Regulation of reproductive dominance hierarchies in *Apis mellifera capensis* workers

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Contents

1. Introduction

1.1 Reproduction and regulation of reproductive dominance

The transfer of genes from one generation to the next generation is realized in various ways ranging from solitary breeding to reproduction in social groups. An extreme form of society is giving up personal reproduction in favour of helping to rear the offspring of other colony members. Species, in which the partitioning of reproduction, and additionally the overlap of generations within the society and the cooperation in broodcare are realized, are termed eusocial (Batra 1966, Michener 1974, Wilson 1971). Eusociality has been found in many Hymenoptera species like *vespinae* wasps, ants and some bees as well as in other species e.g. aphids (Aoki 1987), thrips (Crespi 1992, Crespi and Mound 1997), in shrimps (Duffy 1996), in mole rats (Jarvis et al. 1994), and in ambrosia beetles (Kent and Simpson 1992)

Due to Darwins theory of evolution the existence of non-reproductives poses an evolutionary paradoxon between sterility and natural selection (Bourke and Franks 1995). Darwin (1859) solved this problem in arguing that the natural selection in social insects acts rather on the colony than on the individual level (Moritz and Southwick 1992). About 100 years later Hamilton (1964a, b) provided his kin-selection theory which was based on genetical theories of the evolution of sociality. His theory is based on the inclusive fitness, taking the relation of cost (reduction of survival or production of own offspring) and benefit (enhancement of production or survival of offspring of another individual) to relatedness into account (detailed description in Moritz and Southwick 1992). In other words, as long as the colony members are sufficiently related to each other the existence of non-reproductives is in accordance with the theory of evolution.

In honey bees, reproduction is often considered to be channelled exclusively through the queen (Seeley 1989). The workers, usually reproductively degenerated and unable to mate, are raising the queens offspring. Nevertheless, workers are able to produce offspring as well in queenless as in queenright colonies, but here in very small number (Ratnieks 1993, Visscher 1995; 1996).

At this point, the question about the factors determining the regulation of reproduction arises.

The suppression of worker reproduction is very common in social Hymenoptera (reviewed by Brian 1979; 1980, Fletcher and Ross 1985, Hölldobler and Bartz 1985).

Within the eusocial hymenopteran species different strategies both pheromonal and behavioural for the regulation, of the reproduction exist (Bourke 1988). E.g., the halictid bee *Lasioglossum zephyrum* displays agonistic behaviour towards other nestmates (Brothers and Michener 1974). The reproduction is regulated by antagonistic hierarchies among the nestmates with the occurrence of aggressive interactions. Also the monopolization of reproduction by one individual through aggressive interactions is found in *Polistes dominulus* (Röseler 1991), but pheromonal signals seem to play a role as well (Hölldobler and Carlin 1987).

In honey bees workers are prevented from developing ovaries and egg laying through a combination of brood and queen pheromones (Free 1987, Winston 1987). Those substances produced by the mandibular glands are regulating many activities in the social organisation of the colony, but are particularly important signals for queen-worker (Kaminski et al. 1990) interactions. Mated queens have three aliphatic compounds: 9-keto-2(E)-decenoic acid (9-ODA), 9-(R/E/-(-)-keto-2(E)-decenoic acid and 9-hydroxy-2(e)-decenoic acid (9-HDA). Furthermore they have two aromatic ones, methyl p-hydroxybenzoate (HOB) and 4-hydroxy-3-methoxyphenylethanol (HVA)(Winston and Slessor 1992). This is called the queen mandibular complex (QMP). These compounds may act together as a whole, e.g. for inducing retinue response (Slessor et al. 1988), or the various components are involved in separate functions. The major component of the QMP in queens is the 9-hydroxy-2 (E)-decenoic acid (9-ODA) which acts as a primer pheromone since it inhibits queen rearing by workers (Winston et al. 1990), inhibits ovarian development of workers (Hepburn 1992), and inhibits juvenile hormone III biosynthesis in workers (Kaatz et al. 1992). 9-HDA plays an important role in regulation, movement, cohesion and stability of swarms. (Winston et al. 1982). In contrast, Seely (1985) revealed a compelling evidence that the pheromones released by the queen function not as a drug inhibiting the development of the worker's ovaries but instead as a signal indicating the presence of the queen (Seeley 1985, Keller and Nonacs 1993). Also Jay (1972) found that the ovary activation of workers is not completely blocked by the queen when there was no brood present. Especially in periods around swarming, when there is little or no brood, workers were found to activate their ovaries (Verheijen-Voogd 1959, Kropácová and Haslbachová 1970).

This might be due to the risk that the colony becomes queenless, when the old queen swarmed and the new queen might not return from her mating flight (van der Bloom 1991).

Beside chemical signals other factors prevent worker reproduction in queenright colonies.

A phenomenon called worker policing, first proposed by Starr (1984) and Seeley (1985), and more rigorously by Woyciechowski and Lomnicki (1987) and Ratnieks (1988), is said to have an influence on worker reproduction. Each worker should try to prevent other workers in her colony from reproducing, either by destroying worker laid eggs or by showing overt aggression towards workers attempting to lay eggs (Greeff 1996). The explanation is that the workers are more related to the queen sons (r=0,25) than to sons of a randomly chosen worker (r=0,125+0,25/n, where n=the number of males mated with the queen).
Furthermore, the costs involved in mutual prevention of reproduction may lead to self-policing, i.e. workers should not even try to reproduce when this might decrease colony efficiency (Liebig 1998). Nevertheless, workers might forgo personal reproduction, with its attendant cost to group efficiency, in order to maximize their inclusive fitness (Seeley, 1985), especially under queenless conditions. Thus, it can be stated that, as long as the queen and brood are present worker reproduction is exceedingly rare.

But what happens if the colony becomes queenless?
Typically laying workers appear when additional to queen loss there is no brood in the colony. As described before, orphanage could occur if the old queen dies during winter season when there is no brood for her replacement, or during summer if a young queen failed to return from the mating flight having previously killed her royal sisters (Bourke 1988).
Typically honey bees produce parthenogenetically drone offspring. The adaptive significance of the reproductive strategy is that worker produced drones are able to transfer their genes into the next generation, but the colony is inevitably doomed (Hepburn and Radloff 1998).
Nevertheless the production of drones is an obvious fitness advantage compared to colonies that do not produce laying workers (Moritz and Southwick 1992).
In *Apis mellifera capensis* the scenario of worker reproduction is different to that found in all other races. The short latency time of 4-6 days (Ruttner and Hesse 1981), the ability to produce a queen-like mandibular gland secretion and the production of female offspring make the *Apis mellifera capensis* unique for studying worker reproduction. I will return to this problem in detail after having shortly introduced the Cape honey bee.

1.2 The Cape honey bee (*Apis mellifera capensis* Esch.)

Apis mellifera capensis is the only race of *Apis mellifera* that colonized a temperate climate in the southern hemisphere (Hepburn and Radloff 1998). The Cape honey bee was described by different authors (De Geer 1778, Eschholtz 1821, Lepeletier 1836) assigning different names (Hepburn 1998). The term „*Apis mellifera capensis*“ used today is that given by Escholtz (1821) and was supported later by Ruttner (1975; 1977). Alpatov (1933; 1940) separated *A. m. capensis* from its northern neighbour, *Apis mellifera scutellata*. Various authors describe the distribution and the regions where these two races appear. *A. m. capensis* has been found the winter rain fall region of South Africa (Kerr and Portugal-Araújo 1958, Anderson 1963, Guy 1976, Ruttner 1976 a, b, c). Others have described wider distributions (Tribe 1983; Hepburn and Crewe 1990 a; b; 1991).*Capensis* is said to be specially adapted to the Cape fynbos biome (Tribe 1983, Hepburn and Jacot Guillarmod 1991) with brood rearing (Hepburn 1992) and swarming/absconding (Hepburn 1993) being tied to floral events. Hepburn and Crewe (1991) defined the geographic locations of *capensis* and *scutellata* and a hybrid zone based on the number of ovarioles and the sex ratio in the offspring of laying workers and Hepburn et al. (1998) refined these results with a morphometric data set. Due to their findings *A. m. capensis* is distributed from the western Cape to Port Elisabeth (Fig. 1.1).

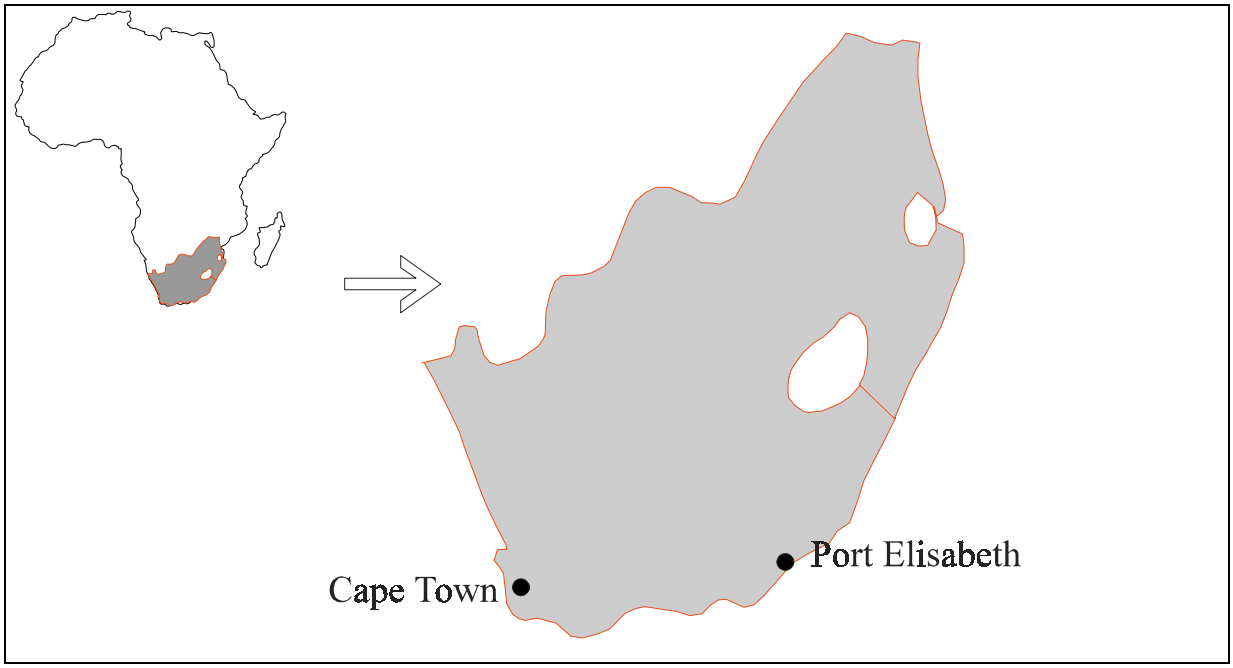

Fig. 1.1 Distribution of *A. m. capensis*

In spite of their major differences in internal morphology and biology *A. m. capensis* is nothing but a geographic variety of *Apis mellifera*. This race is able to hybridize with various

other races including those of Europe with fully vital and fertile offspring (Onions 1912, Tribe 1981; 1983, Kerr and Portugal-Araújo 1958, Kauhausen 1984, Ruttner 1988, Hepburn and Crewe 1990b).

1.2.1 Worker reproduction in *Apis mellifera capensis* Esch.

Three typical characters appear to be unique for queenless *A. m. capensis* workers. The high frequency of parthenogenetic female eggs (a process called thelytoky; see below) laid by workers following queen loss, high numbers of ovarioles (Anderson 1963), twice as much as *Apis mellifera scutellata* (Hepburn and Crewe 1991) and the production of queen-like substances in the mandibular glands (Crewe 1982). With this signal dominated by high levels of the queen substance (9-ODA [9-hydroxy-2 (E)-decenoic acid]), the workers have the ability to reproductively dominate workers of their own and of other races (Crewe and Velthuis 1980). *Capensis* workers also seem to develop their chemical signals before they lay eggs (Hepburn and Allsopp 1994). Often many eggs are deposited into one cell (Fig. 1.2) and a few larvae may simultaneously hatch, but only one will be allowed to develop to the imaginal stage (Moritz and Southwick 1992).

Fig. 1.2 Cells with eggs from laying workers of *A. m. capensis*

Production of parthenogenetic female eggs - thelytoky

Verma and Ruttner (1983) showed that the secondary oocyte fuses with a polar body in the content of the unfertilized oocyte during meiosis. This automictic mechanism was suggested by Tucker (1958) and allows no combination of loci in the offspring, unless crossing over mediates the exchange of linkage groups. Moritz and Haberl (1994) could not detect crossing over in the formation of these diploid offspring, hence all offspring of a single capensis worker are genetically identical, mother and daughter therefore form a genetic clone. Ruttner (1988) claimed that only a single recessive allele, *th*, at one locus determines workers to perform thelytokous parthenogenesis. The thelytokous parthenogenesis has been explained as an adaptation to the harsh, wet, windy conditions where the queen is much more on a risk getting lost during her mating flights (Tribe 1983, Moritz and Kauhausen 1984, Moritz 1986). But paradoxically, the highest frequencies of matings occurs in months in which the winds are most intense (Allsopp and Hepburn 1997). Referring to the high mating frequency detected in *A. m. capensis* (Estoup et al. 1994, Moritz et al. 1996, Kryger 1997) the mating risk of the queens seems not to be higher than in European races.

After queen loss in *A. m. capensis* colonies several possible events can arise (Fig. 1.3A). If the colony has young larvae a new queen can be reared (Onions 1912, Mowbray 1916). Or a queen is reared but soon replaced (Onions 1912, Gough 1928, Cooke 1981, Hepburn et al. 1988). Another possibility is the construction of queencells, which are immediately destroyed (Onions 1912, Pullinger 1922 Anderson 1963). This can be repeated several times and may end in rearing a queen from a worker laid egg (Onions 1912, Pullinger 1922, Gough 1928, Lundie 1929; 1954, Morkel 1946, Ormsby 1958, Anderson 1961; 1963; 1965). Many more possibilities arise when the colony is broodless (Fig 1.3B). The different pathways of worker development result in the production of pheromones (worker-like or queen-like), ovarian development and the production of eggs.

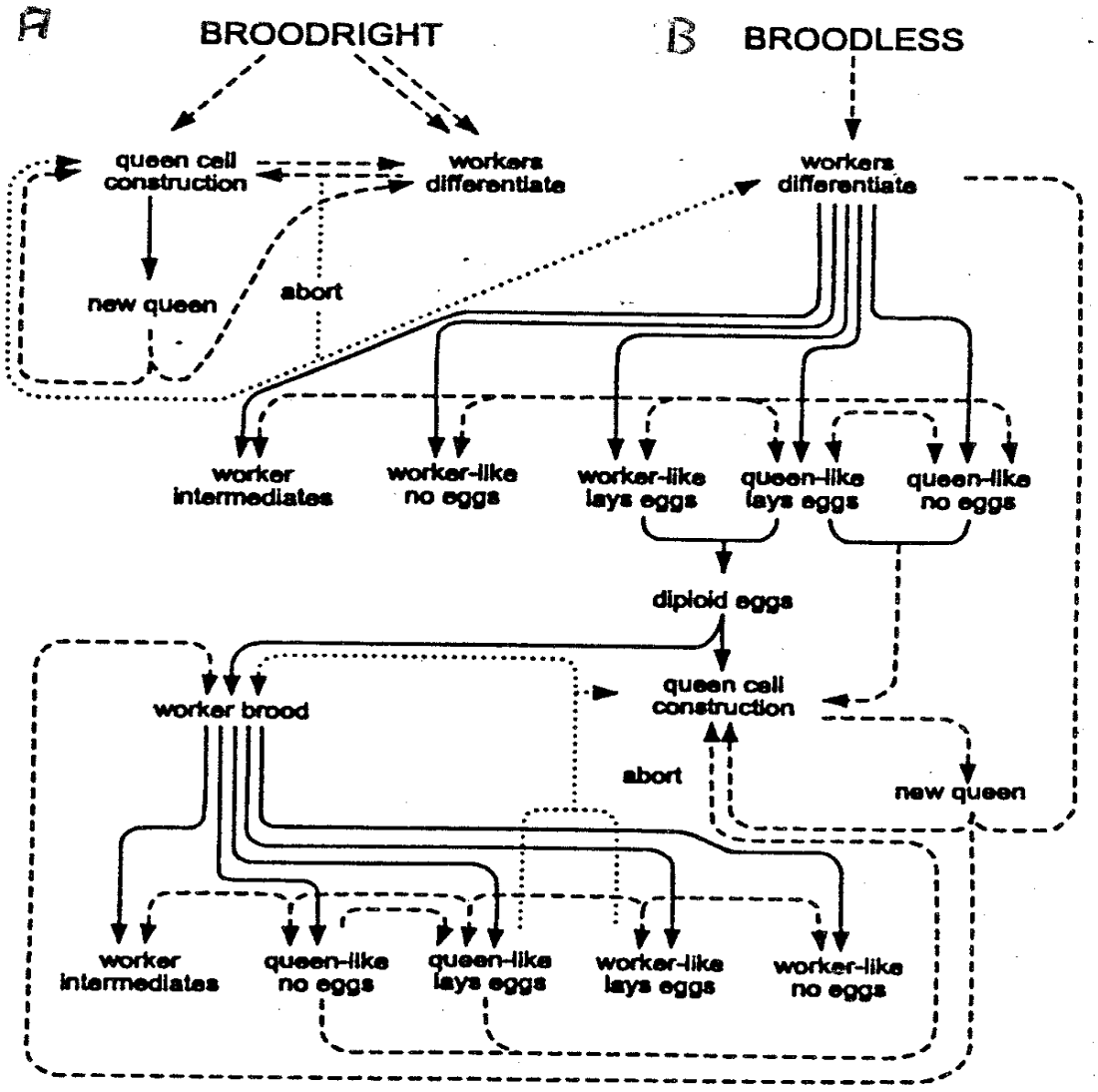

Fig. 1.3 Different pathways of *A. m. capensis* workers after queen loss. A colonies with brood, B colonies without brood. Solid lines= the step follows naturally the previous one, dashed lines= pathways of inhibitory effects following from a primary process, dottet lines= secondary effects that may lead to additional cycles (changed after Hepburn and Radloff 1998).

1.3 Reproductive dominance hierarchies in *Apis mellifera capensis* Esch. workers

Worker oviposition is clearly done by a small minority of workers (Page and Robinson 1994, Hepburn and Allsopp 1994). Workers might forgo personal reproduction, with its attendant cost to group efficiency, in order to maximize their inclusive fitness (Seeley 1985).

What forces a worker into one of the different pathways ?
In this thesis the interaction and the relevance of three factors, namely pheromones, trophallaxis, and genetics, for the regulation of reproductive dominance hierarchies are taken into account.

A. Pheromones

Under queenright conditions the mandibular gland secretion of workers is characterized by two major components 10-hydroxy-2(E)-decenoic acid (10-HDA) which is a regioisomeric form of 9-ODA, and 10-hyderoxydecanoic acid (10-HDAA) (Winston and Slessor 1992, Plettner et al. 1993). Queenless conditions and the absence of brood lead to changes in the pheromonal bouquet (Hemmling et al. 1979). *A. m. capensis* workers produce a queen-like pheromonal signal. The production of 9-ODA has also been found in other races but Cape bees produce it in much larger amounts (Crewe and Velthuis 1980, Crewe 1988, Velthuis et al. 1990, Plettner et al. 1993). Nevertheless the pheromonal composition among queenless workers can range from worker-like (dominated by 10-HDA and 10-HDAA) to queen-like signals (dominated by 9-ODA) (Fig. 1.3). Age has been shown to be an important factor, affecting pheromone and egg production (Crewe 1988, Hepburn and Radloff 1998). It is the young workers which develop into reproductives due to physiological constraints (Engels and Imperatriz-Fonseca 1990, Velthuis et al. 1990).
9-ODA forms about 75% of the total mandibular gland secretion of laying workers (Crewe and Velthuis 1980, Saiovici 1983). Like queens, workers with queen-like pheromones can suppress queencell construction and even cause queencell destruction (Hepburn et al. 1988), can lead to the abortion of queen rearing (Hepburn 1994), can inhibit the development of other workers ovaries (Hepburn et al. 1991). Generally queen mandibular pheromone seems to be associated with reproductive dominance (Hillesheim et al. 1989). Possibly every colony member produces its own mandibular gland signal, which is also transmitted to other colony members.

This could imply that the relative rate of the secretion of pheromones of a worker determines the degree to which it is able to regulate the other colony members and would lead to a higher degree of domination over the others. However, Ruttner et al. (1976) found a contrary result. Laying workers of *A. m. capensis* produce abundant 9-ODA but there was no apparent correlation between 9-ODA production and oviposition. Thus, the role of 9-ODA in the regulation of worker reproduction is not very clearly. Simple test systems are needed to evaluate its impact. Moreover, it may as well not only a single chemical component but a blend of chemical signals controlling reproduction, which has been postulated by Slessor et al. (1988) and Winston and Slessor (1998).

In **chapter 2** the ontogenetic pattern of the mandibular gland signal of 1-4 day old workers under queenless conditions is investigated. It was to evaluate if and when the mandibular gland components are developed and at what stage the signal changes from worker to queen-like. This is of major interest because it is the young workers who develop mostly into reproductives which should correspond to the mandibular gland pheromone composition. This has been done on e.g. *Apis mellifera intermissa* (Crewe and Moritz 1989). They investigated workers of different ages and found an age dependent mandibular gland pheromone production. There are data on *A. m. capensis* but of various ages (Velthuis et al.
1990, Crewe 1988).

A continuous determination of the early days of adult life of queenless *A. m. capensis* workers are, which appear to be crucial for the reproductive potential, still missing. Of further importance is the context under which the workers do produce the signal. Silverstein et al. (1966) and Velthuis (1970) found that the specifity in the blend of the different components may be highly context dependent because it can produce effects on the individual and on the colony level. Also group size has a considerable influence on the reaction of individuals to pheromones (Moritz and Bürgin 1987).

Chapter 3 covers these aspects. Worker bees were either kept isolated or in pairs of two see if there are qualitative and quantitative differences in the production of the mandibular gland pheromones. It has been respectively stated that 9-ODA was found to suppress ovary development but considerably less effectively than a queen extract (Butler et al. 1961, Velthuis and van Es 1964). 9-ODA alone has been found not to inhibit the ovarian development (Winston and Slessor 1998). However if the ovarian development is suppressed, no 9-ODA should be produced by this worker, as both of these factors co-vary (Hepburn 1992). Usually young workers are exposed to the queen pheromone from earliest stages of life in queenright colonies. My working hypothesis is that bees might „compete“ with

pheromone signals suppressing others and escaping suppression by others up to certain individual thresholds. Furthermore it is supposed that the restriction of the contact should have an influence on the production of the workers mandibular gland signal. In a queenright colony retinue bees have been claimed to function as messenger bees by contacting and contaminating other workers with the queen pheromones (Verheijen-Voogd 1959, Velthuis 1972, Seeley 1979). This way the queen pheromone is distributed among thousands of workers (Free 1978, Seeley 1979, Ferguson and Free 1980, Naumann et al. 1991).

B Trophallaxis

Trophallaxis is the transfer of food from one individual to another by oral contact, generally found in social insects (Free 1959). It is assumed to be a central cue for the evolution of sociality (Wheeler 1918; Roubaud 1916; Rüschkamp 1921). The trophallactic behaviour in social insects often reflects the hierarchical structure of individuals in the colony (Wilson 1971, Franks and Scovell 1983). In more primitive societies like *Polistes* but also in *Vespula* trophallactic interactions usually occur in an agonistic context, in which the donor is regarded as the submissive, the receiver as the dominant individual, which implies no random give and take (Pardi 1948, Montagner 1971). Workers of honey bee societies are often involved trophallactic interactions, the contacts between the individuals usually occur without overt aggression or submission, the colony has been quoted to have a „communal stomach“ (Wilson 1971, Gould 1988, Korst and Velthuis 1982). Korst and Velthuis (1982) developed a bio-assay for evaluating trophallactic dominance of bees (TD).

$$TD = \frac{\text{(asking + being offered)- (offering + being asked)}}{\text{total number of interactions}}$$

It was found that some workers were more frequently fed than others concurring with physiological dissimilarities between workers like ovarian development and rectum content (Korst and Velthuis 1982). Also workers after leaving the retinue and probably loaded with queen pheromone made food gains in their contacts with other bees (Velthuis 1972; Seeley 1979).

Under queenless conditions the welly fed dominant workers apparently mainly invest in their ovarial development, and are more likely to become laying workers than submissive ones (Korst and Velthuis, 1982). Consequently, not only the production of pheromones (see above) but also trophallactic interactions seem to play a role in the establishment of dominance

hierarchies (Wilson 1971; Franks and Scovell 1983, Korst and Velthuis 1982, Seeley 1979, Moritz and Hillesheim 1985).

Thus the purpose of a part of **chapter 5** was to monitor the trophallactic behaviour in groups of two bees to see if dominance hierarchies are established in the smallest possible group, and if, that these hierarchies concur with the production of the 9-ODA. The hypothesis is that dominance hierarchies are primary caused by the production of 9-ODA and because of the signal are favoured to be fed.

C. Genetics

The members of a eusocial monogynous colony (one queen per colony) are genetically diverse. The main reason for genetic variation among members of a colony results from the recombination of paternal and maternal chromosomes during meiosis in the queen's gametes (Moritz and Southwick 1992). Another factor is the multiple mating of the queen. A set of 10 to 17 fathers sire the colony (Adams et al. 1977) or even more like up to 50 (Estoup et al. 1994, Moritz et al. 1995, Oldroyd et al. 1995). This results in large sets of subfamilies (Laidlaw and Page 1986, Robinson and Page 1988) or patrilines (Getz 1991) which coexist in a colony. As a consequence, colony members are related to each other by various degrees (Fig. 1.4).

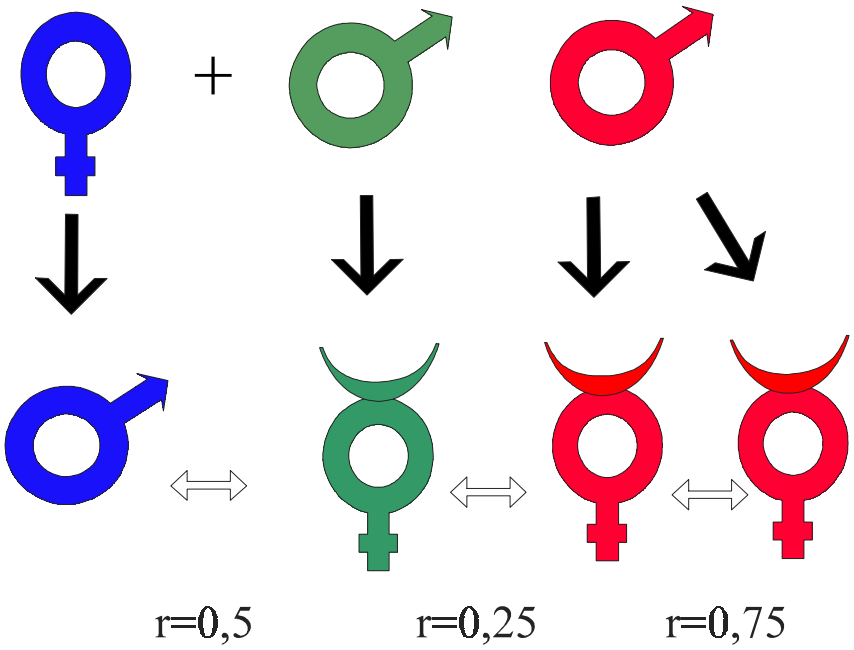

Fig. 1.4 Relationships of colony members headed by a queen mated with unrelated drones

Members of the same subfamily are called „super sisters“ (Page and Laindlaw 1988). They share both a queen mother and a drone father and, assuming mating of unrelated queens and drones, have on average 75% of their genes in common by descent. Half sisters are individuals that belong to different subfamilies as they derived from unrelated drone fathers and share in common 25% of their genes (Moritz and Southwick 1992). The genetic variability is therefore expected to be higher between the patrilines than within a patriline.

Genotypic variability also exists among individuals of a subfamiliy due to the recombination in queens. This within subfamily genotypic variability should also generate behavioural variability among individual colony members. The ability of colonies to respond to changes in environmental and social conditions, by altering the ratio of individuals, performing various tasks within a given age group, may be in part a consequence of intracolonial genetic variation in worker behaviour (Robinson and Page 1989). Several investigations in queenright colonies have shown that the genetic variability among workers influence colony division of labour in honey bees, e.g. larval care (Page et al. 1989), and allogrooming (Frumhoff and Baker 1988), egg laying in queenless colonies (Visscher 1996), oophagy, oviposition and larval care in queenless colonies (Page and Robinson 1994), egg laying in queenright colonies (Oldroyd et al. 1994), pollen versus nectar gathering (Dreller et al 1995) and trophallaxis (Hillesheim et al. 1989). Thus the genetic variance of the trophallactic behaviour and the production of 9-ODA is to be determined to see if certain subfamilies are favoured in the establishment of dominance hierarchies, carried out in small units of two bees. Moritz et al. 1996 have shown that certain subfamilies are favoured in reproduction. In this study we want to investigate if it is trophallaxis or 9-ODA which forms the basis for the hierarchy.

In **chapter 6** it should be shown if the combination of all of these factors mentioned above finally lead to reproductive hierarchies. The establishment of laying workers under queenless conditions is known to be attended by highly aggressive interactions between the workers (Velthuis 1976). This aggression is orientated toward half sister rather than to full sisters (Getz and Smith 1983; Evers and Seeley 1986). In *A. m. capensis* inclusive fitness arguments predict more conflicts between workers over reproductive dominance, especially when the colony becomes queenless (Greeff 1996).

Therefore, small units of bees were kept in boxes without any brood. The expectation is that after a certain period of time some of the workers will develop into reproductives, and that there is a relation between the production of 9-ODA, the ovarian development, the production of eggs and the affection by genetic variance.

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2. The ontogenetic pattern of mandibular gland signals in queenless worker bees (*Apis mellifera capensis* Esch.)

2.1 Abstract

Apis mellifera capensis workers are able to change their worker-like mandibular pheromone composition to a queen-like signal with high 9-ODA levels. This plays a major role in the development of reproductive hierarchies under queenless conditions. Mostly the young workers develop into reproductives thus they are expected to change their mandibular composition very quickly. The quantity and composition of the six major mandibular gland components of young workers were determined. The total amount of the six components increased with age. Also the relative quantities of changed, four day old workers were found to produce a mandibular gland signal, dominated by 9-ODA.

2.2 Introduction

Many activities of social insects are regulated by bouquets of semiochemicals. Their full chemical identities and behavioural effects are, in most instances, unknown (Free 1987, Winston 1987). Most insect semiochemicals have been proven to be complex mixtures (Silverstein and Young 1976, Hölldobler and Carlin 1987), and the honey bee (*Apis mellifera* L.) queen mandibular gland pheromone complex (QMP) is no exception (Slessor et al. 1988). The substances produced by the mandibular glands of the honey bee are components with which many activities in honey bee social organisation are controlled. In both female castes the secretions of this gland regulate a variety of key functions acting both as primer and releaser pheromones (Free 1987). Secretions of the mandibular glands provide particularly important signals for queen-drone (Gary 1962) as well as for queen-worker (Kaminski et al. 1990) interactions. Glands of mated queens contain among other three aliphatic compounds:(E)-9-keto-2-decenoic acid (9-ODA), (R,E)-(-) and (S,E)-(+)-9-hydroxy-2-decnoic acid (9-HDA). Furthermore two aromatic ones, methyl p-hydroxybenzoate (HOB) and 4-hydroxy-3-methoxyphenylethanole (HVA) (Slessor et al. 1988, Winston and Slessor 1992; 1998). This blend is a releaser pheromone that attracts nearby workers to the queen, resulting in a retinue of workers around the queen (Slessor et al. 1988) and among other effects inhibits queen rearing by workers (Winston et al. 1990, Winston and Slessor 1998).

The queen pheromone also is a primer pheromone, since it inhibits the ovarian developement of the workers (de Groot and Voogd 1954, Butler 1959, Verheijen-Voogd 1959, Butler et al. 1962, Butler and Fairy 1963, Velthuis and van Es 1964, Velthuis 1970, Hepburn et al. 1991) and, its major compound 9-ODA inhibits juvenile hormone III biosynthesis in workers (Kaatz et al. 1992). The components 9-HDA and 9-ODA play a role in stabilizing swarm clusters and attraction of drones (Winston et al. 1991). Queen mandibular gland secretions play important roles in regulation the movement, cohesion and stability of swarm, 9-ODA is a powerful sex pheromone that readily attract drones. Under queenright conditions the mandibular gland secretion of workers is characterized by two major components, 10-hydroxy-2(E)-decenoic acid (10-HDA) which is a regioisomeric form of 9-ODA and 10-hyderoxydecanoic acid (10-HDAA) (Winston and Slessor 1992, Plettner et al. 1993). When a worker emerges, the development of her glandular system is extremly dynamic. The complex pattern effects the changes in the bees behaviour over her life time, related to the tasks she is performing (Lindauer 1952, Sakagami 1953, Free 1965, Wilson 1971, Michener 1974, Seeley 1982, Robinson and Page 1989). The mandibular secretion of *Apis mellifera* workers appear to be involved in food preservation and larval nutrition. The hydroxy acids and the corresponding diacids are found in royal jelly (Weaver et al. 1968), where they may act as antiseptics (Blum et al. 1959). 10-HDA inhibits the germination of pollen, that is important for pollen storage (Winston 1987), it is an important larval nutrient that prevents larvae from pupating precociously and is most abundant in workers of foraging age (Plettner et al. 1997). Generally the amount of volatiles per gland was found to increase with age in queens (Engels et al. 1997) as well as in workers (Crewe and Moritz 1989).Queenless conditions and the absence of brood lead to changes in the pheromonal bouquet (Hemmling et al. 1979).

Especially in queenless workers of *A. m. capensis* the development of a pheromonal bouquet with more queen-like characters (Crewe and Velthuis 1980) plays an important role because it is known to be related to the reproductive stage (Moritz and Hillesheim 1985, Hillesheim et al. 1989, Hepburn 1992, see chapter 6). Also workers of other races are able to produce a queen-like signal (Plettner et al. 1993, Plettner et al. 1997). However *A. m. capensis* workers produce it much faster which corresponds to the short latency period of 4 to 6 days (Ruttner and Hesse 1981) starting with queen loss to oviposition of workers in *A. m. capensis*.

The aim of this study is to reveal the development and the change of the major mandibular gland components and their composition of young workers.

Mainly the young workers develop into reproductives (Velthuis et al. 1990), therefore the key for dominance hierarchies may lay in the first days after emergence. Especially these workers ought to be able changing their signal from worker to queen-like very quick due to the reasons described above.

2.3 Materials and Methods

Freshly emerged worker bees were sampled from a brood frame kept in an incubator (34°C, 60% rel. humidity). Four boxes, each with 20 bees were kept in an incubator (34°C, 60% rel. humidity) with honey and water ad libitum. They bees decapitated after one, two, three or four days. The head was given into a vial containing dichloromethane.

Gas chromatography

The head was removed from the dichloromethane, which was evaporated with N_2 just to dryness. The residue was redissolved with an 20µl internal standard (± 1mg of each octanoic acid and tetradecane in 4ml dichloromethane) and 20µl BSTFA (bis-trimethylsilyl)trifluoroacet-amid). 1µl of this solvent was injected into a gas chromatograph (Hewlett Packard 5890) fitted with a split-splitless inlet and a 25mX0.32mm methyl silicone coated fused silica capillary column. The carrier gas was hydrogen with a flow rate of 1ml/min, the oven temperature was as follows; 60°C for 1min, then heated at 50°C/min to 110°C, then 3°C/min from 110°C to 220°C and held at 220°C for 10 min. Chromatograms were recorded and peak area quantified using HP ChemStation software.

The relative mass ratio ([R.M.R.], Gehrke and Leimer 1971) of the components of interest (see below) were measured relative to tetradecane and the absolute amount determined. An internal standard solution was run every second day to ensure that RMR's were within the limit of variability found in the series of standard runs (Crewe and Moritz 1989).

Six of the mandibular gland components were determined.

1) methyl p-hydroxy benzoate (HOB)
2) 4 hydroxy-3-methoxy phenylethanol (HVA)
3) 9-keto-2(E)-decenoic acid (9- ODA)
4) 9-hydroxy-2-(E)-decenoic acid (9-HDA)
5) 10-hydroxydecanoic acid (10-HDAA)
6) 10-hydroxy2(E)-decenoic acid (10-HDA)

2.4 Results

The fatty acids in the mandibular gland extracts of the workers of different ages showed qualitative and quantitative differences (Fig. 2.1 A-D and Tab.1.1).

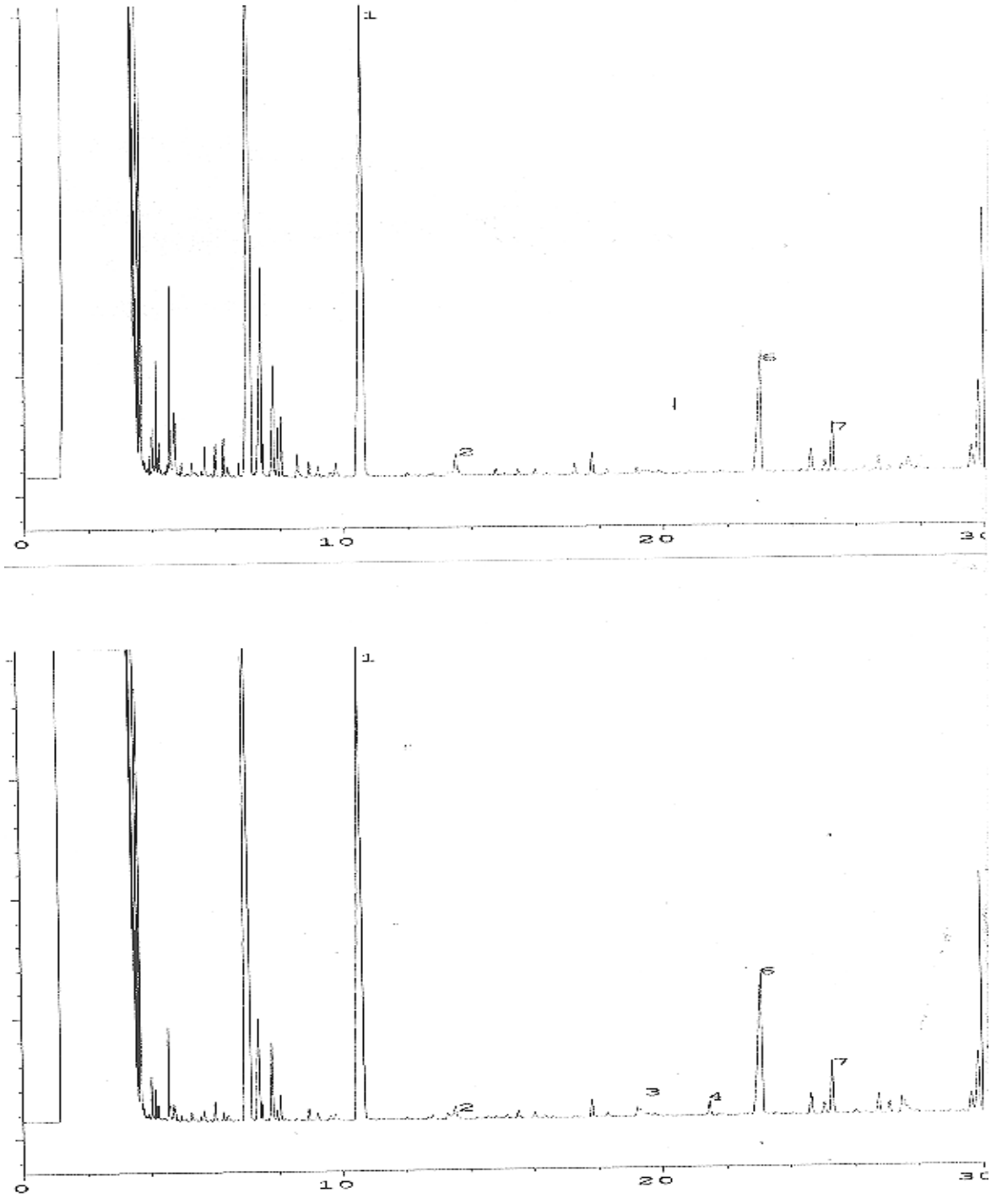

Fig. 2.1 A-B Typical gas chromatograms of dichloromethane extracts of mandibular glands of *A. m. capensis* workers one (A), two (B) days old. 1= Tetradecane, 2= HOB, 3= 9-ODA, 4= HVA, 5= 9-HDA, 6= 10-HDA, 7= 10HDAA

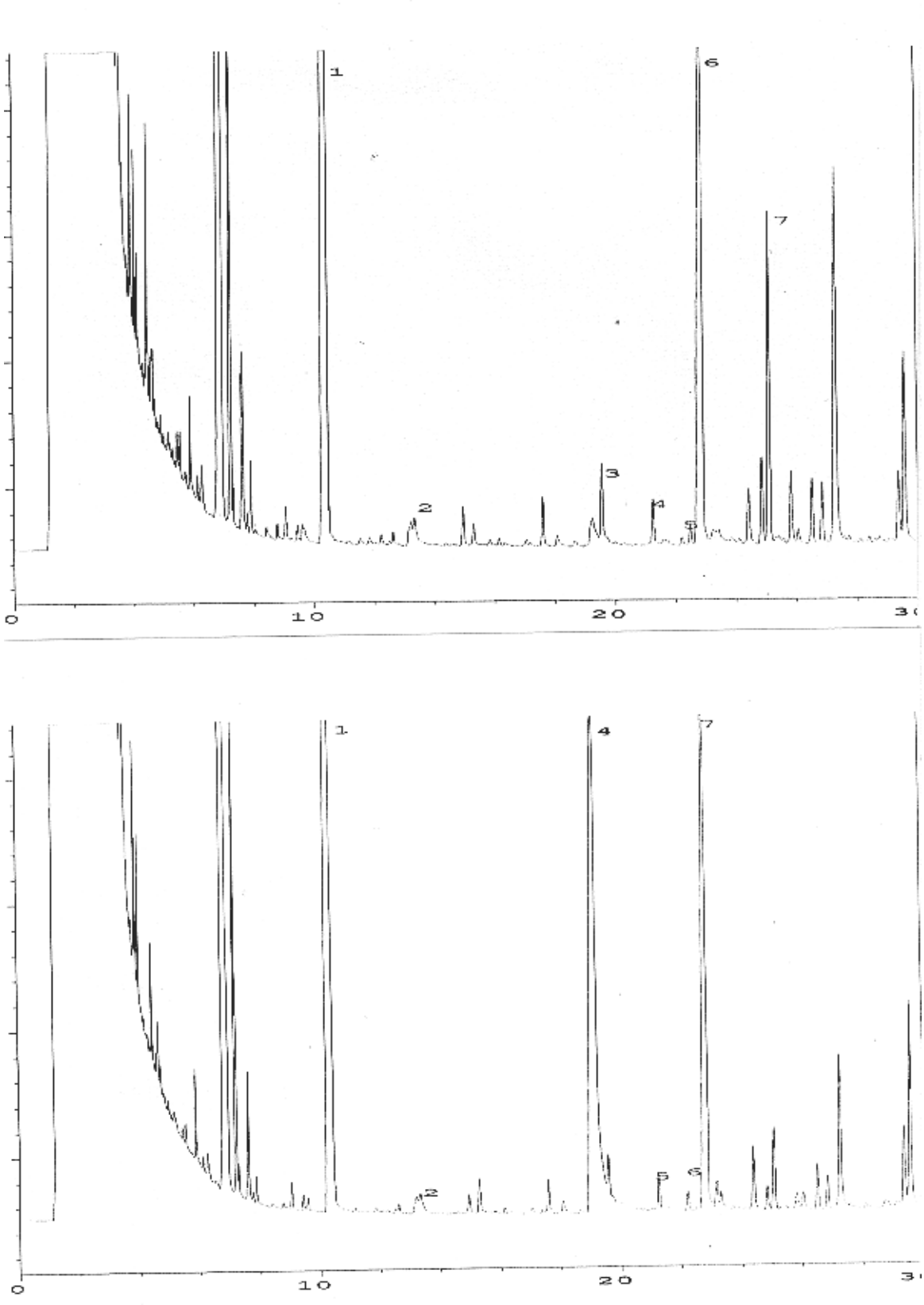

Fig. 2.1 D-C Typical gas chromatograms of dichloromethane extracts of mandibular glands of *A. m. capensis* workers three (C) and four (D) days old. 1= Tetradecane, 2= HOB, 3= 9-ODA, 4= HVA, 5= 9-HDA, 6= 10-HDA, 7= 10HDAA

The total amount of all components increased about the order of magnitude ten times from day one to day four. The large standard deviations indicate that the composition of the individual extracts were highly variable.

	day 1 N=23	day 2 N=22	day 3 N=20	day 4 N=18
substance	Mean (µg)	Mean (µg)	Mean (µg)	Mean (µg)
HOB	0,13±0,26	0,06±0,12	0,13±0,14	0,56±1,66
9-ODA	0,18±0,65	0,22±0,71	2,02±4,02	8,01±6,8
HVA	0,04±0,15	0,05±0,09	0,07±0,08	0,24±0,41
9-HDA	0	0	0,03±0,04	0,47±0,59
10-HDAA	1,38±1,50	1,62±0,93	2,42±1,91	6,47±3,12
10-HDA	0,76±1,22	0,46±0,38	0,50±0,35	7,90±15,92
total	2,49±3,28	2,41±1,53	5,16±4,95	23,65±21,36

Tab. 2.1 Mandibular gland extracts composition of *A. m. capensis* workers of ages one to four days. HOB=methyl p-hydroxy benzoate, HVA= 4 hydroxy-l-methoxy phenylethanol, ODA= 9-keto-2(E)-decenoic acid, 9-HDA= 9-hydroxy-2-(E)-decenoic acid, 10HDAA= 10-hydroxydecanoic acid, 10HDA= 10-hydroxy-2(E)-decenoic acid

The percentage of each component was determined for every age. The extracts from the one day old workers was dominated by 10-HDAA (62,7±23.3%), 10-HDA (27,0±18,0%) and ODA (6,7±22,49). The two old day bees showed a very similar fatty acid composition. Mandibular gland extracts of three days old queenless workers were still dominated by 10-HDAA (57,18±22,48%) followed by ODA(23,55±26,06%) and 9-HDA. Four day old worker extracts were dominated by 9-ODA (45,14±17,16%) and 10-HDAA (40,13±15,56%). The amount of ODA and 9-HDA increased with age whereas the amounts of HOB, 10-HDAA and 10-HDA decreased with age (Fig. 2.3). Between the workers one and two day old and between those three and four days old were no significant differences in the produced amounts. The significantly different components between the other days are presented in table 2.2.

day	1	2
3	10-HDA p<0,05	
4	9-ODA p<0,001 9-HDA p<0,001 10-HDAA p<0,05 10-HDA p<0,05	ODA p<0,001 9-HDA p<0,001 10-HDAA p<0,05

Tab. 2.2 The pheromone components that differed significantly between the one and two to three and four old day workers (Tukey test for unequal sample size).

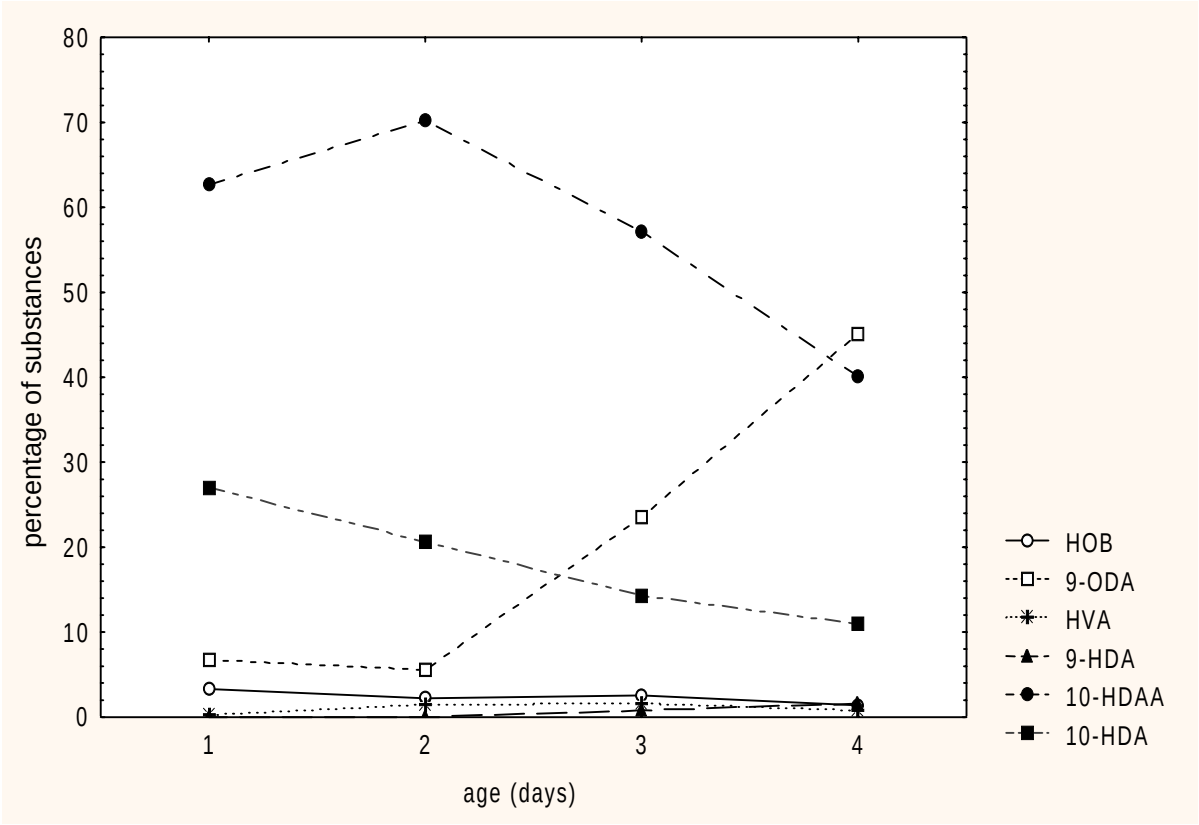

Fig. 2.3 Age dependent amount (%) of the six different mandibular components of *A. m. capensis workers*.

2.5 Discussion

Our data clearly show that the mandibular secretions of queenless *A. m. capensis* workers change with age. Similar studies on other *Apis* races revealed that the pattern of the mandibular gland secretions also changes during imaginal lifetime (Crewe and Moritz 1989, Engels et al. 1997). But the production of the different components is depending on the race (Crewe 1988, Plettner et al. 1993, Plettner et al. 1997). In a study on *Apis mellifera intermissa*, the ontogenetic pattern of the mandibular products showed that these are also able to produce the queen substance 9-ODA (Tab. 2.3). It is also present in four day old workers and is present in relative large quantities at eight days. Even 21 days old workers the 9-ODA content is still only 1,13±0,09µg, which is eight times less than the content in four day old *capensis* workers. *Intermissa* workers produced 9-HDA from the first day whereas this particular component was present in *capensis* bees from day three. The *capensis* workers produced much larger amounts of 10-HDAA and 10-HDA from the beginning of their lives. C*apensis* workers have less 9-HDA, more 10-HDAA and more 10-HDA which is not present et all in four day old *intermissa* workers.

	day 1	**day 2**	**day 3**	**day 4**	day 1	day 2	day 4
substance	*A.m.c N=23 (µg)*	*A.m.c. N=22 (µg)*	*A.m.c. N=20 (µg)*	*A.m.c. N=18 (µg)*	A.m.i* N=8 (µg)	A.m.i* N=8 (µg)	A.m.i* N=8 (µg)
HOB	*0,13±0,26*	*0,06±0,12*	*0,13±0,14*	*0,56±1,66*	n.a	n.a	n.a
9-ODA	*0,18±0,65*	*0,22±0,71*	*2,02±4,02*	*8,01±6,8*	0	0	0,16+0,43
HVA	*0,04±0,15*	*0,05±0,09*	*0,07±0,08*	*0,24±0,41*	n.a	n.a	n.a
9-HDA	*0*	*0*	*0,03±0,04*	*0,47±0,59*	0,45±0,24	0,53±0,39	1,9+2,8
10-HDAA	*1,38±1,50*	*1,62±0,93*	*2,42±1,91*	*6,47±3,12*	0,16±2,1	0,33±0,44	3,01+6,9
10-HDA	*0,76±1,22*	*0,46±0,38*	*0,50±0,35*	*7,90±15,92*	0,14±0,17	0,06±0,21	0

Tab. 2.3 Six analyzed mandibular gland components of *A. m. capensis* **(A.m.c)** and *Apis mellifera intermissa* (A.m.i.*) by Moritz and Crewe 1989, n.a.= not analysed, mean and SE (µg)

The mandibular gland composition of the four day old workers coincide with the amounts found in *A. m. capensis* workers with partially developed ovaries (Crewe and Velthuis 1980, see chapter 3). Plettner et al. (1993) investigated different workers of the „North American“ honey bee. The mandibular content of the four day old *capensis* workers shows a partly

different mandibular gland composition in comparison to all of the workers tested by Plettner et al. (1993). Although the amount of 9-ODA (8,01±6,8) of the four day old *capensis* workers is similar to that found in the false queens of the North American race (5,63±1,3). But the 9-HDA, 10-HDA and 10-HDAA amounts are totally different. The hydroxy acids of four day old *capensis* bees are more comparable to that of laying workers of the North American race.

In queenright colonies the composition of the mandibular gland content coincides with the task the workers perform and with age (Lindauer 1952, Sakagami 1953, Free 1965, Wilson 1971, Michener 1974, Seeley 1982, Robinson and Page 1989). During the first days of her life the workers create primary as cell cleaners (Seeley 1982). After about 2 days they functions as nurse bees feeding the brood (Seely 1982). As the hydroxy acids are found in royal jelly, the nurse bees are expected to produce these in their mandibular glands (Plettner et al. 1997). This seems also to be the case for three days old queenless workers of *A. m. capensis*. Their mandibular content is still dominated by 10-HDAA (57,18±22,48%). But these queenless workers produces significant more 9-ODA (8,01±6,8µg) than queenright workers (1,59±2,52) (Simon unpublished data).

The switch from a worker-like signal to a more queen-like signal with large amounts of 9-ODA (45,14±17,16%) takes place on day four. Nevertheless the so called worker substances 10-HDAA and 10-HDA are also present in large amounts. The *capensis* workers might still produce large amounts of 10-HDAA to mimic a „normal“ worker and to get the chance of developing the ovaries without being detected. This was reported by Plettner et al. (1997) and Crewe (1982) for virgin queens. This chemical camouflage may enable the prospective laying workers to minimize aggression by other workers until their ovaries are fully developed. The time to develop into a reproductive worker is very short in capensis bees. From the beginning of queenlessness to the establishment of laying workers takes only 4-6 days in *capensis* bees (Ruttner and Hesse 1981). During this period the subfertile nurse bees (Velthuis et al. 1990) should develop into a worker with sufficiently developed ovaries to lay eggs and produce consequently a pheromonal signal which is strong enough to dominate nestmates, which has been shown by Hillesheim et al. (1989).

In summary our results indicate that quality and quantity of the production and composition of mandibular gland pheromones of queenless worker bees depend on their developmental program and on race.

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3. Social context and fatty acid signal production in honey bees (*Apis mellifera capensis* Esch.)

3.1 Abstract

The social context of a group seems to be a general factor, affecting not only behavioural but also various physiological conditions in honeybees. Using gas chromatography the content of three main „worker“ and „queen“ mandibular gland components in isolated and pair wise kept bees were determined. The paired bees had three times higher amounts in their mandibular glands than the isolated bees. Also the secretion composition between pairs and isolated differed. Paired bees produce more mandibular secretions with more queen-like signal. Thus there seemed to be a social effect on the production and composition of the mandibular gland components. Groups of two bees provide a sufficient social context to achieve mandibular gland pheromone levels similar to that of bigger groups.

3.2 Introduction

The social cohesiveness in eusocial insect societies is often maintained primarily through the utilisation of pheromones, which transmit information among colony members (Naumann et. al. 1991). In honey bees a suite of pheromones has been identified to be essential for stabilisation, homeostasis, and integration in everyday functioning of the colony.

Numerous glands, mainly of abdominal origin have been identified to produce a wide variety of pheromones (Michener 1974, Wilson 1971, Free 1987, Winston 1987). The mandibular glands are developed in both queens and workers and are used for several key functions.

The secretion of these glands is composed of several fatty acids with a highly caste specific composition. These compounds may act together as a whole like the queen mandibular complex (QMP) inducing retinue response (Slessor et al. 1988), or the various components are involved in separate functions. The major component of the QMP in queens is the (E)-9-keto-2-decenoic acid (9-ODA) which acts as a primer pheromone since it inhibits queen rearing by workers (Winston et al. 1990). It inhibits ovarian development (Hepburn 1992), and juvenile hormone III biosynthesis in workers (Kaatz et al. 1992). The mandibular gland secretion from workers is characterized by two major components 10-hydroxy-2(E)-decenoic

acid (10-HDA) which is a regioisomeric form of 9-ODA and 10-hydroxydecanoic acid (10-HDAA) (Plettner et al. 1993).

Both workers and queens are competent to produce the other caste's dominant hydroxylated compound, as both isomers can be detected in queens and workers (Naumann et al. 1991, Plettner et a. 1997).

A special situation appears in queenless *Apis. mellifera capensis* colonies. These workers are able to produce a pheromonal bouquet that is very similar to that of the queen (Crewe and Velthuis 1980). Queenless workers change their typical worker signal dominated by 10-HDAA to a queen-like signal with high 9-ODA content within few days (see chapter 2). Also non African honey bees have been shown to produce 9-ODA but less quantity than in *capensis* bees (Crewe and Velthuis 1980, Crewe 1988, Velthuis et al 1990, Plettner et al. 1993).

Like queens, workers with queen-like pheromones can suppress queencell construction and even cause queencell destruction (Hepburn et al. 1988), can lead to the abortion of queen rearing (Hepburn 1994), can inhibit the development of other workers ovaries (Hepburn et al. 1991), and the queen mandibular pheromone seems to be associated with reproductive dominance (Hillesheim et al. 1989). The specificity in the blend of the different components can produce effects on the individual and on the colony level, and thus may be highly context dependent (Silverstein et al. 1966, Velthuis 1970). Also group size has a considerable influence on the reaction of each individual in the group. Moritz and Bürgin (1987) showed that isolated honey bee workers had weak responses to the alarm pheromones whereas the reaction per individual rose with increasing group size.

Different studies have revealed the pheromone production of *A. m. capensis* workers under various conditions and different group sizes (Crewe and Velthuis 1980, Crewe 1982, Hepburn et al. 1988). The impact of the social context for the mandibular gland secretions has not been tested. Here we studied the production of three mandibular gland component, the two worker substances (10-HDAA, 10-HDA) and the „queen substance" 9-ODA in socially deprived isolated and grouped bees.

3.3 Materials and Methods

Worker bees of *A. m. capensis* were obtained from a colony in Stellenbosch, South Africa. A brood comb was kept in an incubator (35°C, 60% rel. humidity), and freshly emerged bees were sampled. The bees were either housed in groups of two or were kept isolated in a petridish and provided with water and honey ad libitum.
The petridishes were placed in the incubator (35°C and 60% rel. humidity). After four days the workers were decapitated and the head was transferred into dichloromethane.

Gas chromatography

The head was removed from the dichloromethane, which was evaporated with N_2 just to dryness. The residue was redissolved in 20µl internal standard (±1mg of each octanoic acid and tetradecane in 1ml dichloromethane) and 20µl BSTFA (bis-trimethylsilyl)trifluoroacet-amid).
1µl of this solvent was injected into a gas chromatograph (Hewlett Packard 5890) fitted with a split-splitless inlet and a 25mX0.32mm methyl silicone coated fused silica capillary column.. The carrier gas was hydrogen with a flow rate of 1ml/min, the oven temperature was as follows; 60°C for 1 min, then heated at 50°C/min to 110°C, then 3°C/min from 110°C to 220°C and held at 220°C for 10min. . Chromatograms were recorded and peak area quantified using HP ChemStation software.
The relative mass ratio ([R.M.R.], Gehrke; Leimer, 1971)) of 9-ODA, 10-HDAA and 10-HDA in each of the samples was measured relative to tetradecane and the absolute amount determined.
A mandibular gland standard solution was composed out of ±1mg of tetradecane, octanoic acid, decanoic acid, methyl p-hydrobenzoic acid, p-hydroxybenzoic acid, 2-(3-methoxy-4-hydroxyphenyl)ethanol, (E)-9-ketodecanoic acid, and 10-hydroxydecanoic acid dissoluted in 4ml dichloromethane and revealed the retention times for 9-ODA and 10-HDAA. Since a standard solution of 10-HDA was missing the retention times were calculated to information from Robin Crewe (pers. communication). The internal standard solution was run every second day to ensure that RMR's were within the limit of variability found in the series of standard runs (Crewe and Moritz 1989).

3.4 Results

The main mandibular gland components were identical for both isolated and paired bees but there were significant differences in the produced amount (Tab. 3.1, Fig. 3.1).

substance	isolated bees N=40	paired bees N=37
9-ODA	0,81±0,30	9,29±1,19
10-HDAA	5,10±0,60	6,54±0,73
10-HDA	1,37±0,73	9,36±6,47

Tab. 3.1 The amounts of the three determined mandibular gland products (µg), mean ±SE

The average amount of the three analysed components 7,28±1,26µg in isolated bees and 25,18±6,67µg in pair bees. It is not only the amount of the components which differed but also their composition (Fig. 3.1). In pair bees 9-ODA was the most dominant compound (44,21±3,35%), whereas in isolated bees the worker substance 10-HDAA dominated the secretion (80,82±3,32%). The percentage of 9-ODA and 10-HDAA differed significantly between the isolated and the pair bees (Mann-Whitney U test: ODA U=91, N1=40, N2=37, $p<0,01$; 10-HDAA U=121, N1=40, N2=37, $p<0,01$), whereas no significant difference was estimated for 10-HDAA.

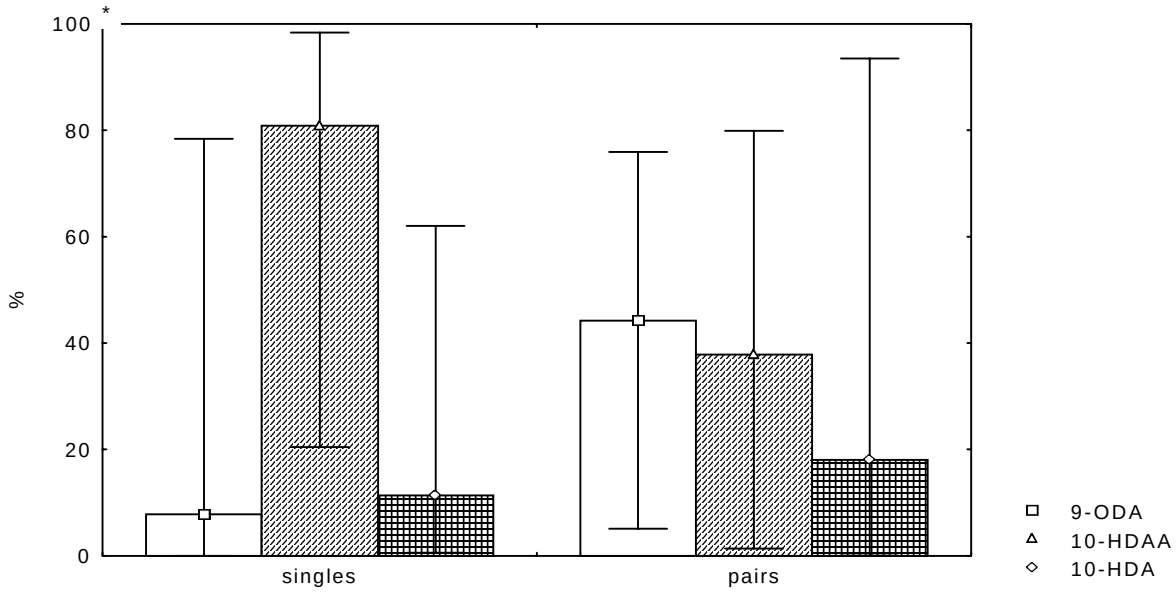

Fig. 3.1 Composition of the three analysed mandibular gland components 9-ODA, 10-HDA, 10-HDAA. The percentage of 9-ODA and 10-HDAA differed significantly between the isolated and the in pairs kept bees. *100%= 7,28µg for singles, 25,18µg for pairs.

3.5 Discussion

Queen and worker bees of *A. m. capensis* produce a caste specific blend in the mandibular glands (Fig. 3.2)

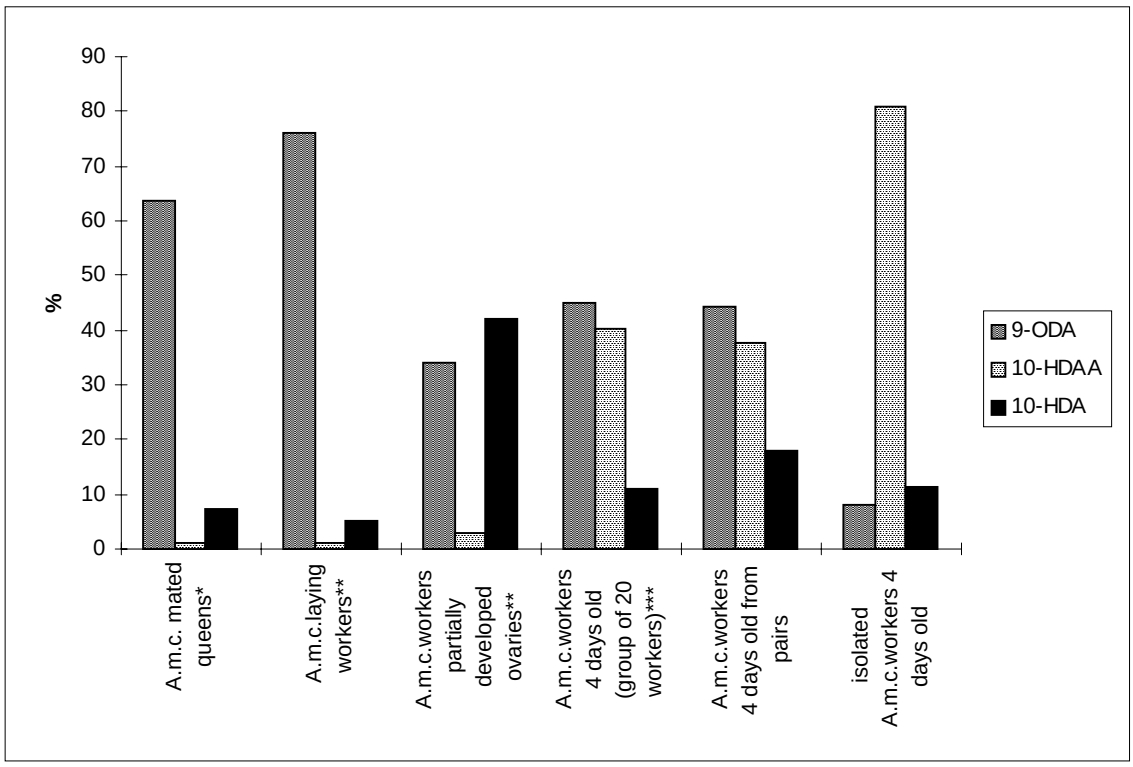

Fig.3.2 The composition of 9-ODA, 10-HDA and 10-HDAA of different *A. m. capensis* (A.m.c.) worker and queen types. *Crewe 1982, Hepburn et al. 1988, **Crewe and Velthuis 1980, *** see chapter 2

The mandibular glands of mated queens contains the most 9-ODA and only slight amounts of 10-HDAA and 10-HDA (Crewe 1982, Hepburn et al. 1988). Laying workers have a very similar composition of the components (Crewe and Velthuis 1980), whereas workers with partially developed ovaries have a composition which is dominated by the 10-HDA and 10-HDAA (Crewe and Velthuis 1980). We found that the percentage of 9-ODA of the paired bees corresponds to the percentage present in laying workers. The 9-ODA content (9,29µg) is half of that found in laying workers (16,72µg). Thus, the amount present in four day old paired bees was more similar to that found in workers with partially developed ovaries (7,48µg). But if the sum of the as well percentage and amounts of hydroxy acids of the pair bees are taken into account it can be seen that the secretion was dominated by them, which

also corresponded more to that of a worker with partially developed ovaries despite the fact that the relation between 10-HDAA and 10-HDA was different.

The amount of 10-HDAA was more than 10 times higher in the pair wise kept workers whereas the amount of 10-HDA was 2,5 times less than in the workers with partially developed ovaries.

The bees kept in pairs show a similar gland composition to that of workers kept in groups of 20 bees (see chapter 2), where the blend was dominated by ODA (45,14±17,16%, 8µg) and 10-HDAA (40,13±15,56%, 6,47µg).The isolated kept bees showed a totally different composition dominated by the hydroxy acids with 81% which is not comparable to any of the shown *A. m. capensis* examples in Fig. 3.2 and also no data could be found in the literature.

The data suggest that the more a worker develops into a reproductive individual the more the amount of the hydroxy acids decreases and the amount of 9-ODA increases.

9-ODA is known to play an important role for worker reproductive dominance hierarchies (Moritz and Hillesheim 1985, Hillesheim et al. 1989, see chapter 6). The more the worker is able to produce a more queen-like signal with high 9-ODA rates the more the worker could dominate other worker bees finally leading to the suppression of the ovarian development of nestmates (Hillesheim et al. 1989).

All individuals produced 9-ODA, 10-HDAA and 10-HDA under our experimental conditions. But the content of the three mandibular gland components was three times higher in paired bees than in isolated kept bees. Additionally, the composition differed, e.g. isolated bees produced on average 7,9% of 9-ODA whereas bees kept in groups produce 44,2%. However, there seems to be a high degree of social effects on the production and composition of the mandibular gland components. Apparently groups of two bees provide a social context to achieve a pheromone level similar to that of bigger groups such as 20 bees (Fig. 3.2). It is the interaction with a fellow bee which stimulates the pheromone production of the mandibular glands. Whether signal production is a sign of true worker-worker competition or just a phenomenon of general physiological retardation under isolations remains to be determined.

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4. The inhibition of queen substance production of worker bees (*Apis mellifera capensis* Esch.) by 9-ODA

4.1 Abstract

The reproductive dominance of the fertile queen is mediated through pheromones of the mandibular glands. There is a controversy over the influence of 9-ODA (9-keto-2(E)-decenoic acid), the major component of the queen mandibular complex (QMP). The exposure to a 9-ODA signal from the beginning of a workers life hinders them to produce an own 9-ODA signal.

A restriction of physical contact between worker and pheromone source did not affect the production of mandibular gland pheromones. We suggest that the „airborne“ 9-ODA is sufficient to regulate mandibular gland pheromone production in workers.

4.2 Introduction

The mandibular glands of honey bees have been coined to be the „social organ“ (Naumann et al. 1991) to control a variety of key functions. It’s compounds may act together as a bouquet signal like the queen mandibular complex (QMP) (Slessor et al. 1988, Winston and Slessor 1998) or alternatively the various components can be individually involved in separate functions.

The queen produced blend has releaser as well as primer pheromone qualities, e.g. it attracts nearby workers to the queen, given rise to a retinue of workers around the queen (Slessor et al. 1988) or inhibits queen rearing by workers (Winston et al. 1990) among other effects (Winston and Slessor 1992). The major component 9-ODA has been claimed to inhibit the ovarian development of the workers (de Groot and Voogd 1954, Butler 1959, Verheijen-Voogd 1959, Butler et al. 1962, Butler and Fairy 1963, Velthuis and van Es 1964, Velthuis 1970, Hepburn et al.1991), and inhibits juvenile hormone III biosynthesis in workers (Kaatz et al. 1992). However Butler et al. (1961) and Velthuis and van Es (1964) found that 9-ODA suppresses the ovary development of workers considerable less effective than a queen extract. Velthuis (1972) found that it was sometimes necessary to provide 30µg or even 150µg to 50 queenless bees every three days before any inhibition occurred. Hence it seems that other components must be important and several of them have been identified (Winston et al. 1990,

Winston and Slessor 1992, Plettner et al. 1995). The workers in a queenright honey bee colony are provided and affected by the signals of the queens from the beginning of their lives. The degeneration of the ovariole anlagen for example begins already in the forth larval instar and continues till emergence (Engels and Imperatriz-Fonseca 1990). Laboratory experiments have shown that strongest response on queen extracts occurred within one to five day old bees (Pham et al. 1982, Pham-Delegue et al 1991).

If the queens signal decreases for different reasons (Free 1987) and if there is no brood present some of the workers start to develop ovaries. In *Apis mellifera capensis* this process is very dramatic, and connected to a change in the composition of the mandibular glands from worker to queen-like pattern. 9-ODA levels are higher than in any other race which is why workers are called pseudoqueens (Crewe and Velthuis 1980, Crewe 1988, Velthuis et al. 1990, Plettner et al. 1993). Also pseudoqueens produce less amounts of 9-ODA than queens (Velthuis et al. 1990), they are able to inhibit the ovarian development in other workers and establish dominant hierarchies (Hillesheim et al. 1989).

The first purpose of this study was to investigate if production of 9-ODA by workers is affected by the concentration of synthetic 9-ODA they are exposed to. The workers „compete“ with the given signal up to a certain concentration and/or their own 9-ODA production might be suppressed.

Additionally the influence of physical contact between workers on their respective pheromone production was investigated. The detection of the mandibular components are mostly through antennation or gustation (licking) (Velthius and van Es 1964, Naumann et al. 1991;1992) and by direct worker-worker contact (Free 1978, Seeley 1979, Ferguson and Free 1980, Naumann et al. 1991). It is known that socially isolated workers produce significantly less 9-ODA than workers in groups (see chapter 3). Thus the social context seems to play a major role in the production of 9-ODA. Similar results were found by Southwick and Moritz (1985) for alarm pheromones. They reported that the defensive response per bee (as measured by a metabolic test) increased with the number of bees.

The question arises if the restriction of physical contact influences the reaction and thus the production of 9-ODA.

4.3 Materials and Methods

Freshly emerged bees were sampled from a brood comb which was kept in an incubator (32°C, 60% re. humidity).

Experiment I: Reaction of single workers to different amounts of 9-ODA

Dead worker bees were extracted with ethanol absolute (at room temperature for 2 days and 4 subsequent changes of ethanol) to obtain an odourless control individual. One of these extracted workers and a freshly emerged bee were housed in a petridish with honey and water ad libitum. Four solutions with 9-ODA concentrations of 0,1µg, 10µg, 50µg and 100µg were prepared. These quantities were chosen according to the production of 9-ODA of *A. m. capensis* workers under experimental conditions (see chapter 3). 0,1µg is equivalent to the minimal, 10µg is the average and 50µg the upper limit of the 9-ODA content found in capensis workers. Concentration IV is about equivalent to queen produced amounts (Naumann et al. 1991). The 9-ODA was diluted in ethanol. Every 12h 10µl of each concentration was dripped with a pipette on the head and the corps of the dead bee (according to the rate of decomposition of the pheromone (Naumann et al. 1991, Winston et al. 1989). As a control group 7 petridishes were set up with a freshly emerged bee and an extracted dead bee. After four days in an incubator (32°C, 60% rel. humidity). Live bees were chilled in a freezer for a few minutes to immobilize them and then decapitated. The heads were given into a vial containing 50µl of dichloromethane.

Experiment II: Effect of physical contact on 9-ODA production

Different experiments on bees as well as on ants (Hess 1942, Free 1987, Liebig 1998) have shown that the restriction of physical contact influences the behaviour and the physiology of individuals. To investigate the impact of physical contact on the production of 9-ODA of *A. m. capensis* workers, special petridishes were designed as follows. Each petridish was divided into two segments and separated by a double copper wire mesh with a distance twice as long as the antennae of the bees (~1,5cm). This design allows free air circulation but restricts physical contact. A bee was placed in each of the both parts of the petridish. Secondly two bees were housed together in one petridish without separation. All of the bees were supplied with honey and water ad libitum and kept in an incubator (32°C, 60% rel. humidity).

Gas chromatography

The head of the bee was removed from the dichloromethane, which was then evaporated with a stream of N_2. The redissolvation of the residue was done with an internal standard (± 1mg of each octanoic acid and tetradecane in 4ml dichloromethane) and 20µl BSTFA (bis-trimethylsilyl)trifluoroacet-amid). 1µl of this solvent was injected into a gas chromatograph (Hewlett Packard 5890), which was fitted with a split-splitless inlet and a 25mX0.32mm methyl silicone coated fused silica capillary column. The carrier gas was hydrogen with a flow rate of 1ml/min. The oven temperature was as follows: 60°C for 1 min, heated at 50°C/min to 110°C, then 3°C/min from 110°C to 220°C and held at 220°C for 10 min. Chromatograms were recorded and peak area quantified using HP ChemStation software. Peaks of the 9-ODA were identified by their retention times relative to tetradecane.
The relative mass ratio ([R.M.R.], Gehrke and Leimer 1971) of 9-ODA in each of the samples was also measured relative to tetradecane and the absolute amount determined.

4.4 Results

Experiment I: Reaction of single workers to different amounts of 9-ODA

All the workers of the groups I-IV produced very small amounts of 9-ODA (Fig.4.1). The 9-ODA content was not significantly different (Kruskal-Wallis test H= 4,79 N=53 p>0,05). Workers in contact with a dead extracted untreated bee produced 3,4 ±l,2µg 9-ODA, whereas workers in contact with the 9-ODA treated dead workers showed a significant reduced 9-ODA production (Mann-Whitney U test, U=55, N1=7, N2=53, p<0,01).

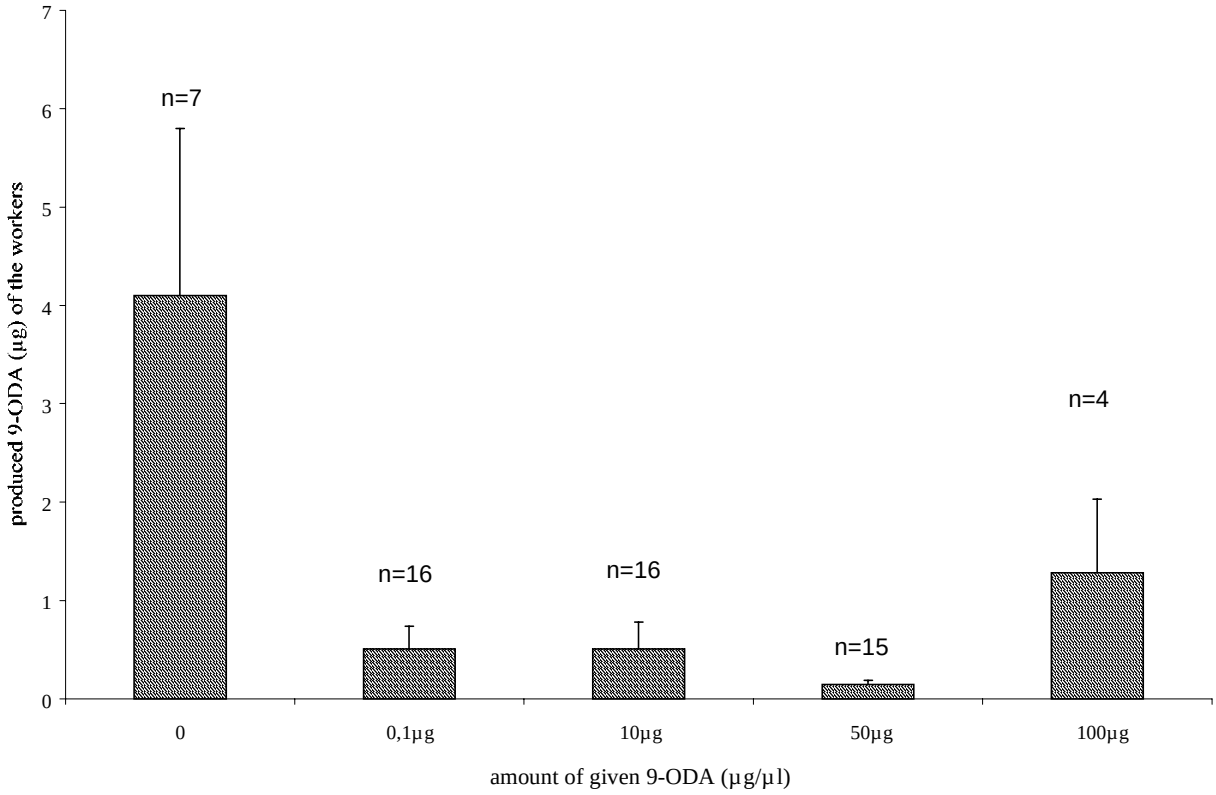

Fig.4.1 The 9-ODA content in the mandibular glands of workers which were exposed the different concentrations of 9-ODA.

Experiment II: Effect of physical contact on 9-ODA production

The worker bees which were separated by the double screen produced 6,0 µg±7,0µg of 9-ODA. The workers kept with a dead bee show a reduced but not significantly different amount of 9-ODA in comparison to workers kept in pairs (Kruskal-Wallis- test and Schaich-Hamerle post hoc comparison) (Fig 4.2).

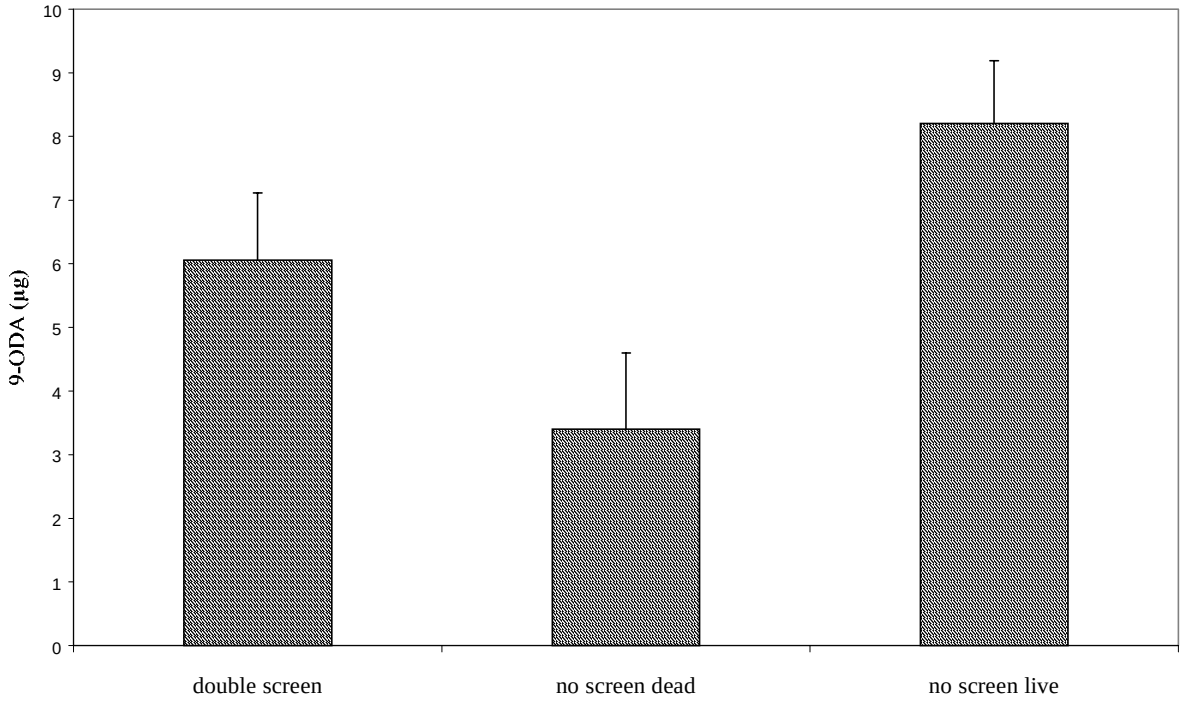

Fig. 4.2 The 9-ODA production of workers kept under different conditions

4.5 Discussion

Experiment I: Reaction of single workers to different amounts of 9-ODA

9-ODA is critical to the workers as a recognition cue for the presence of the queen (Butler and Simpson 1967, Slessor et al. 1988). In a normal colony approximately 0,5µg 9-ODA (Naumann et al. 1991) is maintained on the body surface of the queen which is mostly removed by the retinue workers surrounding the queen. These retinue contacts facilitate the transport of queen pheromones throughout the colony (Verheijen-Voogd 1959, Velthuis 1972, Seeley 1979, Winston and Slessor 1998). The amount of queen pheromone is distributed among thousands of workers (Free 1978, Seeley 1979, Ferguson and Free 1980, Naumann et al. 1991), and it decreases from individual to individual by internalisation of the

pheromone by the worker itself or the diffusion into wax among other factors (Naumann et al. 1991). The amount which finally reaches the even the „last“ receiver seems to be strong enough to signal the presence of the queen at least as the group size is not to big (Winston and Slessor 1998). But the impact of 9-ODA on the suppression of worker reproduction and consequently on the production and composition of the mandibular gland compounds of workers is discussed very controversially.

9-ODA was found to be considerably less effective than a queen extract (Butler et al. 1961, Velthuis and van Es 1964), hence it seemed that other components must be important. Free (1987) and Winston (1987) stated that workers are only prevented from developing ovaries and egg laying through a combination of brood and queen pheromones. Also the queen mandibular complex (QMP) alone was reported to have no role in the suppression of worker ovary development and egg laying (Willis et al. 1990, Plettner et al. 1993).

In our experiments the production of an own 9-ODA signal was significantly reduced by any of the 0,1-100µg tested 9-ODA concentrations. Now we have evidence that the exposure to a 9-ODA signal from the beginning of life inhibits a worker bee to produce an own 9-ODA signal. This reflects the situation found in worker bees in queenright colonies (Simon unpublished data). Consequently *A. m. capensis* workers don’t produce a signal as long as the queen with a sufficient 9-ODA signal or just the 9-ODA signal is present. But if the signal decreases due to queen loss (Free 1987) and the colony is broodless (for details see Hepburn and Radloff 1998) especially the young workers (Velthuis et al. 1990, see chapter 3) are supposed to change the composition of their mandibular glands from worker like to queen-like with high 9-ODA levels (Crewe and Velthuis 1980, see chapter 3). The amount of 9-ODA co-varies with the development of ovaries (Hepburn 1992, see chapter 6). Thus if the bees do not produce a signal they are unlikely to develop their ovaries and become reproductive.

Experiment II: Effect of physical contact on 9-ODA production

This result seems to be very surprising at the first glance as one might expect a reduction of the pheromone production due to the restricted physical contact. We suggest that the workers within one petridish detect the presence of the other bee by the secreted volatile signals, therefore produce similar amounts of 9-ODA as bees kept pair wise.

The mandibular gland pheromone composition exists not only of non-volatile components such as 9-ODA which are mainly dispersed through direct worker-worker contact but also of volatile components which are circulating through the air (Free 1987, Moritz and Southwick 1986). The perception of the volatile components is likely, pheromones are removed from circulation by being internalized into workers (Naumann et al. 1992). It is known that bees are able to perceive the odour of a queen and are attracted to it in an olfactometer (Butler 1960, Moritz and Crewe 1988; 1991) and Sladen (1901; 1902; 1905) was the first to suggest that the odour is dispersed by fanning. Worker honey bees posses olfactory cells (sensilla placodae) on their antennae that specifically react to odours of a queen and to 9-ODA (Beetsma and Schoonhoven 1966, Kaissling and Renner 1968, Allan et al. 1987). Gaschromatographic analysis of mandibular gland extracts have shown that 40-50 components are present (Brockmann et al. subm.) or even more than 100 compounds could be identified (Engels et al. 1997). It could be shown that workers responded on some of the components for example 9-keto-2(E)-decenoic acid (9-ODA), 9-hydroxy-2(E)-decenoic acid (HDA), 4-hydroxy-3-methoxy phenylethanol (HVA), methyl p-hydroxy benzoate (HOB) and 10-hydroxydecanoic acid (10HDAA) given as airborne signals (Moritz and Southwick 1986, Moritz and Crewe 1991, Brockmann et al. subm.,). Moritz and Southwick (1986) found that volatile queen odours release 46,8% of the reaction of worker groups compared to combination of volatile and non-volatile factors. But in enclosed environments the bees react as well on 9-ODA in spite of its low volatility (Moritz and Crewe 1988) and this might be comparable to the situation in a petridish.

It might be also possible that not only the volatile components of the mandibular glands, but also those of other glands, might play a role in signalling the presence of other bees as well (Moritz and Crewe 1991). We have no evidence that the restriction of physical contact affects the production of mandibular gland pheromones. These findings are in contrast to the work of Hess (1942) who divided a colony by a single wire mesh screen, where no ovary development did not occur, in either the part with or without the queen. But when a double mesh was used so that the bees could not contact each other, the ovaries of workers developed in the queenless part. In our experiments we can’t exclude this mechanism because we have no data concerning the status of the ovaries.

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5. Genetic variance of trophallaxis and queen substance production in worker bees (*Apis mellifera capensis* Esch.)

5.1 Abstract

Hierarchical dominance structure among honey bee workers (*Apis mellifera*) are established by behavioural, physiological and genetic mechanism. To test the influence of trophallactic interactions on pheromone production and the impact of genetical variance on the behavioural and physiological traits experiments in small groups were carried out.
The development of a trophallactic dominance hierarchy structure between two bees was determined. The production of the queen substance 9-ODA [(E)-9-ketodec-2enoic-acid] had no influence on the feeding behaviour. The workers were genetically determined with three polymorphic microsatellite loci. On the basis of subfamily analysis a significant genetic variance for the production of 9-ODA but no variance for trophallactic behaviour was found.

5.2 Introduction

Worker reproduction is almost non existent in queenright honey bee colonies. Only 0,01% of workers have a developed egg in their ovaries (Ratnieks 1993), and only 0,12% of the male offspring originates from worker bees (Visscher 1989). Nevertheless, workers start to develop their ovaries and lay eggs when the queen is absent (van der Blom 1991, Visscher and Dukas 1995). Potentially all of the workers could develop into reproductives, but it is only a small minority (Page and Robinson 1994, Hepburn and Allsopp 1994).
A special situation appears in queenless *Apis mellifera capensis* colonies. The loss of a queen can result in various options for a worker to develop (see Hepburn and Radloff 1998). One of these options is the development into laying workers or pseudoqueens (Velthuis et al. 1990). These are able to thelytokously produce female offspring, which has a dramatic impact on the kin structure of the colony. As a consequence, inclusive fitness arguments predict conflict between workers over reproductive dominance (Greeff 1996).
The question about the factors influencing the reproduction hierarchy is still debated. One possibility is a bias on reproduction by trophallactic behaviour. Montagner and Pain (1971) argued that contents of the honey stomachs flow freely between the individual bees. The concept of the „social stomach“ has been often referred to. Korst and Velthuis (1982),

however, found that some workers are more frequently asking individuals while others tend to specialize in offering. Trophallactic dominant workers are more likely to become laying workers in queenless colonies than others. This was the first report on individual intracolonial selection among workers. Montagner and Pain (1971) also suggested that the transport direction of food might be triggered by chemical stimuli such as pheromones. Workers loaded with queen pheromone after leaving the retinue made food gains in their contacts with other bees (Velthuis 1972; Seeley 1979).

Queenless *A. m. capensis* workers are able to produce a queen-like signal with high levels of (E)-9-ketodec-2-enoic-acid, which is known as the queen substance (9-ODA) (Crewe and Velthuis 1980, Crewe 1982, Velthuis et al. 1990, Plettner et al. 1993, see chapter 6). These can be transmitted to other colony members and determine the degree of domination of nestmates. Consequently they are able to suppress the ovary development in other workers (Hepburn et al. 1991). Thus both trophallaxis and 9-ODA have been reported to affect ovary development.

Genotypic variability among individuals for performing specific tasks have been demonstrated several times: egg laying in queenless colonies (Visscher 1996), oophagy,

oviposition and larval care in queenless colonies (Page and Robinson 1994), egg laying in queenright colonies (Oldroyd et al. 1994) and pollen versus nectar gathering (Dreller et al. 1995). Moritz and Hillesheim (1985) found a remarkable estimate for trophallactic dominance (h^2=0,32) in a population of *A. m. capensis* and they found a strong genetic

component in the determination of reproductive workers.

The development of the hierarchical dominance structure among the workers is thus established by all three behavioural, physiological and genetic components. The trophallactic interactions, the production of pheromones, and the genotypical composition of a colony are taken into account in this study. The goal is to clarify if trophallactic behaviour and/or the production of 9-ODA and the genetic variance of both of these factors mediates the establishment of dominance hierarchies.

5.3 Materials and Methods

A. m. capensis workers were obtained from a colony in Stellenbosch (Plant Protection Institute), South Africa. A brood comb was placed in an incubator (35°C, 60% rel. humidity). Pairs of freshly emerged bees were housed in a petridish and provided with water and honey ad libitum. Water and honey were changed daily.

Observation of trophallactic interactions

One bee was marked with a paint dot on the thorax to distinguish the bees in a petridish.
Sets of 45 petridishes were kept in the incubator (32°C, 60% rel. humidity). These 45 petridishes were split in 3 groups of 15. Each group was observed over a 20 minutes period then returned to the incubator. The interactions of the bees were detected 6 hours a day for 4 consecutive days. In total 180 pairs were observed. Workers could either ask for or offer food. Events of receiving food for every bee within each pair in a petridish were counted.
A bee of a pair was considered dominant if it was more frequent fed. Therefore, a relative number of being fed was calculated for each bee taking into account the total numbers of interactions per petridish (after Korst and Velthuis 1982).
After four days the bees were decapitated and the heads transferred into a vial containing dichloromethane. They were used for the gas chromatography to determine the amount of 9-ODA in the mandibular glands. The rest of the body was placed into ethanol abs. to store it for the DNA extraction.

Gas chromatography

The head extracts were analyzed by removing the head from the solvent, then evaporating the solvent just to dryness with a stream of N_2. The residue was then redissolved in 20µl internal standard (±1mg of octanoic acid and tetradecane in 1ml dichloromethane) and 20µl BSTFA (bis-trimethylsilyl)trifluoroacet-amid). 1µl of the solution was injected into a gas chromatograph (Hewlett Packard 5890). This was fitted with a split-splitless inlet and a 25mX0.32mm methyl silicone coated fused silica capillary column. Hydrogen was used as carrier gas with a flow rate of 1ml/min and the oven temperature was as follows: 60°C for 1 min, heated at 50°C/min to 110°C, then 3°C/min from 110°C to 220°C and held at 220°C for

10 min. . Chromatograms were recorded and peak area quantified using HP ChemStation software. Peaks of 9-ODA were identified by their retention times relative to the two internal standards. The relative mass ratio ([R.M.R.], Gehrke and Leimer 1971) of 9-ODA in each of the samples was measured relative to tetradecane and the absolute amount determined.

Genetic analysis

The DNA was extracted using routine protocols (Moritz et al. 1994).

Paternity of the workers was determined by genotyping with 3 polymorphic microsatellite loci. The primer sequences of A28, A43, B124 were taken from Estoup et al (1994, 1995). PCR was performed in 10µl solution containing 5-10ng DNA template, 1,5-1,7 mM $MgCl_2$, 400nM of each primer, 75µM dCTP, dGTP and dTTP, 6µM dATP 0,7µCi[α^{35}S) dATP, 0,4u *Taq* polymerase, 1X reaction buffer. The PCR was performed by denaturating the DNA for 3 min at 94°C, 33-35 cycles of 30s at 94°C, 30s at 54°C or 55°C and 30s at 72°C. The final step was an elongation time of 10 min at 72°C. The PCR with the primers for A43 and B124 was performed in a multiplex reaction. 7µl of each PCR reaction were mixed with 5µl of formamide solution. 2µl of this mixture was heated for 5min at 94°C and then run on a 6% polyacrylamide sequencing gel. The banding pattern was revealed with a X-ray film.

The genotype of the queen and the males she had mated with, were derived from the worker samples. For each worker the paternal alleles were considered to be those not carried by the queen. If both alleles of a worker at a given locus were identical to those of the mother, both were considered to be potentially of male origins with a 50% chance. These workers were determined using the other loci.

5.4 Results

Trophallactic behaviour

The frequency of the trophallactic interactions increased during the course of the experiments. Most feeding interactions could be observed on day four (38%) whereas on the first day trophallaxis was infrequent (Fig.5.1).

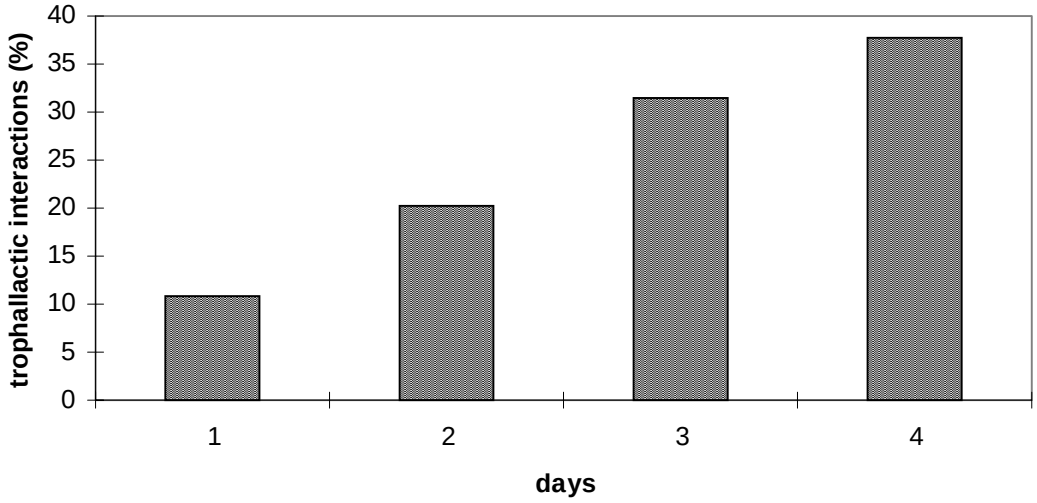

Fig. 5.1 The percentage of trophallactic interactions of all pairs for each of the four observation days. 100%=1214 interactions

The trophallactic behaviour often changed within a pair over the observation time. The „offering“ bee on one day was frequently observed to be the fed bee the next day.
A χ^2-test was performed for each petridish in which the bees had 10 or more interactions. In the tested pairs 34% differed significantly ($p<0,05$) indicating a hierarchy concerning the trophallactic interactions.

The 9-ODA production

The quantity of 9-ODA, detected in the extracts of 4 day old queenless workers, showed a considerable variation .The mean amount found was 9,8 ± 8,9µg (range 0,16- 47,7µg).
There was no significant correlation (Pearson and Spearman) between the 9-ODA content and the trophallactic behaviour on none of the four days.

Patriline distribution of the tested bees

The 168 bees which could be determined with the three loci belonged to 43 subfamilies with a different number of individuals in a range of one to 14 (Fig. 5.2).

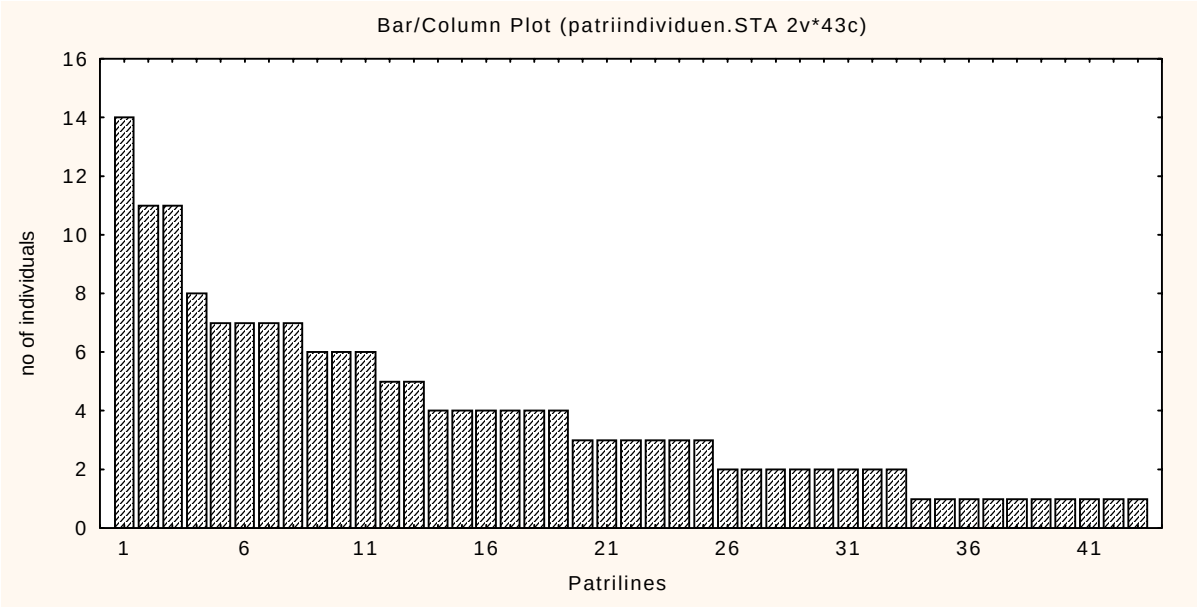

Fig. 5.2 Frequency distribution of the patrilines determined with three microsatellite loci.

The interaction of trophallactic behaviour, 9-ODA and the genotypes

The mean food receiving of the various patrilines over all four days was calculated for each subfamily (Fig. 5.3).

Patrilines with fewer than two individuals were skipped for the analysis to avoid erroneous results. Genetic variance was not significantly larger than zero (Tab. 1.1). Although patrilines 11, 12, 28 are less fed and 17 has a high rate of getting food, but the variability within these subfamilies is high.

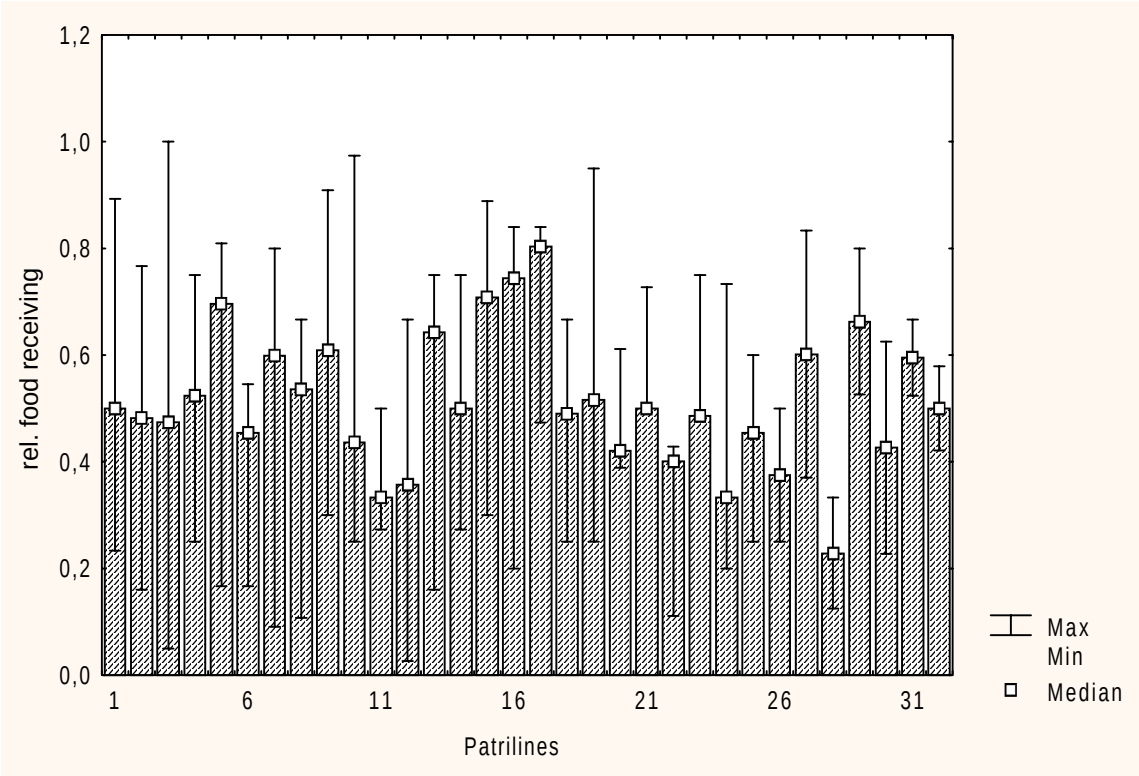

Fig. 5.3 The relative number of food receiving for each subfamily with ≥ 2 individuals. There are no significant differences between the patrilines (ANOVA with Tukey test for unequal N) and the genetic variance between the subfamilies is not significantly larger than zero.

For trophallaxis and the production of 9-ODA an estimation of variance components in a single classification ANOVA with unequal sample size was performed (Tab. 1.1).

Source of variance

	9-ODA			**Trophallaxis**		
	df	mean square	**h^2**	df	mean square	**h^2**
between subfamilies	33	80,08	**0,13**	33	,041	**<0**
within	124	57,21		124	,049	

Tab. 1.1 Genetic variance estimated via analysis of variance (intraclass correlation), devided by the relationship of the individual for the production of 9-ODA and trophallactic interactions.

Also for the production of 9-ODA the genetic variability within the subfamilies is high (Fig. 5.4). Up to 13% of the 9-ODA production are heritable and selectable variation (Tab1.1).

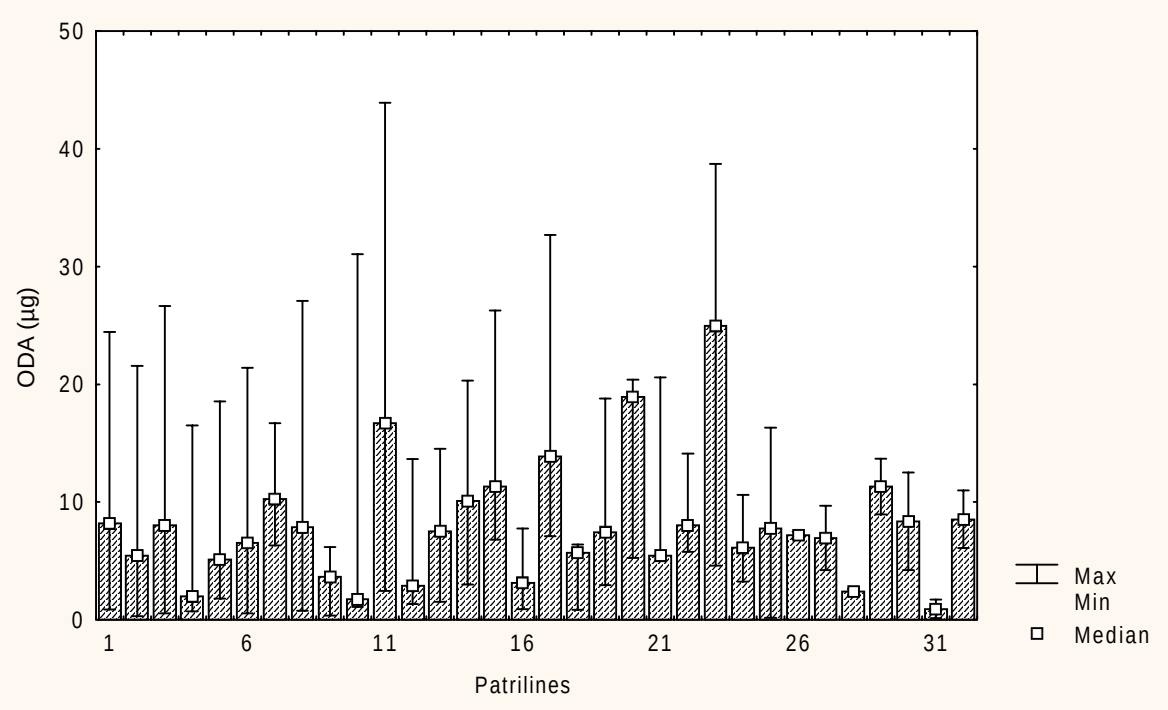

Fig. 5.4 The median queen substance content of the different subfamilies ≥ 2 individuals. The variability within and between patrilines a high. The heritability for the production of 9-ODA is 13%.

The mean 9-ODA amount and the mean frequency of receiving food for day one to four for each of the 43 patrilines was calculated. No significant correlation could be found for the produced amount of queen pheromone and the received food within the subfamilies.

5.5 Discussion

As described by Korst and Velthuis (1982) a trophallactic hierarchy was quickly established within the small experimental groups of honey bees. Some of the workers received significantly more often food than others. An aggressive behaviour was never seen during the entire observation time. Therefore, the hierarchy was not due to physical contact among workers like in bumble bees (Röseler and Röseler 1977) or even in honey bees (Korst and

Velthuis 1982). Korst and Velthius (1982) concluded from their experiments that whether a worker asks or offers depends on more than merely her feeding stage.

As hypothesized by Montagner and Pain (1971) the trophallactic interaction could be triggered by chemical signals. 9-ODA could play a major role since it is responsible for dominance hierarchies (Moritz and Hillesheim 1985, see chapter 6). But our data did not show a direct correlation between the dominant fed bee and the produced 9-ODA signal. We have no evidence that the increased feeding behaviour triggers a strong 9-ODA signal.

Differences in the workers behaviour and the production of 9-ODA is known to be influenced by genotypes (Moritz and Hillesheim 1985). We found no evidence that the trophallactic behaviour or the production of 9-ODA among the two individuals in one petridish is influenced by the genotypical composition. This does not necessarily mean that there is none. The bees were randomly taken from the brood frame and given into the petridishes. After determining the patrilines of the pairs, it became obvious that the combination of the different patrilines occurred only once therefore replicates were lacking. Only two patriline pairs were tested more than once, resulting in poor estimates, if non linear individual interactions are strong. Also no correlation was found on the level of average trophallactic behaviour and the production of 9-ODA of the different patrilines.

The transmission of a trait from one generation to the next and for predicting the short-term response to selection, is the narrow sense heritability h^2, the ratio of additive variance to phenotypic variance. We found no heritable variation for the trophallactic behaviour, but for the production of 9-ODA (h^2=0,13). Moritz and Hillesheim (1985) found a high estimation for trophallactic dominance (h^2=0,32) and the production of 9-ODA (h^2=0,89) in *A. m. capensis*. This might be controversially at the first view but can be explained by their experimental design. They tested offspring of laying *A. m. capensis* workers, and therefore genetic clones which lack all genetic recombination (Moritz and Haberl 1994). All additive, dominance and epistatic gene effects cause similarities in such an analysis which overestimates additive selectable gene effects. Since dominance in reproduction is related to the production of 9-ODA, a high genetic variance for this trait is consequently expected. This supports the suggestion of Velthuis and van Klerk (1988) that the production of 9-ODA is under genetic control and hence may have been selected for.

In our study worker offspring of a naturally mated queen result in a high number of patrilines and recombination. Our result is in line with Crnokrak and Roff (1995) who stated that traits

for a population at equilibrium are predicted to have low additive genetic variance. It is assumed that selection has moulded the traits to an optimum (Hegman and Dingle 1982, Lynch and Sulzbach 1984).

The genetic variance, acquired through polyandry increases colony fitness (Oldroyd et al. 1997). Concrete evidence on this point was reported by Moritz (1989), Hillesheim et al.(1989), Fuchs and Schade (1994). Hillesheim et al. (1989) found that colony efficiency is negatively correlated to the proportion of dominant bees in the colony. Subordinate bees are needed in the colony to rear brood. Egg laying alone is not sufficient to transmit genes into the next generation. But they also stated that it seems to be profitable on the colony selection level to invest in a low proportion of dominant mostly idle, but potentially reproductive bees. A colony structured like this would be favoured under queenless conditions because the dominant bees can swiftly gain reproductive status and all members profit through inclusive fitness benefits.

The occurrence of 9-ODA in workers of orphaned colonies of arrhenotokous races (Crewe 1988, Plettner et al. 1993) has been interpreted as a result of selection for dominance (Page and Robinson 1994). In *A. m. capensis* the selection for such dominance is much higher due to their ability to produce female offspring and the resulting in alteration of kin-structure (Greeff 1996). This workers are able to monopolize reproduction and can secure their own daughters to be the next queen (Greeff 1996), which is a major selective advantage. The subordinate workers help to raise the offspring of their half or super sisters respectively. The evolutionary way in which helping behaviour can be favoured was outlined by Hamiltons kin selection theory (Hamilton 1964 a, b). Consequently, these subordinate bees benefit of the survival of the colony, and the dominant workers benefit of the brood rearing of their offspring and all the other tasks performed for colony needs by the subordinate bees.

Acknowledgements

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6. Reproductive dominance in experimental groups of *Apis mellifera capensis* (Esch.)

6.1 Abstract

Apis mellifera capensis workers were kept queenless in small experimental groups. The fitness benefits in becoming a laying worker are supposed to be very high, therefore fitness models predict more conflict between the workers of the different subfamilies. A lot of studies were undertaken to detect the mechanism which finally result in the dominance of the laying workers. In this study the 9-ODA content of the mandibular glands, the development of ovaries, the production of eggs and the genotypes of the workers were determined. The production of 9-ODA seemed to play an important role for the reproductive state of a worker. But no correlation could be found between the amount of produced 9-ODA and the frequency of laid eggs. A high estimation for the reproductive dominance (h^2=0,7) is found.

6.2 Introduction

In honey bees the females are clearly divided into two specific morphological castes, the larger queen and the workers (Moritz and Southwick 1992). Usually the queen is the reproductive dominant female, suppressing the ovary development in workers through a pheromonal blend of fatty acids (de Groot and Voogd 1954, Butler 1959, Verheijen-Voogd 1959, Butler et al. 1962, Butler and Fairy 1963, Velthuis and van Es 1964, Velthuis 1970, Hepburn et al. 1991). Worker reproduction is exceedingly rare as long as the queen is present, only 1% of the workers have sufficiently developed ovaries to lay eggs (Ratnieks 1993, Visscher 1995). Typically laying workers appear in queenless colonies when there is no brood. In a process of intracolonial selection few workers develop into reproductively dominant individuals which have been called pseudoqueens (Velthuis et al. 1990). The majority of the workers remain sterile (Hepburn and Allsopp 1994, Page and Robinson 1994, Moritz et al. 1996).

In *A. m. capensis* worker reproduction is particularly frequent under queenless conditions and the latency time between queen loss and the onset of worker oviposition is shorter than in any other *Apis mellifera* race (Ruttner and Hesse 1981). The reproductive gain of laying *capensis* workers seems to be particularly strong, because they can produce thelytokously female

offspring. Therewith a colony can requeen itself from the offspring of a laying worker (Greeff 1996).

Many studies were undertaken to detect the mechanisms that finally result in the dominance of laying workers and a number of explanations have been hypothesized to explain this phenomenon. Series of experiments established that worker dominance is considerably genetically based (Moritz and Hillesheim 1985, Hillesheim 1987, Hillesheim et al. 1989). Moritz et al. (1996) found that workers of certain subfamilies in the colony were able to produce offspring whereas others remained sterile. The proximate factor for a worker to become a reproductive bee might be influenced by the production of queen-like pheromones.

Laying *A. m. capensis* workers produce a queen-like pheromone signal in their mandibular glands with high 9-ODA (E)-9-keto-2-decenoic acid) levels (Crewe and Velthuis 1980).

Although Slessor et al. (1988) argued that it is not exclusively the 9-ODA, but a mixture of five compounds of the mandibular secretions, the queen mandibular complex (QMP) the workers react to (Winston and Slessor 1998). Nevertheless 9-ODA seems to play a major role for the establishment of the dominance hierarchies among workers (Moritz and Hillesheim 1985). And Moritz and Hillesheim (1985) reported a strong genetic component in the determination of reproductive „dominant“ workers after artificial selection. Such dominant workers produce 9-ODA (Hemmling et al. 1979, Crewe and Velthuis 1980, Velthuis et al. 1990) and suppress ovary development in non reproductive „subordinate“ workers (Hillesheim et al. 1989). In contrast Plettner et al. (1993) found no relation between the pheromone secretions of workers and the ovarian development. This may be to the different race of their tested bees, which express less pronounced dominance hierarchies than *capensis*.

This study was undertaken to search for both ultimate and proximate mechanism which determine the reproductive rank of a *capensis* laying worker of naturally mated queens.

6.4 Materials and Methods

A. m. capensis workers were shaken from brood combs of broodright colonies. The bees were chilled for a few minutes to immobilize them. About 100 individuals were given into each of 11 small boxes (10x15x5cm), containing an empty piece of comb (4x11cm). The bees were supplied with water and honey ad libitum and kept in an incubator (34ºC, rel. humidity 65%). After 10 days all surviving workers were collected and decapitated. The head was given into a vial containing 500µl dichloromethane and the rest of the body was given into an Eppendorf tube with ethanol abs. The combs containing the eggs were stored in a freezer at -80ºC.

Control group

To reveal the distribution of the patrilines in the colony, workers were sampled at the same time for genotyping.

Gas chromatography

The head extracts were analyzed by removing the head from the solvent. The dichloromethane was evaporated just to dryness with a stream of N_2. The residue was then redissolved in 20µl internal standard (1±mg octanoic acid and tetradecane in 1ml dichloromethane) and 20µl (bis-trimethylsilyl)trifluoroacet-amid (BSTFA). 1µl was injected in a gas chromatograph (Hewlett Packard 5890) fitted with a split-splitless inlet and a 25mX0.32mm methyl silicone coated fused silica capillary column. The split-splitless injection technique was used with hexane as the solvent plug. The carrier gas was hydrogen with a flow rate of 1ml/min and the oven temperature was as follows; 60°C for 1 min, then heated at 50°C/min to 110°C, then 3°C/min from 110°C to 220°C and held at 220°C for 10 min. Chromatograms were recorded and peak area quantified using HP ChemStation software. Peaks were identified by their retention times relative to tetradecane. The relative mass ratio of 9-ODA ([R.M.R.], Gehrke; Leimer 1971) of 9-ODA in each of the samples was measured relative to tetradecane.

Genotyping of workers and eggs

DNA extraction

The extraction of the workers (control and experimental groups) DNA was done with the Chelex method (Walsh et al. 1991) using the following modifications. The flight muscles of the bees were dissected and given into an Eppendorf tube, the ethanol residues were evaporated in a heater at 80°C for a few minutes. 200µl Wilson buffer (100mM Tris, 10mM EDTA, 100mM NaCl) and 10µl proteinase K were added and incubated for 2 hours at 55ºC. 10µl of the homogenate was mixed with 200µl of Chelex (5%) and incubated for 30min at 56ºC. The mixture was vortexed at high speed for 5-10s. The proteinase K in the homogenate was inactivated at 95ºC for 8 min. The samples were vortexed again for 5-10s and centrifuged at 13.000xg. 1µl of the supernatant was taken for the PCR reactions.
The eggs were removed from the cells with a fine brush and transferred directly into 50µl of Chelex (5%). The sample was heated for 10min at 95ºC, vortexed for 5-10s and centrifuged for 3 min at 13.000xg. 1µl was taken from the top of the solution to be used in the polymerase chain reaction (PCR).

PCR

Paternity determination was done with five polymorphic microsatellite loci (Estoup et al. 1994, Estoup 1995). The annealing temperature and the concentration of $MgCl_2$ was changed according to changed conditions for the runs on a sequencer (ABI 310). The PCRs were conducted as multiple reactions with a triplett and a pair of loci. PCRs were performed in 10µl volume containing 50mM KCl, 10mM Tris HCl (pH8,3), 1,5mM MgCl 2, 200µM of each dNTP, 333nM of each of the 5 primers (one of each pair labeled at the 5`end with one of the fluoreszent dyes 6-FAM, HEX and TET (Perkin Elmer), 0,5 µl Golden Taq polymerase (Perkin Elmer) and 1µl of DNA extract. The PCR products of both of the multiplex reactions were mixed after the runs. 2µl of the mixture were given into 10µl of formamide containing 0,3µl of the TAMRA 500 standard (Perkin Elmer). The samples were run on a DNA sequencer (ABI 310) with the POP4 polymer and a capillary (ϕ 50µm, 47cm), 5s injection and a total running time of 21min. The genotype of the fathers siring a subfamily were determined by comparing the queens genotype with that of the worker offspring. Worker alleles which were not present in the queen were interpreted as paternal alleles. If both alleles

of a worker at a given locus were identical to those of the mother both were considered to be potentially of male origins with a 50% chance.

Ovary dissection

The ovaries oft the genetically determined bees were dissected and scored at four different categories.

0= completely undeveloped (thread like with no contents)

1= small ovaries but with slightly visible contents

2= large ovaries but no visible ova

3= as 2 but with visible separated ova

6.4 Results

In total 188 workers and 231 eggs out of 11 experimental groups and 117 workers of the control sample were genetically determined. The colony was composed of 30 subfamilies. The distribution of the workers and the eggs for each patriline is presented in Fig. 6.1-6.4.

Patriline 30 was not detected in the control sample. 13 patrilines were only represented by one individual. Also in the bees from the cages six subfamilies had only one individual. Some subfamilies were present in the worker samples but no eggs were found of these patrilines.

The frequency of the patrilines of the random sampled workers from the colony (Fig. 6.1) was compared with the frequency of workers which survived in the experimental groups (Fig. 6.2). The distribution of the frequency differed significantly (χ^2 =117,95 df=29, p<0,01).

The comparison with the frequency of laid eggs for each subfamily (Fig. 6.3) and the subfamilies of the workers still present in the groups (Fig. 6.2) showed that only 18 of the remaining subfamilies were present in the egg samples. Not all of the workers from one patriline became laying workers. Fig 6.4 shows the percentage of egg layers within a subfamily.

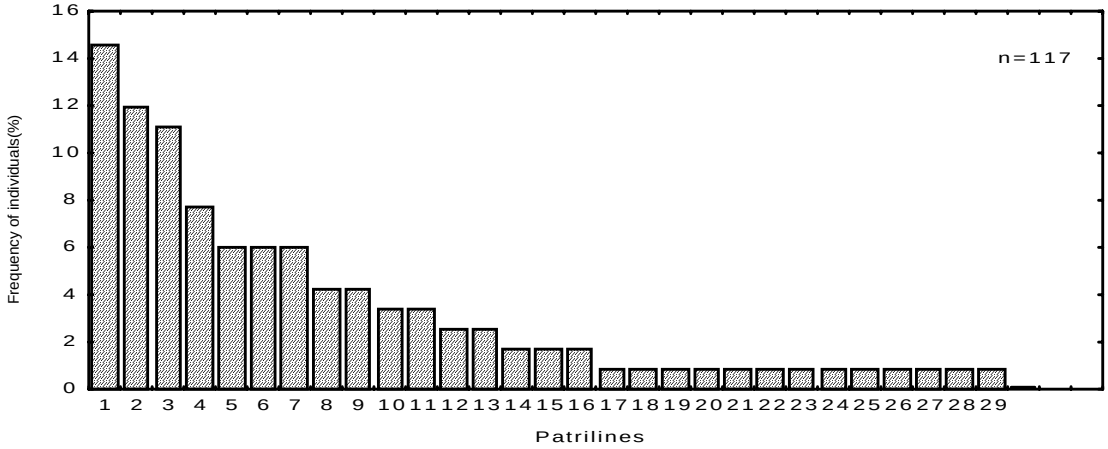

Fig. 6.1 Frequency (in %) of the various patrilines found in the workers that were sampled from the colony. Patriline 30 was not present in the sample.

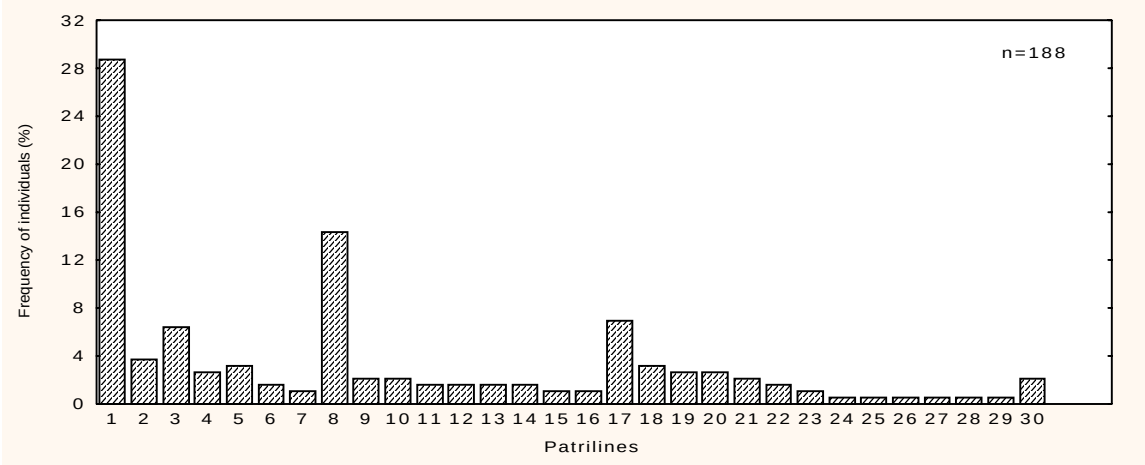

Fig. 6.2 Frequency (in %) of the patrilines of all workers survived in the 11 boxes.

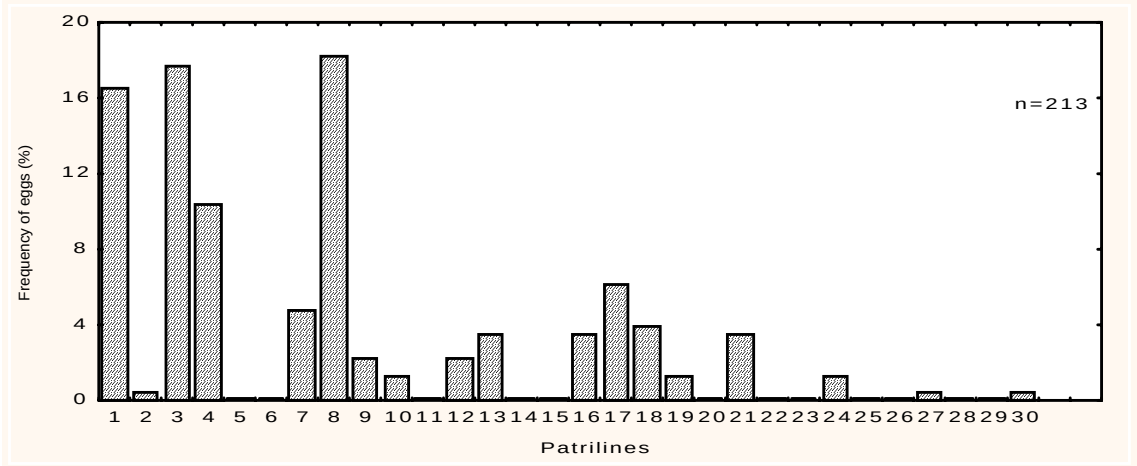

Fig. 6.3 Frequency (in %) of the laid eggs in all of the 11 boxes per patriline. 18 of the 30 subfamilies were present in the egg samples.

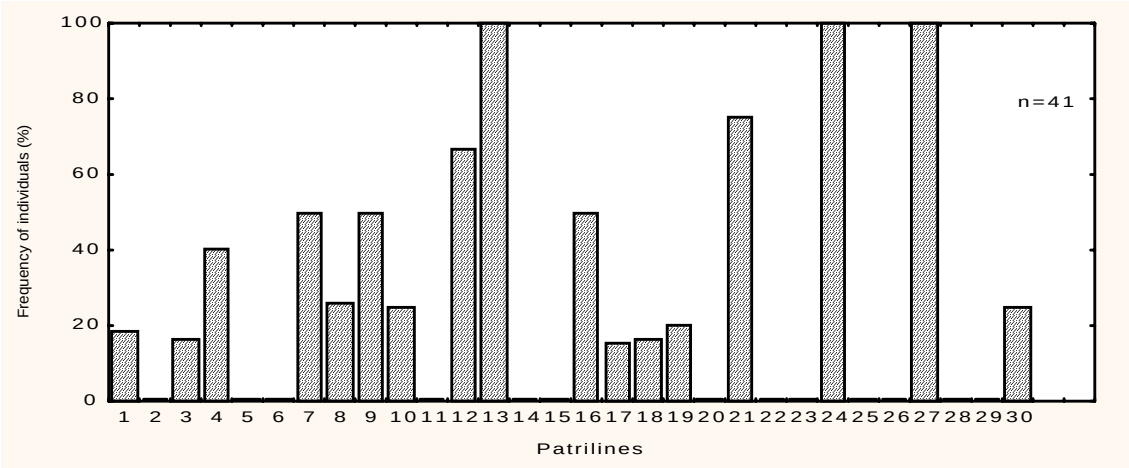

Fig 6.4 Frequency (in %) of the laying workers within the different patrilines.

The genetic variance estimated via analysis of variance (intraclass correlation) reveals an estimation of h^2=0,7 for egg laying (Tab. 6.1)

Source of variance

	df	mean square	h^2
between subfamilies	16	0,152	0,7
within subfamilies	26	0,039	

Tab. 6.1 Analysis of variance on the reproductive success of the various subfamilies in the 11 experimental groups.

Relation between the production of 9-ODA and the ovary status

The ovary status of each worker from the groups was determined and the amount of produced 9-ODA calculated. The amount of 9-ODA with the various ovary status differed significantly among the workers (Kruskal-Wallis analysis (H=15,9; N=154; p=0,012). A Mann-Whitney U test revealed significant differences between group 0 and 1 (U=649; p=0,13), 0 and 2 (U=339; p=0,005) and 0 and 3 (U=397; p=0,0002).

A Spearman Rank correlation showed a significant positive correlation between the amount of 9-ODA and the ovary status (r=0,293, N=149 p<0,01), suggesting that 8,4 % of the variation is due to the ovary status (Fig. 6.5).

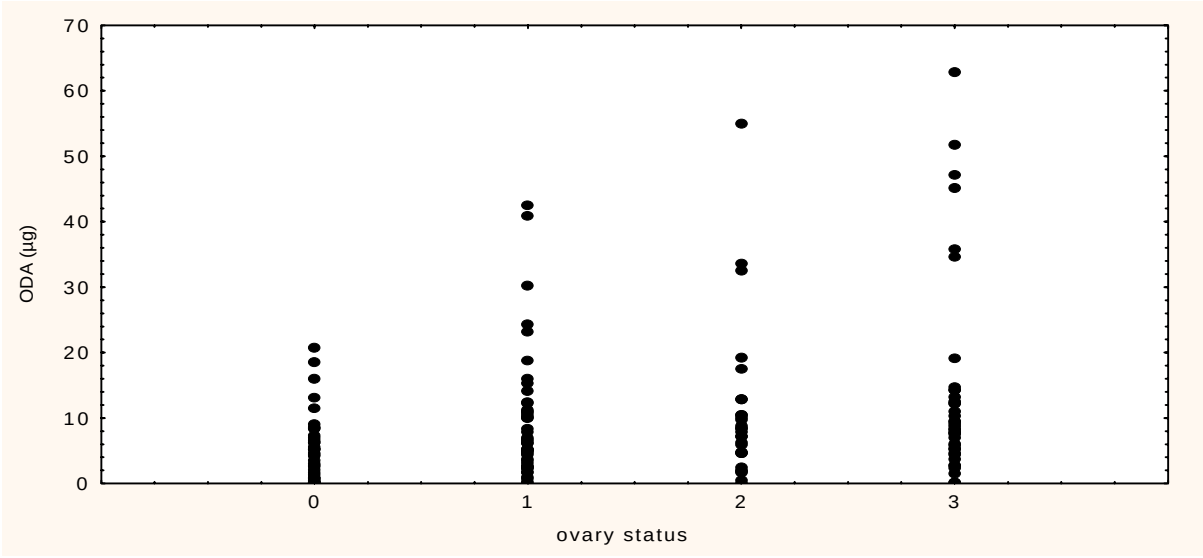

Fig. 6.5 Scatter plot of the correlation (r=0,293, N=149 p<0,01) between the ovary status (0-3) of the workers and their produced amount of the queen pheromone (ODA).

The group of bees with ovary status 3 were divided into those which were (group 3A) and those which were not represented in the egg sample (group 3B).The amount of produced 9-ODA between these two groups differed significantly (Mann-Whitney U test, U=108, N1=16, N2=23, p<0,05) (Fig. 6.6).

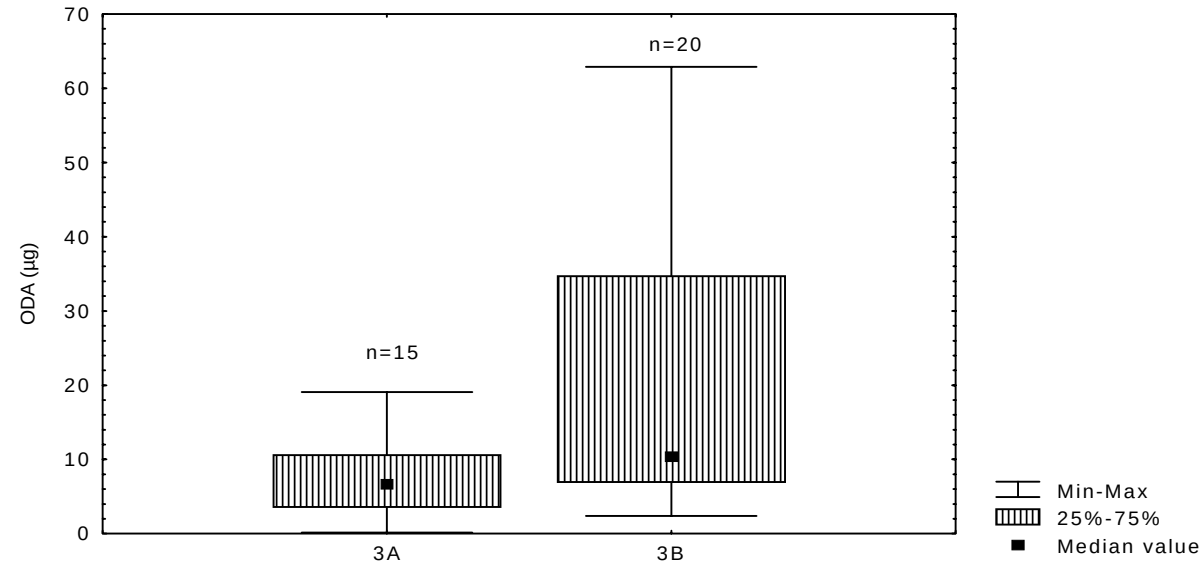

Fig. 6.6 The amount of produced 9-ODA in two groups of workers possessing ovaries of status three. Group 3A are workers which did not lay any eggs, group 3B were workers which had offspring in the egg samples.

Within group 3 no significant correlation between the amount of 9-ODA and the frequency of produced eggs could be found.

6.5 Discussion

Workers have various possibilities to requeen the colony if a *A. m. capensis* colony loses the queen (Hepburn and Radloff 1998). Under our experimental condition with no brood, we forced the workers to develop into reproductives and non reproductives. Laying workers of *A. m. capensis* are able to produce an abundant amount of the queen pheromone (Crewe and Velthuis 1980) and like queens they can suppress queencell construction (Hepburn et al. 1988) and inhibit the development of other workers ovaries (Hillesheim et al. 1989, Hepburn et al. 1991). The results obtained in this experiment support these views. The workers with the most developed ovaries (3) had significantly more queen pheromone than all other groups (0-2).
Secondly the 9-ODA production of possibly reproductives with ovary status 3 was significantly different between those workers which produced eggs and those without eggs. The reproductive individuals which laid eggs, produced on average 18,lµg 9-ODA which is equivalent to the amount found of Velthuis and Crewe (1980) in laying workers. Our data support Hepburn (1992), who reports that the ovarial development and becoming pheromonally queen-like co-vary. The production of 9-ODA seems to play an important role for the reproductive state of a worker in spite of a lack of a correlation between the amount of produced 9-ODA and the frequency of laid eggs. Even in species such as honey bees where group level co-ordination is highly developed, individual selection continue to play a role in shaping colonial phenotypes, even at a possible cost to group efficiency.

The selection for reproductive dominance operates through three distinct processes.

1. worker survival

In *A. m. capensis* inclusive fitness arguments predict more conflict between workers over reproductive dominance. They often show extreme aggression towards each other, resulting in a high number of killed bees in orphaned colonies (Greeff 1996). Velthuis et al. 1990 found that the workers which develop their ovaries and start producing a pheromonal signal are attacked by other workers. This might be the explanation for the change in the

composition of the subfamilies from the colony level to the workers surviving in the boxes (Fig 6.1/6.2).

2. worker reproduction

After a period of time the situation seems to become stabilized with a certain number of reproductive dominant and subordinate bees (Hillesheim et al. 1989). In a second step we could show that not all of the bees of the surviving subfamilies were represented by laying workers (Fig. 6.3). Only 18 of the 30 subfamilies were found to have produced eggs, with a selectivity estimate of 70%. These results are in line with previous observations (Moritz and Hillesheim 1985, Moritz et al. 1996) revealing a high genetic variance and strong intracolonial selection in dequeened experimental groups and colonies.

This selection between level 1 and 2 could be explained by selective trophallaxis (Moritz and Hillesheim 1985), and antagonism toward or killing of reproducing or ovary developing workers (Ratnieks 1988). This selection between level 1 and 2 could be explained by selective trophallaxis (Moritz and Hillesheim 1985). Also worker policing is said to have an influence on worker reproduction (Starr 1984, Seeley 1985, Woyciechowski and Lomnicki 1987, Ratnieks 1988). Each worker should try to prevent other workers in her colony from reproducing, either by destroying worker laid eggs or by showing overt aggression towards workers attempting to lay eggs (Greeff 1996).

3. laying workers

Thirdly a selection within a subfamily could be detected. In some experimental groups more than one individual from a certain patriline was identified , but not all of them were laying (Fig. 6.4). A worker will maximize her own fitness most by producing her own clonal daughters. If she fails, she only gains indirect fitness by rearing nieces from the same or a different patriline.

The reduction of patrilines between levels 2 and 3 might be explained by selective oophagy or removal of brood. Allsopp (1988) found that the absence of brood and developing queencells in queenless colonies depresses ovarian development and leads to changes in the pheromonal blend of the mandibular glands (Hemmling et al. 1979).

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7. Conclusion

Trophallactic interaction, pheromones and genotypes are known to play a major role for the establishment of reproductive dominance hierarchies of *Apis mellifera capensis* worker bees under queenless conditions. But the relation between these three factors were still discussed very contrarily in the literature. The aim of this thesis was to look for the interactions between these factors on the level of small groups to reveal and maybe understand the basic mechanism that are responsible for the development of dominance hierarchies.

Pheromones

A lot is known about the amounts and the composition of the pheromonal components of the mandibular glands of workers and queens (Hemmling et al. 1979, Crewe 1988, Crewe and Moritz 1989, Velthuis et al. 1990, Engels et al. 1997, Plettner et al. 1993; 1996; 1997, Winston and Slessor 1992; 1998). In a queenright colony the mandibular gland composition of workers changes related to the tasks they are is performing (Lindauer 1952, Sakagami 1953, Free 1965, Wilson 1971, Michener 1974, Seeley 1982, Robinson and Page 1989). Different studies have revealed the mandibular gland pheromone production of *A. m. capensis* workers under various conditions and different group sizes (Crewe and Velthuis 1980, Crewe 1982, Hepburn et al. 1988).

However the development of the mandibular gland secretions during the early adult phase of queenless *A. m. capensis* workers were neglected so far. In chapter 2 it could be shown that the amount of six mandibular gland components increase with age. And their composition changed as well. This has been of major interest because the space of time for a worker to develop into a laying worker with a corresponding mandibular gland signal is due to the latency period very short (see Introduction). Thus this signal has to be developed quickly and in amounts sufficient to dominate nestmates. We could show that in *A. m. capensis* the mandibular gland composition of four day old bees is already dominated by 9-ODA, although the worker specific substances (10-HDA, 10-HDAA) are present in high amounts. The *capensis* workers might still produce large amounts of 10-HDAA to mimic a „normal“ worker and to get the chance of developing the ovaries without being detected. This was reported by Plettner et al. (1997) and Crewe (1982) for virgin queens. This chemical camouflage may enable the workers to minimize aggression by other workers till the ovaries

are fully developed. If it is like this workers have to use subtle tactics to weight their chance to become a laying worker against being attack or if it comes to the worst killed beforehand.
On the other hand no overt aggression appeared in the groups during the experimental course. This could alternatively explained by the fact that the workers ovaries were not differentiated far enough to be detected by other workers. An indication for this is the composition of the mandibular gland components of the four day old workers, which is comparable to that of a worker with partially developed ovaries (Crewe and Velthuis 1980).

Social context

We analysed the content of three main „worker“ and „queen“ mandibular gland components in isolated and in paired bees (chapter 3). The mandibular gland amounts in paired bees were three times higher than in isolated bees. Also the composition differed e.g. isolated bees produced on average 7,9% of 9-ODA whereas bees kept in groups produce 44,2%. Therefore the production and composition of the mandibular gland components seemed to be affected socially. The question arises why bees produce almost no 9-ODA being isolated. It might be that 9-ODA is a „social pheromone“. Without a „receptor“ there is no need to produce a signal to e.g. regulate or dominate somebody else. Producing expensive but senseless signals ought to be maladaptive. This result might even make more sense taking the statement from Hess (1942) into account. He found that single bees never reproduce thus don’t develop ovaries so what for producing a regulating signal. The surprising result in the light of Hess (1942) data is that paired bees are producing a 9-ODA signal.
Two different possibilities arise to interpret the mandibular gland pheromone data. Either the total amounts or the composition of the tested components are taken into account. One has to be careful with the interpretation of these results since they might lead to different conclusions. The percentage of the content of 9-ODA was similar to that found in laying workers (Crewe and Velthuis 1980), but the total amount was just half of it. Velthuis et al. (1990) investigated the production of 9-ODA *A. m. capensis* workers with workers of either the same race or with *Apis mellifera carnica* . They stated that it is rather the composition of the mandibular gland components than their amount which reveals an advantage over the other colony members. We suggest that both ways of interpreting should be taken into account related to the goal of the experiments.

Physical contact

In a queenright colony the pheromonal signals of the mandibular glands are mostly distributed through worker-worker contacts (Seeley 1979, Velthuis 1970, Naumann et al. 1991). It has often been found that the restriction of physical contact in social insects can affect individuals as well behaviourally as physiologically (Liebig 1998). Thus we wanted to know whether the restriction of the physical contact between two bees does change the production of the 9-ODA, having in mind that the restriction of the physical contact might simulate isolated conditions (see chapter 3). Our results give clearly evidence that the restriction of physical contact does not inhibit the production of 9-ODA. This leads to the conclusion that chemical components of the mandibular glands but maybe also those of other glands are dispersed through the air (Brockmann et al. subm., Moritz and Crewe 1991, Moritz and Southwick 1986). They might furthermore play a role in signalling the presence of other bees, thus having a social component. This might be adaptive if we look at the number of individuals living in a honey bee colony. To reach many conspecifies within a short time airborne signals are much more useful than direct contact. It was also found that the presence of a dead bee is sufficient to simulate a „social context“ (chapter 3), because bees kept with a dead bee produce similar amounts of 9-ODA than those kept with a live bee.

In summary these results might be explainable by the evolution of eusocialty in social insects. With the increase in group size the division of labour became more differentiated (Bourke 1988) and the partitioning of reproduction was evolved to a stage where just one individual monopolises the reproduction like the queen in honey bees does. She increases her fitness maximally but just as long as there are workers taking care of her and the colony needs, and helping to rear the offspring. A queen alone would never succeed in funding a colony. Thus honey bees are dependent on the social context for their reproduction. Therefore the mechanism regulating the reproduction might only be developed in a suitable situation. It would be very interesting to investigate of which group size the bees not only produce a queen-like signal but also develop ovaries and succeed in rearing a replacement queen.

9-ODA in reproductive cues

The importance and influence of 9-ODA has been discussed very contrarily in the reproductive context. It was found to suppress ovary development of workers (de Groot and Voogd 1954, Butler 1959, Verheijen-Voogd 1959, Butler et al. 1962, Butler and Fairy 1963, Velthuis and van Es 1964, Velthuis 1970, Hepburn et al.1991). But it is considerably less effectively than queen extracts (Butler et al. 1961, Velthuis and van Es 1964) and other factors are additionally responsible for the workers inhibition of reproduction (Winston 1987, Winston and Slessor 1998). The contradiction about the impact of 9-ODA on the ovarian development is especially interesting for *A. m. capensis* workers. If the ovarian development can be suppressed by the queens 9-ODA signal, the workers itself should not produce an own 9-ODA signal. Consequently they will not develop their ovaries since the ovarial development and the production of 9-ODA co-vary (Hepburn 1992). We could show that under our experimental conditions the production of an own 9-ODA signal of workers was inhibited by any of the tested 9-ODA concentrations. In summary it could be stated that the bees exposed to a 9-ODA signal „feel“ queenright, thus do not produce a 9-ODA signal. The other „feel“ queenless, the dead bee alone alone does not hinder the bee to produce a 9-ODA signal.
If we try transfer these results to a „normal“ colony situation, we could argue from our experiments that the workers do not produce a 9-ODA signal as long as the queen produces a sufficient amount of 9-ODA. If this would be not the case, the bees would start to compete for the signal. This might result in physical interaction („aggression“), possibly resulting in reduced colony fitness. On the other hand the queen is replaced when she doesn’t lay any longer eggs and her pheromonal signal is decreasing (Free 1987). This might however be related to the period of time the bees are not provided with the queens signal. An experiment with controlled conditions of different exposure times with various 9-ODA concentrations and the determination of the ovarian development could support this hypothesis .

Trophallaxis and Genetics

But pheromones are not the only one factor playing a role in reproductive dominance hierarchies. Also the trophallactic behaviour in social insects often reflects the hierarchy structure of individuals in the colony obtained through physical and/or pheromonal support (Wilson 1971, Seeley 1979, Korst and Velthuis 1982, Franks and Scovell 1983, Moritz and Hillesheim 1985).

In queenright honey bee colonies the direction of food transfer was thought to be triggered by pheromones (Montagner and Pain 1971). This was supported by Velthuis (1972) and Seeley (1979) who found a relation between pheromonal identity and the treatment by nestmates. Workers covered with queen mandibular gland pheromones after leaving the retinue made food gains. We found a hierarchy concerning trophallactic interactions. But our data did not show a direct correlation between the dominantly fed bee and the produced 9-ODA signal.

Within the last years the genetic influence on behavioural and physiological factors became off increasing importance. Series of studies have revealed data for genetic variance of different worker tasks in queenright colonies (Frumhoff and Baker 1988, Page et al. 1989, Oldroyd et al. 1994, Page and Robinson 1994, Dreller et al 1995, Visscher 1996). Also in queenless colonies dominance hierarchies of workers (revealed through trophallactic and pheromonal (9-ODA) data) are considerably genetically based (Moritz and Hillesheim 1985, Hillesheim 1987, Hillesheim et al. 1989). In contrast to their results we found no genetic variance for trophallactic behaviour. The genetic variance of the 9-ODA production was less than calculated by Moritz and Hillesheim (1985). But they tested offspring of laying *A. m. capensis* workers, thus genetic clones which lack all genetic recombination (Moritz and Haberl 1994). We used however offspring of a natural mated queen, resulting in 43 patrilines, thus the genetic variance is expected to decrease (Crnokrak and Roff 1995).

If just a few patrilines are able to produce a queen-like signal it might be still a „honest“ signal for all other patrilines in the establishment of dominance hierarchies. If all of the bees would produce large amounts of 9-ODA there is no advantage, not on the colony level and not on the individual level. The occurrence of 9-ODA in workers of orphaned colonies of arrhenotokous races (Crewe 1988, Plettner et al. 1993) has been interpreted as a result of selection for dominance (Page and Robinson 1994). In *A. m. capensis* the selection for such dominance is much higher due to their ability to produce female offspring and the resulting in alteration of kin-structure.

Selection for reproductive dominance was found to operate on different levels. The distribution of the patrilines changed during the process of the establishment of the reproductive dominance. This is explained by various factors such as selective trophallaxis (Moritz and Hillesheim 1985), antagonism toward ovary developed workers (Ratnieks 1988) or worker policing (Starr 1984, Seeley 1985, Woyciechowski and Lomnicki 1987, Ratnieks 1988). The production of 9-ODA seems to play an important role for the reproductive state of

a worker even if no correlation could be found between the amount of produced 9-ODA and the frequency of laid eggs. We found a remarkable estimate for reproductive dominance (h^2=0,7). Our results are in line with those of Moritz et al. (1996) who also found a high intracolonial selection for reproduction.

The eggs were sampled all at one time. It would be of further interest to look for processes like selective oophagy, which has been found in many hymenopteran species (Bourke 1991; 1994) to reveal data for possible selection of patrilines due to kin recognition cues. Additionally it would be appropriate to remove the dead bees every day. Their pheromonal content of the mandibular glands, the ovarian development and the genotype could be determined to see if there are relations between these factors and the attacking or killing.

All our results show that even in species such as honey bees where group level co-ordination is highly developed, individual genetic interest continue to play a role in shaping interactions, even at a possible cost to group efficiency.

I suggest that the production of a queen-like mandibular gland signal with high 9-ODA levels is a genetically variable crucial for the establishment of reproductive dominance hierarchies. The production of 9-ODA coincide with the ovarial development. Workers which are able to produce a strong signal might suppress the development of a queen-like signal in nestmates.

The traits described above could be interpreted as adaptations to increase worker inclusive fitness.

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8. Zusammenfassung

Die Kap-Honigbiene *Apis mellifera capensis* ist eine in der Kapprovinz verbreitete geographische Varietät von *Apis mellifera* (Ruttner 1988). Sie wurde von Escholtz (1821) beschrieben und benannt (Ruttner 1977). Sie zeichnet sich durch drei Charakteristika aus, die sie von allen anderen Rassen unterschiedet. 1) bei Weisellosigkeit sind Kap-Arbeiterinnen in der Lage thelytokisch weibliche Nachkommen zu erzeugen und 2) ihre Ovariolenanzahl ist doppelt so hoch wie die der ebenfalls in Südafrika vorkommenden *Apis mellifera scutellata* (Hepburn und Crewe 1991) 3) weiterhin sind sie in der Lage ein königinnen-ähnliches Mandibeldrüsensekret zu synthetisieren, welches hohe Mengen an 9-Oxodecensäure (9-ODA) beinhaltet (Crewe 1982). Wird ein *A. m. capensis* Volk weisellos und ist keine Brut vorhanden, so können Arbeiterinnen die Reproduktion übernehmen. Potentiell wären alle Arbeiterinnen in der Lage ihre Ovarien zu aktivieren, es sind jedoch nur einige wenige, die sich zur legenden Arbeiterin entwickeln.

Die Ergebnisse zahlreicher Untersuchungen weisen darauf hin, daß die drei Faktoren Pheromone, Trophallaxis und Genetik bei der Ausprägung von Dominanz eine entscheidende Rolle spielen (Korst und Velthuis 1982, Moritz und Hillesheim 1985, Hilleheim et al. 1989).

Die Interaktion und Relevanz dieser Faktoren für die Etablierung der Dominanzhierarchien der Arbeiterinenreproduktion ist das Thema dieser Promotion.

Es wurden zunächst Daten gewonnen, die Aufschluß über die Entwicklung der Mandibeldrüsensekrete von ein bis vier Tage alten Arbeiterinnen geben sollten (**Kapitel 2**). Das Alter ist von Bedeutung, da es meistens die jungen Arbeiterinnen sind, die sich in reproduktive Individuen differenzieren (Engels and Imperatriz-Fonseca 1990, Velthuis et al. 1990). Spielt das königinnen-ähnliche Manibeldrüsensignal eine Rolle für die reproduktive Dominanz, so ist dessen schnelle Entwicklung von entscheidender Bedeutung. Vom Verlust der Königin bis zur Etablierung von legenden Arbeiterinnen vergeht nur eine kurze Latenzzeit von 4-6 Tagen (Ruttner und Hesse 1981). Hier konnte gezeigt werden, daß die Menge der sechs analysierten Komponenten in den vier Tagen um das Zehnfache ansteigt. Auch der relative Anteil der einzelnen Komponenten verändert sich mit dem Alter der Bienen. Kaparbeiterinnen produzieren ein Mandibeldrüsensekret, welches von 9-ODA dominiert ist bereits im Alter von 4 Tagen.

Die Ausprägung der Mandibeldrüsensekrete ist nicht nur altersabhängig sondern auch abhängig von dem sozialen Kontext. **In Kapitel 3** wurde die Pheromonproduktion von

Arbeiterinnen untersucht, die in der kleinstmögliche Gruppe von zwei Bienen oder isoliert gehalten wurden. Es wurden die typischen „Arbeiterinnensubstanzen“ und die Königinnensubstanz analysiert. Dabei wurde deutlich, daß isoliert gehaltene Bienen eine dreifach reduzierte Pheromonproduktion im Vergleich zu den Paaren aufzeigten. Auch die Zusammensetzung der drei analysierten Komponenten unterschied sich signifikant. Diese Ergebnisse zeigen deutlich, daß die Interaktionen zwischen den Bienen die Produktion von Pheromonen stimuliert. Somit ist die Ausprägung dieser „Signale“ abhängig von dem sozialen Kontext. Im weiselrichtigen Volk werden die Mandibeldrüsenpheromone der Königin durch direkten Kontakt zwischen den Arbeiterinnen verbreitet (Free 1978, Seeley 1979, Ferguson and Free 1980, Naumann et al. 1991). Einige Untersuchungen an Ameisen und Bienen haben gezeigt, daß das Verhindern physischen Kontaktes zwischen den Individuen sowohl zur einer verhaltens- als auch zu einer physiologischen Veränderung führen kann . Wir fanden keinen Hinweis, daß die Verhinderung des physischen Kontaktes zwischen den Arbeiterinnen zu einer veränderten Pheromonproduktion in den Mandibeldrüsen führt. Wie nehmen daher an, daß sowohl Komponenten aus den Mandibeldrüsen als auch aus anderen Drüsen über die Luft übertragen werden und ausreichen, einen sozialen Kontext herzustellen.

Die Wirkung von 9-ODA, der Hauptkomponente des Königinnenmandibeldrüsensekrets (QMP), auf die Unterdrückung von Arbeiterinnenreproduktion wird sehr kontrovers diskutiert. Auf der einen Seite wurde gezeigt, daß 9-ODA für die Unterdrückung der Ovarienentwicklung der Arbeiterinnen verantwortlich ist (de Groot and Voogd 1954, Butler 1959, Verheijen-Voogd 1959, Butler et al. 1962, Butler and Fairy 1963, Velthuis and van Es 1964, Velthuis 1970, Hepburn et al. 1991). Auf der anderen Seite wird berichtet, daß zusätzliche Faktoren notwendig sind um die Reproduktion von Arbeiterinnen zu verhindern (Slessor et al. 1988, Winston and Slessor 1998). Die Ovarienentwicklung der Arbeiterinnen geht einher mit der Ausprägung königinnen-ähnlicher Mandibeldrüsensekrete (Hepburn 1992). In **Kapitel 4** haben wir die Ausprägung von 9-ODA Signalen weiselloser Arbeiterinnen untersucht, die unterschiedlichen Konzentratioen von synthetischen 9-ODA ausgesetzt waren. Es konnte gezeigt werden, daß die Produktion eines eigenen 9-ODA Signals bei jeder gegebenen Konzentration signifikant reduziert wurde. Das Vorhandensein einer toten unbehandelten Biene war ausreichend um einen sozialen Kontext zu simulieren. Die Arbeiterinnen, die mit einer solchen Biene gehalten wurden, produzierten einen 9-ODA Gehalt, der dem der in Paaren gehaltenen Bienen entspricht (Kapitel 3).

Aber die Produktion von Pheromonen ist nicht der einzige Faktor, der die Ausbildung von Dominanzhierarchien beeinflußt. Auch trophallaktische Interaktionen spielen eine Rolle in Bezug auf Dominanz. Weiterhin wird angenommen, daß die Richtung des Futterflusses durch Pheromone moduliert wird. Und ein dritter Faktor, die genetische „Ausstattung“ spielt sowohl für die trophallaktische Dominanz als auch für die Produktion von 9-ODA eine entscheidende Rolle (**Kapitel 5**)

Wir haben die Herausbildung einer Dominanzhierarchie in Bezug auf Trophallaxis gefunden. Jedoch gab es keine Korrelation zwischen dem Futterfluss und dem 9-ODA Gehalt. Die Produktion von 9-ODA ist zu 13% auf genetische Varianz zwischen den Patrilinen zu erklären. Die trophallaktischen Interaktionen waren nicht auf Patrilinien zurückzuführen.

Unsere Daten weisen darauf hin, daß die Produktion von 9-ODA und die Zugehörigkeit zu einer bestimmten Patrilinie die proximaten Faktoren für die Dominanzhierarchien darstellen.

In **Kapitel 6** wurden kleine Gruppen von Arbeiterinnen weisellos und ohne Brut in Versuchskäfigen gehalten. Untersucht wurde ob der Zusammenhang zwischen den Patrilinien und der Produktion von 9-ODA sich im reproduktiven Erfolg widerspiegelt. Es konnte gezeigt werden, daß die Ausbildung von Dominanzhierarchien sich auf drei Selektionsebenen abspielt. Dabei spielt die 9-ODA Produktion eine bedeutende Rolle für den reproduktiven Status einer Arbeiterin. Es konnte jedoch keine Korrelation zwischen dem 9-ODA Gehalt und der Anzahl der gelegten Eier gefunden werden. Die 70% genetische Varianz für die reproduktive Dominanz bestätigen eine hohe intrakoloniale Selektion für die Reproduktion.

Abschließend kann aus unseren Ergebnissen geschlossen werden das die Produktion von 9-ODA als genetische Variable einen entscheidenden Einluß hat auf die Etablierung von Dominanzhierarchien bei *Apis mellifera capensis* Arbeiterinnen.

Lebenslauf

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Studium

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