production of new sexual forms is mediated *via* their rate of oviposition. Thus, only fully fertile queens produce sufficient eggs to inhibit fully the rearing of new sexual forms. Newly-emerged adult queens (either virgin or fertilised) and senile queens are less able to inhibit the production of sexuals because, in each case, their egg-production is markedly less than that of fully fertile queens.

This system is remarkably efficient since it ensures that new queens are produced (and are able to complete development) at times when such production is essential for the continued growth and survival of the colony.

It is probable that workers respond to the presence of queens because they are able to detect the numbers of eggs present in the colony. This might occur if workers were able to 'count' the number of egg-contacts per unit time. Workers do not appear to use chemical signals to recognise the presence or numbers of eggs.

In colonies of *M. pharaonis,* the behaviour of workers towards developing sexual larvae changes depending upon whether or not they detect the presence of fertile queens as signalled by the presence of eggs in the colony. When queens are present, sexual brood stages are attacked and killed before they develop into adults. In the absence of queens or in the presence of less fecund queens (i.e. reduced numbers of eggs) sexual larvae are not attacked by workers, but are reared to adulthood. It is probable that the cannibalism of sexual brood by workers in queen-right colonies occurs when this brood is at an early stage of development - probably close to the time of hatching from the egg. Workers of *M. pharaonis* are able to recognise sexual brood (larvae and pre-pupae) because these stages, unlike the corresponding worker brood, are almost hairless. There does not appear to be any chemical basis by which workers recognise either brood in general, or sexual as opposed to worker brood.

In *M. pharaonis* there is only indirect evidence as to the mechanism of caste determination. However, whatever the mechanism, it is clear that caste is determined at an early stage in development, i.e. in the egg or during the first few days of larval life. On the basis of the results obtained in this study, it is suggested that caste determination in *M. pharaonis* might well occur as a result of pre-determined genotypes. That is, that caste determination in this species is 'monomorphogenic', and queens lay haploid eggs which are genetically determined to develop into males, and two types of diploid egg - one of which develops into queens and one of which develops into workers. Whether or not the male and queen determined eggs survive to adulthood is dependent on caste-regulating factors, i.e. the way in which workers behave towards these stages as influenced by the presence of extant queens.

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