

Evolution of intraspecific social parasitism in honeybee workers (*Apis mellifera capensis* Esch)

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1 Introduction

1.1 Evolution of social insect colonies

One of the most enigmatic of adaptations in nature is the extreme cooperation in social insect societies (ants, bees, wasps and termites), where the vast majority of individuals do not reproduce. While one or a few female reproductives (usually queens) dominate reproduction, non-reproductive workers participate in all other tasks necessary to maintain the colony, such as brood rearing, foraging and nest defence (Wilson 1971). Giving up their own reproduction is in contrast to the individual level selection which Darwin (1859) regarded as potentially fatal to his theory of natural selection. Therefore, he extended his theory to the level of groups, which raised strong controversy in the field of evolutionary biology. It was Hamilton's work (1964a,b) that gave an elegant solution to the problem of workers' reproductive altruism. His kin selection theory provided answers how sterility and self-sacrifice can evolve under the condition of natural selection. Hamilton could show that workers do not reproduce due to their inclusive fitness gain by helping to rear offspring of the related queen. The male haploid system offers a highly plausible explanation why sociality is particularly common in the order Hymenoptera. Depending on the relatedness it is selectively advantageous for workers to reduce their own direct fitness in favour of the queen. However, cooperation in one area of social life does not prevent conflict in another (Crozier and Pamilo 1996). Members of insect societies are often not genetically identical, this results in potentially divergent interests in sex allocation, queen rearing, male production, queen worker caste fate and conflict among totipotent individuals (Ratnieks et al. 2006). The resolution of (most) of these conflicts is allocated to the worker level. Nevertheless, it is a puzzling issue of evolutionary biology which requires to analyse resolution cues triggering the conflict over reproduction in a social insect colony.

1.2 Social parasitism in social insects

Parasitism is a dominant life history strategy in nature (Price 1980) and about 72% of all insect species are parasites (Price 1977). Co-evolutionary interactions with their hosts are important factors influencing the organisation of communities and driving the diversification of life (Thompson 1994). An advanced host-parasite relationship is social parasitism, the exploitation of the accumulated resources of a social insect colony by non-group members (Wilson 1971; Hölldobler and Wilson 1990). Such social parasites share the benefits of the well organised division of labour within the host colony for their own fitness without participating in its work. In all major groups of social insects socially parasitic life strategies have evolved (Schmid-Hempel 1998). Social parasitism may either be found within a single species (intraspecific) or between two species (interspecific; Wilson 1971). A variety of socially parasitic strategies persist to undermine this advanced mode of cooperation (Michener 1974; Hölldobler and Wilson 1990; Bourke and Franks 1995). Social parasites either exploit other species only temporarily during the nest-founding phase (e.g. *Formica exsecta*; Hölldobler and Wilson 1990), or they are

obligatory, dependent on their hosts throughout all stages of their life cycle. One group of obligate social parasites are slave makers. They kill the adults of foreign nests in order to steal the brood which is transported and hatches in the slave maker nest (Hölldobler and Wilson 1990). The hatching foreign workers perform all tasks necessary for the maintenance of the parasite colony. On the other hand, inquiline species such as *Teleutomyrmex schneideri* or the socially parasitic wasp *Polistes sulcifer*, have completely lost the worker caste and are thus totally depending on their host species (Wilson 1971).

In a variety of taxa, many socially parasitic species tend to be close phylogenetic relatives of their hosts (Hölldobler and Wilson 1990). This common phenomenon of close relatedness is known as Emery's rule (Emery 1909). His theory implies that a social parasite can develop from a true social species to a socially parasitic one, which eventually parasitizes its social living ancestor. Both a sympatric as well as an allopatric route followed by assortative mating have been suggested to achieve reproductive isolation from the social host species (Wilson 1971; Buschinger 1986, 1990; West-Eberhard 1986; Ward 1989; Bourke and Franks 1991; Bourke and Franks 1995; Lowe et al. 2002). It is obvious that evolutionary time scales usually impose difficulties to pinpoint the actual circumstances leading to the speciation of a social parasite. Nevertheless, close relatedness between host and social parasite at the species level has been inferred with genetic markers in bumblebees (Pedersen 1996) and in *Myrmica* ants suggesting evidence of a sympatric evolutionary pathway to Emery's rule (Savolainen and Vepsäläinen 2003). An intraspecific host-social parasite system which allows the study of Emery's rule contemporaneously may give further insights into new sympatric barriers to gene flow.

1.3 Worker reproduction in honeybees (*Apis mellifera*)

The Western honeybee, *Apis mellifera*, is endemic to western Asia as well as the European and African continents (Ruttner 1988; Fuchs 1998a,b; Hepburn and Radloff 1998). Honeybee colonies are headed by a single multiple mated queen, which produces a population of several thousand workers (~10,000 to 60,000) and hundreds of male sexuals (= drones; Moritz and Southwick 1992; Seeley 1985). The nest is located in cavities and constructed of wax produced by the honeybee workers (Hepburn 1986). Honeybee workers cannot mate but retain functional ovaries and can lay unfertilised eggs that develop into males (= arrhenotoky; Ruttner and Hesse 1981; Winston 1987; Page and Erickson 1988; Visscher 1989). Normally the presence of both the queen (Butler 1959; Hoover et al. 2003) and brood (Arnold et al. 1994) inhibit worker ovary activation. Thus, the reproductive output of workers in most queen-right colonies is negligible, because only a single worker in 10,000 has full-sized eggs in her ovaries (Ratnieks 1993; Visscher 1996). Nevertheless, these few workers can lay a high proportion (7%) of the total male eggs in a colony (Visscher 1996), but very few worker-laid eggs develop into adult drones. Only about 0.1% of a colony's males are worker derived (Visscher 1989, 1996; Ratnieks 1993). Several mechanisms are probably responsible for this. Multiple mating by the queen results in worker population comprising

of a mixture of super-sisters ($r=0.75$), which share also the same father, and half-sisters ($r=0.25$), which only share the mother, the queen. Thus, workers are more related to their brothers ($r=0.25$; sons produced by the queen) than to the average worker-produced male ($r=0.125$). Individual worker will always prefer to produce her own offspring, since she is more related to her own sons ($r=0.5$) than to any other males produced by the colony, but worker reproduction is not typically tolerated because it reduces the inclusive fitness of non reproducing workers. In order to limit worker reproduction in the colony workers with activated ovaries can be recognized and attacked by their non reproducing nestmates (Visscher and Dukas 1995). In addition, oophagy of worker laid eggs is found (Ratnieks and Visscher 1989) as predicted in the “worker policing hypothesis” of Woyciechowski and Lomnicki (1987) and Ratnieks (1988). However, worker-laid eggs show a lower egg viability than queen-laid ones (Pirk et al. 2004), providing an additional explanation for egg eating and a further reason why worker reproduction is only infrequently in queen right *A. mellifera* colonies.

Attention should be paid to anarchistic *A. mellifera* colonies which represent a very rare example of worker reproduction (Oldroyd et al. 1994; Barron et al. 2001). In these queenright colonies, many workers activate their ovaries and workers’ sons are produced by evading worker policing (Oldroyd and Ratnieks 2000). Anarchistic workers are less responsive to inhibitory signals (Hoover et al. 2005) but do not show a queen-like pheromonal secretion (Oldroyd et al. 1999). The frequency of anarchistic worker reproduction in *A. mellifera* populations is low (Oldroyd et al. 1994).

The rare occurrence of worker reproduction under queen right conditions is in strong contrast to colonies that have hopelessly lost their queen, because worker reproduction is the only way to gain fitness. After the colony becomes queenless, about 10% of the workers will have ripe eggs in their ovaries, and many eggs are laid (Velthuis 1970; Page and Erickson 1988). But not all workers contribute equally to the drone offspring in queenless colonies. Some subfamilies contribute a disproportionately greater number of offspring than others (Martin et al. 2004). This is also the case in queen less colonies of the Cape honeybee, *A. m. capensis* (Moritz et al. 1996).

1.4 The Cape honeybee (*Apis mellifera capensis*)

The Cape honeybee, *A. m. capensis*, native to the fynbos biome of the southern tip of Africa (Alpatov 1933; Tribe 1983; Hepburn and Crewe 1991; Hepburn and Jacot-Guillarmod 1991; Hepburn and Radloff 1998) is characterized by a unique set of genetic, behavioural, and physiological traits related to worker reproduction (Hepburn and Radloff 1998). Laying workers of the Cape honeybee mostly produce diploid female offspring (= thelytoky, Onions 1912; Anderson 1963; Neumann et al. 2000; Lattorff et al. 2005). The meiosis of *A. m. capensis* workers shows an extremely low crossing over frequency (Baudry et al. 2004). A central fusion of the meiotic products during automixis (Verma and Ruttner 1983) restores the former diploid genotype. Thus, the majority of *A. m. capensis* worker’s offspring is female and almost clonal (Moritz and Haberl 1994; Baudry et al. 2004). Recently, has been shown that only a single recessive allele, *th*, at a single locus determines thelytokous parthenogenesis in honeybee workers (Lattorff et al. 2005).

Thelytokously produced worker offspring show the genetic structure of a clone and is thus equally related to the queen laid female offspring irrespective of the degree of polyandry. Therefore, the theoretical assumption of worker policing does not hold for *A. m. capensis* workers (Greeff 1996). But when worker reproduction appears to be costly at the colony level or the worker laid eggs have a lower viability, then worker policing is expressed despite the absence of relatedness benefits (Pirk et al. 2003). Nevertheless, Anderson (1963) and Hepburn et al. (1991) reported that about 2% of *A. m. capensis* workers have partially activated ovaries which is ~200 times higher (see above) than in arrhenotokous *A. mellifera* subspecies (Ratnieks 1993; Visscher 1996). Worker reproduction in the presence of a queen is much more frequent in *A. m. capensis* than in European honeybee subspecies (Moritz et al. 1999; Beekman et al. 2002). In contrast to arrhenotokous worker reproduction, thelytokous worker lineages have no chance to introgress back into the *A. m. capensis* gene pool via drone production (Page and Erickson 1988). But diploid larvae of *A. m. capensis* worker offspring can either develop into workers or queens (Hepburn and Radloff 1998) depending on the diet of the larvae (Winston 1987). Thus, raising sexual reproducing queens from worker-laid eggs is the only possible way for workers to achieve fitness relevant to the Cape honeybee population level.

Cape honeybee workers can also develop into an inter-caste phenotype the so-called pseudoqueen (Crewe and Velthuis 1980; Velthuis et al. 1990). Pseudoqueens reproduce exclusively parthenogenetically because they cannot mate, but they have a spermatheca and a high number of activated ovarioles in their ovaries. This ovary activation (~5 d latency) is very fast under queenless conditions (Ruttner and Hesse 1981), compared to other honeybee subspecies such as *A. m. carnica* with up to 30 d latency (Ruttner and Hesse 1981). Laying Cape honeybee workers show considerable longevity, 3-5 months; (Velthuis et al. 1990); up to five months and more, (Tribe and Allsopp 2001), which is approximately five fold longer as the life expectancy of non-reproducing workers (Winston 1987). The pheromonal secretion of such workers is queen-like in their mandibular glands (Ruttner et al. 1976; Hemmling et al. 1979; Crewe and Velthuis 1980; Moritz et al. 2000, 2004; Simon et al. 2005) in their tergal glands (Wossler and Crewe 1999a,b) and finally in their Dufour's gland (Martin and Jones 2004). Cape honeybee pseudoqueens truly resemble queens and can e.g. suppress queen rearing and ovarian development (Hepburn et al. 1988) as well as induce retinue behaviour in other workers (Anderson 1968). But not only *A. m. capensis* workers have a substantial pheromone secretion even the queens produce a stronger mandibular pheromonal secretion compared to those of other honeybee subspecies (Crewe 1988; Wossler 2002).

A set of behavioural predispositions for social parasitism persist in *A. m. capensis* workers (see Neumann and Hepburn 2002 for a review). Cape honeybee workers disperse into foreign colonies more frequently than *A. m. scutellata* workers (Neumann et al. 2001). Cape honeybee workers get preferentially fed in colonies of other *A. mellifera* subspecies (Beekman et al. 2000). Within the host colony drifted workers are more idle and more often observed in areas away from the queen (Neumann et al. 2003a). Furthermore, workers of the Cape honeybee which avoid the queen can express a queen-like pheromonal secretion (Moritz et al. 2002) and achieve successful reproduction (Neumann et al. 2003b). Indeed, female worker laid brood commonly

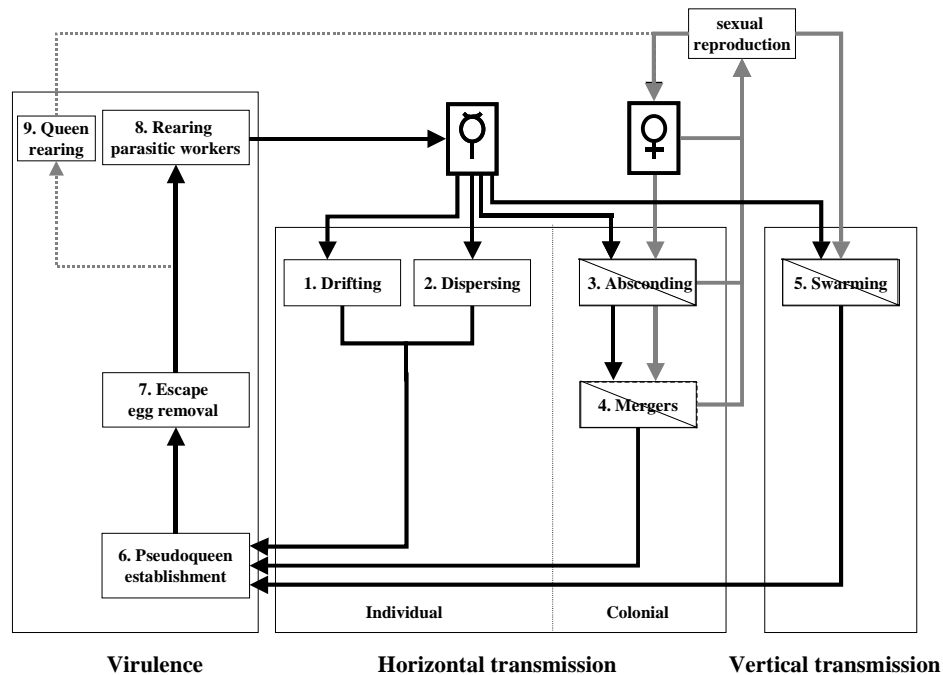
occurs above the queen excluder in routine *A. m. capensis* beekeeping (Petty 1922). Thus, *A. m. capensis* workers show beside the high reproductive capacity of pseudoqueens also behavioural pre-adaptations for a socially parasitic life strategy (Neumann and Hepburn 2002).

1.5 Socially parasitic workers and the “Dwindling Colony Syndrome”

The potential of worker selfishness to undermine the evolution of cooperation, is an interesting issue for the evolution of eusociality. The identification of workers which achieve direct fitness due to the invasion of foreign colonies is a new scientific challenge in the field of worker reproduction. In order to maximise their individual fitness arrhenotokous laying workers of the bumble bees *Bombus terrestris* (Lopez-Vaamonde 2004), *Bombus occidentalis* and *Bombus impatiens* (Birmingham et al. 2004) reproduce at the expense of conspecific host colonies. Interspecific social parasitism by workers also takes place between nests of *Vespula consobrina* and *Vespula atropilosa* wasps, where male-producing reproductive workers invade hosts' nests (Akre et al. 1976). Recently naturally occurring worker social parasitism in *Apis florea* was discovered (Nanork et al. 2005). When an *A. florea* queen dies, unrelated workers move into her colony and lay their own eggs. After queen loss more non-nestmate worker had activated ovaries and produced more male offspring than nestmate workers did (Nanork et al. 2005).

Hamilton (1964) stated that the production of female offspring may open a road for selfish selection in workers. Indeed, thelytokous production of females by workers has important consequences for within-colony relatedness and the occurrence of conflict over reproduction (Greeff 1996). Thus, there is no relatedness benefit for raising the queen's offspring over other worker's offspring. In addition, thelytokous parthenogenesis, which results in the production of female worker offspring enhances the individual fitness of a worker due to the lack of the genetic cost of meiosis (Williams 1975). Thelytoky sets the stage for the establishment of parasitic worker lineages, because pre-adapted gene complexes are not disturbed by genetic recombination (Crow and Kimura 1965; Dobzhansky 1970). These parasitic lineages can persist until they find new host colonies to infest (Neumann and Hepburn 2002). Workers of the ant *Pristomyrmex punctatus* (Tsuji 1995; Sasaki and Tsuji 2003) as well as workers of the Cape honeybee *Apis mellifera capensis* (Johannsmeier 1983; Velthuis et al. 1990; Neumann and Moritz 2002; Neumann and Hepburn 2002) can become thelytokous social parasites.

Fig. 1: (taken from Neumann and Hepburn 2002) Minimum configurations for the reproductive cycle of social parasitic Cape honeybee workers. Shaded boxes and grey lines represent the normal sexual reproductive pathway of *A. m. capensis*. Blank boxes and black lines represent the social parasitic pathway of laying workers. Shaded/blank boxes represent steps, which are involved in both the sexual reproductive and parthenogenetic laying worker pathway. Dotted lines represent rare events (see Swart et al. 2001 and Martin et al. 2002b).



Usurpations by socially parasitic *A. m. capensis* workers of colonies of other honeybee subspecies (*A. m. ligustica*) are long known (Onions 1912). Intraspecific social parasitism by Cape honeybee workers has also been confirmed for a variety of other honeybee subspecies such as *A. m. scutellata*, *A. m. carnica*, *A. m. caucasica* and *A. m. mellifera* (Koeniger and Würkner 1992; Woyke 1995). The most prominent example for Cape honeybee worker social parasitism is the “dwindling-colony-syndrome” of *A. m. scutellata* colonies (Allsopp and Crewe 1993). Usurpation by socially parasitic workers results in the death of the host colony in about nine weeks after initial infestation (Neumann and Hepburn 2002). As soon as many social parasites are in a colony, the host queen is lost probably due to lethal fights with parasitic pseudoqueens (Moritz et al. 2003). The surviving *A. m. capensis* workers monopolize the colony reproduction which eventually causes the collapse of the host colony (Neumann and Hepburn 2002; Swart 2003). If the majority of *A. m. scutellata* workers are replaced by parasitic workers, regular brood rearing and foraging ceases (Martin et al. 2002b), because most *A. m. capensis* workers invest only in individual reproduction (Hillesheim et al. 1989). As a result, eggs are laid but no parasites are reared to the adult stage. In contrast to usurpations by queenright *A. m. scutellata* swarms in the Americas (Schneider et al. 2004) the infestation by *A. m. capensis* workers results in the death of the entire colony. Figure 1 shows the socially parasitic life cycle of Cape honeybee workers as described in

Neumann and Hepburn (2002). The “dwindling colony syndrome” is the result of a large scale introduction of *A. m. capensis* colonies into the range of the adjacent subspecies *A. m. scutellata* by professional beekeepers in 1990. Since then, socially parasitic workers have caused considerable harm to the *A. m. scutellata* based apiculture, resulting in significant losses and even the complete loss of all managed colonies of large scale beekeepers in every season for the last decade (Allsopp and Crewe 1993; Hepburn and Allsopp 1994; Greeff 1997; Swart et al. 2001; Neumann and Moritz 2002; Swart 2003). A recent genetic study by Baudry et al. (2004) has shown that a single clonal lineage, derived from one individual worker by uninterrupted generations of thelytokous parthenogenesis caused the mass extinctions of managed *A. m. scutellata* colonies in vast regions of northern South Africa. Furthermore, multivariate discriminant analyses of morphometric characters confirmed that this clonal lineage originated from the *A. m. capensis* distribution range in the Western Cape (Neumann et al. 2002). Parasitic Cape honeybee workers can evade worker policing (Martin et al. 2002a) and they get preferentially fed by the *A. m. scutellata* host workers (Calis et al. 2002; Allsopp et al. 2003), resulting in a parasitic pseudoqueen phenotype with high reproductive potential. But only a small proportion of socially parasitic workers have fully developed ovaries (Martin et al. 2002b). Thus, during the course of infestation it remains unclear how reproduction is partitioned among initial infesting *A. m. capensis* social parasites and their offspring generation.

1.6 Evolution of *A. m. capensis* worker social parasitism

Under thelytoky, evolutionary theory predicts strong selection for traits related to worker reproduction (Hamilton 1964; Greeff 1996). These kind of traits showing a considerable selective value in *A. m. capensis* workers (Moritz and Hillesheim, 1985). Furthermore, on the level of intracolony selection Moritz et al. (1996) demonstrated that after queen loss reproductive competition among laying workers occurs, resulting in the dominance of only a few reproducing worker subfamilies. This strong intracolony selection is most likely mediated through the queen-like pheromone secretion of reproducing workers. Due to its function as primer pheromone (Kaatz et al. 1992; Winston and Slessor 1998; Hoover et al. 2003) the queen substance 9-keto-2-(E)-decanoic acid (9-ODA) is a key factor in establishing dominance hierarchies among workers (Moritz et al. 2000, 2004; Simon et al. 2005). Indeed this major component of the queen mandibular gland pheromone (QMP) is secreted from *A. m. capensis* workers in large quantities (Ruttner et al. 1976; Hemmling et al. 1979; Simon et al. 2005). The large scale introduction of *A. m. capensis* into the endemic range of *A. m. scutellata* was the first step in the selection of the socially parasitic lineage (Baudry et al. 2004). In the new environment, intracolony selection will favour the most adapted parasitic worker genotype. This worker genotype must show a low response to the QMP secretion of the *A. m. scutellata* host queen, as well as a swift development of a own queen like pheromone signal and activated ovaries. Thus, socially parasitic workers should have a queen-like pheromone signal with large quantities of 9-ODA in their mandibular glands to establish their reproductive dominance. But until now nothing is known about their

mandibular gland secretion. The predicted reproductive potential of socially parasitic pseudoqueens in combination with the uniform genetic background further serve as a model system to analyse the regulation of the (9-ODA) biochemical pathway and its possible function in this highly reproductive worker lineage.

1.7 Aims of the study

To investigate and characterise social parasitism by reproductive honeybee workers is the central aim of this work. The Cape honeybee *Apis mellifera capensis* will serve as a study system in this thesis. Worker reproduction by *A. m. capensis* workers is a common phenomenon. Some important pre-adaptations and ultimate mechanisms of social parasitism by laying *A. m. capensis* workers, such as thelytoky and the development of pseudoqueens are identified. On the other hand, several proximate mechanisms of the socially parasitic pathway are only poorly understood. Surprisingly, comprehensive information about the biology of the parasite is not known and data about the parasites' interactions with their conspecific hosts are widely lacking. Furthermore, the possible persistence of naturally occurring social parasitism among *A. m. capensis* colonies, the influence of socially parasitic workers on the native *A. m. scutellata* host population and further aspects of the socially parasitic life cycle were missing but are investigated in this thesis. The manuscripts/papers on which this thesis is based are further addressing the evolution of a queenless socially parasitic honeybee lineage. In particular, reproductive barriers to gene flow as well as the impact that queen-like pheromonal secretion could have on this process are studied in detail.

1.8 References

- Akre RD, Garnett WB, MacDonald JF, Greene A, Landolt P (1976) Behavior and colony development of *Vespula pensylvanica* and *V. atropilosa* (Hymenoptera: Vespidae). *J Kans Entomol Soc* **49**: 63-84.
- Alpatov WW (1933) South African bees biometrically investigated. *Bee World* **14**: 62-64.
- Allsopp MH, Crewe R (1993) The Cape honeybee as a Trojan horse rather than the hordes of Jenghiz Khan. *Am Bee J* **133**: 121-123.
- Allsopp MH, Calis JNM, Boot WJ (2003) Differential feeding of worker larvae affects caste characters in the Cape honeybee, *Apis mellifera capensis*. *Behav Ecol Sociobiol* **54**: 555-561.
- Anderson RH (1963) The laying worker in the Cape honeybee, *Apis mellifera capensis*. *J Apic Res* **2**: 85-92.
- Anderson RH (1968). The effect of queen loss on colonies of *Apis mellifera capensis*. *S Afr J Agric Sci* **11**: 383-388.
- Arnold G, LeConte Y, Trouiller J, Hervet H, Chappe B, Masson C (1994) Inhibition of worker honeybee ovaries development by a mixture of fatty-acid esters from larvae. *Comptes Rendus De L Academie Des Sciences Serie III* **317**: 511-515.

- Barron AB, Oldroyd BP, Ratnieks FLW (2001) Worker reproduction in honey-bees (*Apis*) and the anarchic syndrome: a review. *Behav Ecol Sociobiol* **50**: 199-208.
- Baudry E, Kryger P, Allsopp M, Koeniger N, Vautrin D, Mougél F, Cornuet J-M, Solignac M (2004) Whole-genome scan in thelytokous-laying workers of the Cape honeybee (*A. m. capensis*): Central fusion, reduced recombination rates and centromere mapping using half tetrad analysis. *Genetics* **167**: 243-252.
- Beekman M, Calis JNM, Boot WJ (2000) Parasitic honeybees get royal treatment. *Nature* **404**:723.
- Beekman M, Good G, Allsopp MH, Radloff S, Pirk CWW, Ratnieks FLW (2002) A non-policing honey bee colony (*Apis mellifera capensis*). *Naturwissenschaften* **89**: 479-482.
- Birmingham AL, Hoover SE, Winston ML Ydenberg RC (2004) Drifting bumble bee (Hymenoptera: Apidae) workers in commercial greenhouses may be social parasites. *Can J Zool* **82**: 1842-1853.
- Bourke AFG Franks NR (1991) Alternative adaptations, sympatric speciation and the evolution of parasitic, inquiline ants. *Biol J Linn Soc* **43**: 157-178.
- Bourke AFG, Franks NR (1995) *Social Evolution in Ants*. Princeton University, New Jersey.
- Buschinger A (1986) Evolution of social parasitism in ants. *Trends Ecol Evol* **1**: 155-160.
- Buschinger A (1990) Sympatric speciation and radiative evolution of socially parasitic ants – Heretic hypotheses and their factual background. *Z Zool Syst Evolutionsforsch* **28**: 241-260.
- Butler CG (1959) The source of the substance produced by a queen honeybee (*Apis mellifera*) which inhibits development of the ovaries of the workers of her colony. *Proc R Entomol Soc Lond* **34**: 137-138.
- Calis JNM, Boot WJ, Allsopp MH, Beekman M (2002) Getting more than a fair share: nutrition of worker larvae related to social parasitism in the Cape honey bee *Apis mellifera capensis*. *Apidologie* **33**: 193-202.
- Crewe RM (1988) Natural history of honeybee mandibular gland secretions: development of analytical techniques and the emergence of complexity. In: *Africanized Honey Bees and Bee Mites* (G.R. Needham, R.E. Page, M. Delfinado-Baker and C.E. Bowman, (Eds.). Ellis Horwood, Chichester. pp. 149-158.
- Crewe RM, Velthuis HHW (1980) False queens: a consequence of mandibular gland signals in worker honeybees. *Naturwissenschaften* **67**: 467-469.
- Crow JF, Kimura M (1965) Evolution in sexual and asexual populations. *Am Nat* **99**: 439-450.
- Crozier RH, Pamilo P (1996) *Evolution of social insect colonies*. Oxford University Press, Oxford.
- Darwin C (1859) *On the origin of species*. John Murray, Albemarle Street, London.
- Dobzhansky T (1970) *Genetics of the Evolutionary Process*. Columbia Uni. Press, New York.
- Emery C (1909) Über den Ursprung der dulotischen, parasitischen und myrmekophilen Ameisen. *Biol Zentralbl* **29**: 352-362.
- Fuchs S (1998a) Visualizing the geographic pattern of morphometric Variation in *Apis mellifera* L. *Apidologie* **29**: 468-469.

- Fuchs S (1998b) Die Oberurseler Datenbank in Farbe. *Die Biene* **8**: 17.
- Greeff JM (1996) Effects of thelytokous worker reproduction on kin-selection and conflict in the Cape honeybee, *Apis mellifera capensis*. *Phil Trans R Soc Lond [B]* **351**: 617-625.
- Greeff JM (1997) The Cape honeybee and her way north: an evolutionary perspective. *S Afr J Sci* **93**: 306-308.
- Hamilton WD (1964a,b) The genetical evolution of social behaviour, I, II. *J Theor Biol* **7**: 1-52.
- Hemmling C, Koeniger K, Ruttner F (1979) Quantitative Bestimmung der 9-oxododecensäure im Lebenszyklus der Kapbiene (*Apis mellifera capensis* Escholtz). *Apidologie* **10**: 227-240.
- Hepburn HR (1986) *Honeybees and Wax*. Springer-Verlag, Heidelberg
- Hepburn HR, Crewe RM (1991) Portrait of the Cape honeybee, *Apis mellifera capensis*. *Apidologie* **22**: 567-580.
- Hepburn HR, Jacot-Guillarmod A (1991) The Cape honeybee and the fynbos biome. *S Afr J Sci* **87**: 70-73.
- Hepburn HR, Allsopp MH (1994) Reproductive conflict between honeybees: usurpation of *Apis mellifera scutellata* colonies by *Apis mellifera capensis*, *S Afr J Sci* **90**: 247-249.
- Hepburn HR, Radloff SE (1998) *Honeybees of Africa*. Springer Verlag, Berlin Heidelberg New York.
- Hepburn HR, Nefdt RJC, Whiffler LA (1988) Queen loss in the Cape honeybee: the interactions of brood, laying workers (false queens?) and queen cells, *S Afr J Sci* **80**: 363-368.
- Hepburn HR, Magnuson P, Herbert L, Whiffler LA (1991) The development of laying workers in field colonies of the Cape honey bee. *J Apic Res* **30**: 107-112.
- Hillesheim E, Koeniger N, Moritz RFA (1989) Colony performance in honeybees depends on the proportion of subordinate and dominant workers. *Behav Ecol Sociobiol* **24**: 291-296.
- Hölldobler B, Wilson EO (1990) *The Ants*. Springer Verlag, Berlin, Heidelberg, New York.
- Hoover SER, Keeling CI, Winston ML, Slessor KN (2003) The effect of queen pheromones on worker honey bee ovary development. *Naturwissenschaften* **90**: 477-480.
- Hoover SER, Keeling CI, Winston ML, Oldroyd BP (2005) Retinue attraction and ovary activation: responses of wild type and anarchistic honey bees (*Apis mellifera*) to queen and brood pheromones. *Behav Ecol Sociobiol* **59**: 278-284.
- Kaatz HH, Hildebrandt H, Engels, W (1992) Primer effect of queen pheromone on juvenile hormone biosynthesis in adult worker honey bees. *J Comp Physiol B* **162**: 588-592.
- Koeniger G, Würkner W (1992) Die Kap Honigbienen (*Apis mellifera capensis*) Natürliche Verbreitung und die Schwierigkeiten der Haltung unter unseren klimatischen Bedingungen. *Die Biene* **128**: 583-588.
- Lattorff HMG, Moritz RFA, Fuchs S (2005) A single locus determines thelytokous parthenogenesis of laying honeybee workers (*Apis mellifera capensis*). *Heredity* **94**: 533-537.

- Johannsmeier MF (1983) Experiences with the Cape bee in the Transvaal. *S Afr Bee J* **55**: 130-138.
- Lopez-Vaamonde C, Koning JW, Brown RM, Jordan WC, Bourke AFG (2004) Social parasitism by male-producing reproductive workers in a eusocial insect. *Nature* **430**: 557-560.
- Lowe RM, Ward SA, Crozier RH (2002) The evolution of parasites from their hosts: intra- and interspecific parasitism and Emery's rule. *Proc R Soc Lon [B]* **269**: 1301-1305.
- Martin CG, Oldroyd BP, Beekman M (2004) Differential reproductive success among subfamilies in queenless honeybee (*Apis mellifera* L.) colonies. *Behav Ecol Sociobiol* **56**: 42-49.
- Martin SJ, Jones GR (2004) Conservation of Bio synthetic pheromone pathways in honeybees *Apis*. *Naturwissenschaften* **91**: 232-236.
- Martin SJ, Beekman M, Wossler TC, Ratnieks FLW (2002a) Parasitic Cape honeybee workers, *Apis mellifera capensis* evade policing. *Nature* **415**: 163-165.
- Martin SJ, Wossler TC, Kryger P (2002b) Usurpation of African *Apis mellifera scutellata* colonies by parasitic *Apis mellifera capensis* workers. *Apidologie* **33**: 215–232.
- Michener CD (1974) *The Social Behavior of the Bees; a Comparative Study*. Belknap/Harvard Univ. Press, Cambridge.
- Moritz RFA, Hillesheim E (1985) Inheritance of dominance in honeybees (*Apis mellifera capensis* Esch.). *Behav Ecol Sociobiol* **17**: 87-89.
- Moritz RFA, Southwick EE (1992) *Bees as superorganisms. An evolutionary reality*. Springer Verlag, Berlin, Heidelberg, New York.
- Moritz RFA, Habermehl M (1994) Lack of meiotic recombination in thelytokous parthenogenesis of laying workers of *Apis mellifera capensis* (the Cape honeybee). *Heredity* **73**: 98-102.
- Moritz RFA, Kryger P, Allsopp MH (1996) Competition for royalty in bees. *Nature* **384**: 31.
- Moritz RFA, Kryger P, Allsopp M (1999) Lack of worker policing in the Cape Honeybee (*Apis mellifera capensis*). *Behaviour* **136**: 1079-1092.
- Moritz RFA, Simon UE, Crewe RM (2000) Pheromonal contest between honeybee workers. *Naturwissenschaften* **87**: 395-397.
- Moritz RFA, Crewe RM, Hepburn HR (2002) Queen avoidance and mandibular gland secretion of honeybee workers (*Apis mellifera* L.). *Insectes soc* **49**: 86-91.
- Moritz RFA, Pflugfelder J, Crewe RM (2003) Lethal fighting between honeybee queens and parasitic workers (*Apis mellifera*). *Naturwissenschaften* **90**: 378-381.
- Moritz RFA, Lattorff HMG, Crewe RM (2004) Honeybee workers (*Apis mellifera capensis*) compete for producing queen-like pheromone signals. *Proc Soc Lond B Suppl* **271**: S98-S100.
- Nanork P, Paar J, Chapman NC, Wongsiri S, Oldroyd BP (2005) Asian honeybees parasitize the future dead. *Nature* **437**: 829.
- Neumann P, Hepburn HR (2002) Behavioural basis for social parasitism of Cape honeybees (*Apis mellifera capensis* Esch.). *Apidologie* **33**: 165-192.
- Neumann P., Moritz RFA (2002) The Cape honeybee phenomenon: the evolution of a social parasite in real time? *Behav Ecol Sociobiol* **52**: 271-281.

- Neumann P, Hepburn HR, Radloff SE (2000a) Modes of worker reproduction, reproductive dominance and brood cell construction in queenless honeybee (*Apis mellifera*) colonies. *Apidologie* **31**: 479-486.
- Neumann P, Radloff SE, Hepburn HR (2002) Parasitic Cape bees in the northern regions of South Africa: source of the founder population. *S African J Sci* **98**: 404-406.
- Neumann P, Radloff SE, Pirk CWW, Hepburn HR (2003a) The behaviour of drifted Cape honeybee workers (*Apis mellifera capensis*): predisposition for social parasitism? *Apidologie* **34**: 585-590.
- Neumann P, Pirk CWW, Hepburn HR, Moritz RFA (2003b) Spatial differences in worker policing facilitate social parasitism by Cape honeybee workers (*Apis mellifera capensis* Esch.) in queenright host colonies. *Insectes Soc* **50**: 109-113.
- Neumann P, Radloff SE, Moritz RFA, Hepburn HR, Reece SL (2001) Social parasitism by honeybee workers (*Apis mellifera capensis* Escholtz): host finding and resistance of hybrid host colonies. *Behav Ecol* **12**: 419-428.
- Oldroyd BP, Ratnieks FLW (2000) Anarchistic honey bee workers evade worker policing by laying eggs that have low removal rates. *Behav Ecol Sociobiol* **47**: 268-273.
- Oldroyd BP, Halling L, Rinderer TE (1999) Development and behaviour of anarchistic honeybees. *Proc Roy Soc Lond B* **266**: 1875-1878.
- Oldroyd BP, Smolenski AJ, Cornuet JM, Crozier RH (1994) Anarchy in the beehive: a failure of worker policing in *Apis mellifera*. *Nature* **371**: 479.
- Onions GW (1912) South African "fertile-worker bees". *S Afr Agric J* **1**: 720-728.
- Page RE Jr, Erickson EH Jr (1988) Reproduction by worker honey bees (*Apis mellifera* L.). *Behav Ecol Sociobiol* **23**: 117-126.
- Pedersen BV (1996) A phylogenetic analysis of cuckoo bumblebees (*Psithyrus*, Lepeletier) and bumblebees (*Bombus* Latreille) inferred from sequences of the mitochondrial gene cytochrome oxidase. I. *Mol Phylogenet Evol* **5**: 289-297.
- Petty FW (1922) Workers laying in comb of extracting supers, Elsenberg Apiary. *J Dep Agr Union S Afr* **4**: 122-124.
- Pirk CWW, Neumann P, Hepburn HR (2002) Egg removal and egg laying by workers are positively correlated in queenright Cape honeybee colonies (*Apis mellifera capensis* Esch.). *Apidologie* **33**: 203-212.
- Pirk CWW, Neumann P, Ratnieks FLW (2003) Cape honeybees, *Apis mellifera capensis*, police worker laid eggs despite the absence of relatedness benefits. *Behav Ecol* **14**: 347-352.
- Pirk CWW, Neumann P, Hepburn R, Moritz RFA, Tautz J (2004) Egg viability and worker policing in honey bees. *Proc Natl Acad Sci USA* **101**: 8649-8651.
- Price PW (1980) *Evolutionary Biology of Parasites*. Princeton University Press.
- Price PW (1977) General concepts on the evolutionary biology of parasites. *Evolution* **31**: 405-431.
- Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *Am Nat* **132**: 217-236.
- Ratnieks FLW (1993) Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies. *Behav Ecol Sociobiol* **32**: 191-198.

- Ratnieks FLW, Visscher PK (1989) Worker policing in the honeybee. *Nature*: 342:796-797.
- Ratnieks FLW, Foster KR, Wenseleers T (2006) Conflict resolution in insect societies. *Annu Rev Entomol*: in press.
- Ruttner F, Koeniger N, Veith HJ (1976) Queen substance bei eierlegenden Arbeiterinnen der Honigbiene *Apis mellifica* L. *Naturwissenschaften* **63**: 434.
- Ruttner F (1988) *Biogeography and Taxonomy of Honeybees*. Springer-Verlag, Berlin.
- Ruttner F, Hesse B (1981) Rassenspezifische Unterschiede in Ovarentwicklung und Eiablage von weisellosen Arbeiterinnen der Honigbiene *Apis mellifera* L. *Apidologie* **12**: 159-183.
- Sasaki T, Tsuji K (2003) Behavioral property of unusual large workers in the ant, *Pristomyrmex pungens*. *J Ethol* **21**: 145-151.
- Savolainen, R, Vepsäläinen K (2003) Sympatric speciation through intraspecific social parasitism. *Proc Natl Acad Sci USA* **100**: 7169-7174.
- Schmid-Hempel P (1998) *Parasites in social insects*. Princeton University Press Princeton, USA, 409 pp.
- Schneider SS, Deeby T, Gilley DC, DeGrandi-Hoffman G (2004) Seasonal nest usurpation of European colonies by African swarms in Arizona, USA. *Insectes Soc* **51**: 359-364.
- Seeley TD (1995) *The Wisdom of the Hive. The Social Physiology of Honey Bee Colonies*. Harvard University Press, Cambridge.
- Simon U, Moritz RFA, Crewe RM (2005) Reproductive dominance among honeybee workers in experimental groups of *Apis mellifera capensis*. *Apidologie* **36**: 413-419.
- Swart JD (2001) Beginning Beekeeping. Pages 47-52 in Johannsmeier MF (ed.) *Beekeeping in South Africa*. 3rd edition, revised, Plant Protection Research Institute Handbook No. 14, Agricultural Research Council of South Africa, Pretoria, South Africa.
- Swart JD (2003) The occurrence of *Nosema apis* (Zander), *Acarapis woodi* (Rennie) and the Cape problem bee in the summer rainfall region of South Africa, M.Sc. thesis, Rhodes University, Grahamstown, South Africa.
- Thompson JN (1994) *The Coevolutionary Process*. The University of Chicago Press.
- Tribe GD (1983) What is the Cape bee? *S Afr Bee J* **55**: 77-87.
- Tribe GD, Allsopp MH (2001) Honeybee reproduction. In: *Beekeeping in South Africa*. (M.F. Johannsmeier, Ed.) Plant Protection Research Institute, Pretoria, South Africa, pp 40-46.
- Tsuji K (1995) Reproductive conflicts and levels of selection in the ant *Pristomyrmex pungens*: Contextual analysis and partitioning of covariance. *Am Nat* **143**: 586-607.
- Velthuis HHW (1970) Ovarian development in *Apis mellifera* worker bees. *Entomol Exp Appl* **13**: 377-394.
- Velthuis HHW, Ruttner F, Crewe RM (1990) Differentiation in reproductive physiology and behaviour during the development of laying worker honeybees. In: Engels W (ed) *Social Insects*. Springer Verlag, Berlin Heidelberg New York, pp 231-243.
- Verma S, Ruttner F (1983) Cytological analysis of the thelytokous parthenogenesis in the Cape honeybee (*Apis mellifera capensis* Escholtz). *Apidologie* **14**: 41-57.

- Visscher PK (1989) A quantitative study of worker reproduction in honey bee colonies. *Behav Ecol Sociobiol* **25**: 247-254.
- Visscher PK (1996) Reproductive conflict in honey bees: a stalemate of worker egg-laying and policing. *Behav Ecol Sociobiol* **39**: 237-244.
- Visscher PK, Dukas R (1995) Honey bees recognise development of nestmates' ovaries. *Anim Behav* **49**: 542-544.
- Ward PS (1989) Genetic and social changes associated with ant speciation. In *The genetics of social evolution* (eds. Breed MD, Page RE), pp. 123–148. Westview, Boulder.
- West-Eberhard MJ (1986) Alternative Adaptations, Speciation, and Phylogeny (A Review). *Proc Natl Acad Sci USA* **83**: 1388-1392.
- Wilson EO (1971) *The insect societies*. Cambridge, Harvard University Press, London.
- Williams GC (1975) *Sex and Evolution*. Princeton University Press, Princeton.
- Winston ML (1987) *The Biology of the Honey Bee*. Harvard University Press, London.
- Winston ML, Slessor KN (1998) Honey bee primer pheromones and colony organization: gaps in our knowledge. *Apidologie* **29**: 81-95.
- Wossler TC (2002) Pheromone mimicry by *Apis mellifera capensis* social parasites leads to reproductive anarchy in host *Apis mellifera scutellata* colonies. *Apidologie* **33** : 139-163.
- Wossler TC, Crewe RM (1999a) Mass spectral identification of the tergal gland secretions of female castes of two African honeybee races (*Apis mellifera*). *J Apic Res* **38**: 137-148.
- Wossler TC, Crewe RM (1999b) Honeybee queen tergal gland secretion affects ovarian development. *Apidologie* **30**: 311-320.
- Woyciechowski M, Lomnicki A (1987) Multiple mating of queens and sterility of workers among eusocial Hymenoptera. *J Theor Biol* **128**: 317-327.
- Woyke J (1995) Invasion of Capensis bee, in: Magnuson P (Ed.), Proceedings of the First International Electronic Conference on the Cape Bee problem in South Africa, 05-30 June 1995, Pretoria, PPRI, p. 35.

**Social parasitism by Cape honeybee
workers in colonies of their own subspecies
(*Apis mellifera capensis* Esch.)**

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Abstract

Social parasitism is widespread in the eusocial insects. Although social parasites often show a reduced worker caste, unmated workers can also parasitize colonies. Cape honeybee workers, *Apis mellifera capensis*, can establish themselves as social parasites in host colonies of other honeybee subspecies. However, it is unknown whether social parasitism by laying workers also occurs among Cape honeybee colonies. In order to address this question we genotyped worker offspring of six queenless *A. m. capensis* colonies and determined the maternity of the reproducing workers. We found that three non-nestmate workers dominated reproduction in a host colony and produced 62.5% of the progeny. Our results show that social parasitism by laying workers is a naturally occurring part of the biology of Cape honeybees. However, such social parasitism is not frequently found (6.41% of the total worker offspring) probably due to co-evolutionary processes among *A. m. capensis* resulting in an equilibrium between selection for reproductive dominance in workers, colony maintenance and queen adaptation.

Key words: *Apis mellifera capensis*, honeybees, intraspecific social parasitism, intra colonial selection, worker reproduction

Emery's rule in the honeybee (*Apis mellifera capensis*)

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Evolution: to be submitted

Abstract.

Determining the extent and causes of barriers to gene flow is essential for understanding sympatric speciation, but the practical difficulties of quantifying reproductive isolation remain an obstacle to analyzing this process. Social parasites are common in eusocial insects and tend to be close phylogenetic relatives of their hosts (= Emery's rule). Sympatric speciation due to reproductive isolation between host and parasite is a possible evolutionary pathway. Socially parasitic workers of the Cape honeybee, *Apis mellifera capensis*, produce female clonal offspring parthenogenetically and invade the adjacent subspecies *A. m. scutellata*. In the host colony, socially parasitic worker can become pseudoqueens, an intermediate caste with queen-like pheromone secretion. Here we show that over a range of ~275.000 km² all parasitic workers bear the genetic signature of a clone founded by a single ancestral worker genotype. Any gene flow from the host to the parasite is impossible because honeybee workers cannot mate. Gene flow from the parasite to the host is possible since parasitic larvae can also develop into queens. However, we show that despite sympatric coexistence for more than a decade, gene flow between host and social parasite ($F_{st}= 0.32$) and hybridizations (0.71%) are rare, resulting in reproductive isolation. Our data suggest a new barrier to gene flow in sympatry, which is not based on assortative matings but on reproductive division of labor in the eusocial insects, thereby providing a new pathway to Emery's rule.

Key words: barrier to gene flow, division of labor, honeybee, worker social parasitism, sympatric speciation, thelytoky, Emery's rule

**Pheromonal dominance and the selection
of a socially parasitic honeybee worker lineage
(*Apis mellifera capensis* Esch.)**

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Abstract

Self-replicating Cape honeybee workers, *Apis mellifera capensis*, are facultative social parasites of honeybee colonies. Although a large number of Cape honeybee colonies were transported into the range of the neighbouring subspecies *A. m. scutellata*, only a single worker lineage became established as a parasite. This is likely to have been the result of extreme selection among different clonal parasitic worker lineages that were introduced in the host's range. Since honeybee workers compete pheromonally for reproductive dominance, we hypothesised that the selected lineage was able to dominate other parasitic strains and out competed them. With pheromonal contest experiments, we show that this worker lineage dominates in the production of mandibular gland pheromones over all other competitors it has been exposed to. Our results strongly suggest that mandibular gland pheromones have played a key role in the evolution of a single parasitic genotype of *A. m. capensis* workers.

Keywords: social insects, honeybee, *Apis mellifera*, selection, thelytoky, social parasite, worker reproduction, mandibular gland pheromone

Social parasitism by honeybee workers
(*Apis mellifera capensis* Esch.): evidence for
pheromonal resistance to host queen's signals

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Behavioural Ecology and Sociobiology **60**: 785-793.

Abstract

Social parasites exploit their host's communication system to usurp their resources and reproduce. In the honeybee, *Apis mellifera*, worker reproduction is regulated by pheromones produced by the queen and the brood. Workers usually reproduce when the queen is removed and young brood is absent. However, Cape honeybee workers, *A. m. capensis*, are facultative intraspecific social parasites and can take over reproduction from the host queen. Investigating the manner in which parasitic workers pheromonally compete with host queens can help us to understand how such parasitism can evolve and how division of reproductive labour is regulated. In *A. m. capensis*, worker reproduction is associated with the production of queen-like pheromones. With pheromonal contest experiments, we show that *A. m. scutellata* queens do not prevent the production of queen-like mandibular gland compounds by the parasites. Given the importance of these pheromones in acquiring reproductive status, our data suggest that the single invasive lineage of parasitic workers occurring in the range of *A. m. scutellata* was selected for its superior ability to produce these signals despite the presence of a queen. Indeed, such resistance was less frequent amongst other potentially parasitic lineages. Resistance to reproductive regulation by host queens is probably the key factor that enables the evolution of social parasitism by *A. m. capensis* workers. It constitutes a mechanism that allows workers to evade the reproductive division of labour and to follow an alternative reproductive option by acquiring direct fitness in foreign colonies instead of inclusive fitness in their natal nests.

Keywords: honeybee; *Apis mellifera capensis*; Worker reproduction; Social parasitism; Mandibular gland; Pheromonal competition

Dominance hierarchies among clonal socially parasitic workers (*Apis mellifera capensis* Esch.)

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Journal of Comparative Physiology A: to be submitted

Abstract

Dominance hierarchies among honeybee workers, *Apis mellifera*, show a strong genetic variance and are mediated by the production of a suite of fatty acids resulting in a queen-like mandibular gland pheromone (QMP). Subordinate workers produce a different set of fatty acids compared to dominant ones resulting from different biochemical pathways. *A. m. scutellata* host colonies were infested with clonal socially parasitic workers of *A. m. capensis*. 94.4 % of the initially introduced parasites became pseudoqueens with both activated ovaries and queen-like pheromones. However, the offspring of these pseudoqueens had a significantly reduced development although 89 % showed fatty acid synthesis typical of the QMP pathway. The composition was different with the “queen substance” (9-ODA) produced at a much lower level than its precursor (9-HDA). Since 9-ODA is the main active component of QMP, workers stalling the synthesis at 9-HDA are expected to be less dominant. The final oxidative step from 9-HDA to 9-ODA appears to be a second regulatory switch in the biochemical pathway for worker dominance. Our data show that despite the absence of genetic diversity residual QMP variation is sufficient to establish dominance hierarchies among workers, which can be plausibly explained by self-organized interactions based on QMP response thresholds.

Key words:

Biochemical pathway, queen substance, division of labour, honeybee, pheromone.

Infestation levels of *Apis mellifera scutellata* swarms by socially parasitic Cape honeybee workers (*A. m. capensis* Esch.)

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Apidologie 37: 462-470.

Abstract

A single clonal lineage of socially parasitic Cape honeybee workers, *Apis mellifera capensis*, has caused dramatic losses in managed populations of *A. m. scutellata*, raising concerns that wild populations might also be affected. We surveyed *A. m. scutellata* swarms at 27 localities in beekeeping areas (N=87) and in nature reserves (N=79). While eleven swarms were infested in beekeeping areas, we found no infestations in nature reserves. Eight swarms had no symptoms except workers with black tergites. However, DNA data revealed that these workers are not parasitic, showing that diagnoses based on tergite colour alone yield false positive results. Nevertheless, it is practical because we had no false negative diagnoses either. Nature reserves may be important refuges to protect wild *A. m. scutellata* populations against imported honeybees.

Keywords: *Apis mellifera scutellata* / *Apis mellifera capensis* / honeybee / social parasitism / wild population

Social analogue of immune memory in honeybee colonies

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Biol. Letters: to be submitted

Abstract:

Eusocial insect colonies are often referred to as superorganisms consisting of specialized individuals. Such superorganisms can be exploited by a variety of parasites, suggesting the presence of social analogues of immune defences. Indeed, social analogues of fever and encapsulation have been described in honeybees, *Apis mellifera*. Here we show that honeybee colonies reject socially parasitic Cape honeybee workers faster when they have previously been infested. This indicates that a social analogue of immune memory may occur in honeybee colonies, which is usually lacking in individual insects.

Key words: *Apis mellifera*, honeybee, immune system, nestmate recognition, social parasite, worker reproduction

9. Summary

The goal of my thesis was to analyse the social parasitism carried out by laying Cape honeybee, *Apis mellifera capensis*, workers. Intraspecific social parasitism by laying workers was widely unattended or in the case of the honeybee disposed as an artefact of beekeeping. In contrast to that, I have shown in the first part of my thesis that socially parasitic behaviour of workers is not only a man made phenomenon but is indeed an inherent part of the biology of the Western honeybee. In the second part of my thesis, I have analysed in detail the proximate mechanisms by which *A. m. capensis* workers establish themselves as social parasites within *A. m. scutellata* colonies. This thesis, thus, replenishes substantially the more descriptive papers about intraspecific social parasitism by laying workers in bumblebees and dwarf honeybees. Furthermore, the results may help to understand and manage the socially parasitic *A. m. capensis* workers which are a severe pest for apiculture in Northern South Africa.

9.1 Honeybee workers as social parasites

In eusocial insect colonies intraspecific social parasitism by workers is a widely unattended pathway of worker reproduction. This worker behaviour follows an alternative reproductive option by acquiring direct fitness in foreign colonies instead of inclusive fitness in their natal nests. The rare examples of social parasitism caused by workers have only recently been explored. In the bumblebee *Bombus terrestris*, for example, foreign worker reproduction takes place after the competition point (Lopez-Vaamonde et al. 2004). In the dwarf honeybee, *Apis florea*, non-nestmate workers reproduce in orphaned colonies (Nanork et al. 2005). Cape honeybee workers, *A. m. capensis*, have been described several times as social parasites of other honeybee subspecies (e.g. Onions 1912; Lundie, 1954; Johannsmeier, 1983; Koeniger and Würkner 1992; Allsopp and Crewe, 1993; Woyke 1995 among others). These socially parasitic workers cause dramatic changes in the host colony structure. Until now, this complex behaviour is referred to as a man-made phenomenon that arises due to migratory beekeeping management. In my thesis, I addressed the question to whether Cape honeybee workers show socially parasitic behaviour also among colonies of their own subspecies. In this context, I have genotyped worker offspring of six queenless *A. m. capensis* colonies to determine the maternity of the reproducing workers. It turns out that under queenless conditions worker reproduction can be dominated by non-nestmate workers, which suggests that socially parasitism by laying workers is an integral part of the biology of Cape honeybees. Even though the parasitically reproducing workers can give rise to more than half of the offspring within a queenless colony, the overall frequency of parasitic offspring is still low. My results (Härtel et al. 2006a) are in accordance with the recently discovered parasitic workers of bumble bees and dwarf honeybees suggesting that parasitic workers are found during time windows with reproductive competition among individual nestmate workers (Page and Robinson 1994; Moritz et al. 1996; Martin et al. 2004). However, the proneness of worker social parasitism to colony conditions with less group-selected interest and intracolony conflict does not exclude that even queenright *A.*

m. capensis colonies are parasitized. In fact, the “dwindling colony syndrome” (Allsopp and Crewe 1993) shows that queenright colonies of *A. m. scutellata* are highly susceptible to infestations of *Cape honeybee* laying workers. Hence, social parasitism by laying workers among queenright *A. mellifera* colonies most likely naturally exists also within subspecies.

Whenever non-nestmate workers can circumvent worker policing to a certain degree (Martin et al. 2002a) selection at the group level (colony) is not coincident with selection on the individual. In other words, the benefits of individual reproduction to parasitic workers are offset by reductions in the host colony fitness. In accordance to the theory that thelytoky reinforces selfish worker reproduction in the Cape honeybee (Greeff 1996), it must be assumed that the resulting tensions should be present in the *A. m. capensis* population. Indeed, data about worker policing in *A. m. capensis* show the expected variance. Common worker reproduction was found (Petty, 1922; Moritz et al. 1999; Beekman et al. 2002; Neumann et al. 2003) conversely also worker policing in the absence of relatedness benefits occurs (Beekman et al. 2002; Pirk et al. 2003). Moreover, aggression towards workers with developed ovaries was described (Crewe 1984; Anderson, 1968) but successful laying worker activity is reported from queenright *A. m. capensis* colonies (Petty 1922), which points out that this is not an obligate behavioral pattern. The behavioural conflicts in *A. m. capensis* indicate differential conditions at the colony level for social parasitism of reproductive workers. As a consequence, some queenright non-policing *A. m. capensis* colonies may also be susceptible to infestations by socially parasitic workers. Until now, there are no reports of massive colony extinctions due to parasitic *A. m. capensis* worker reproduction in apiaries and in the wild population. Therefore, socially parasitic *A. m. capensis* workers should only be found infrequently at the population level.

My data about socially parasitic behaviour provided the basis for following studies carried out by (Pirk et al. in prep) which challenged the long established view on worker policing (Woyciechowski and Lomnicki 1987; Ratnieks 1988) “...our most important example of kin selection in action” (Beekman and Oldroyd 2005); (Dugatkin 1997, 2004; Whitfield 2002). The authors could show that the influence of nestmate recognition on oophagy is higher than the influence of the caste. Thus, worker policing is most likely in the first instance a behavioural response on foreign worker reproduction and not only a strategy to resolve a queen-worker conflict over reproduction among kin nestmates.

The anarchy syndrome of honeybee workers (Oldroyd et al. 1994) reflects a similar case of selfish worker reproduction. Anarchistic workers reproduce under queenright colony conditions since worker-laid eggs from anarchists are to a great extent accepted and consequently evade worker policing (Oldroyd and Ratnieks 2000). Furthermore, in these anarchistic colonies reduced amounts or altered compositions of brood pheromones produced by larvae that normally signal workers to refrain from reproduction can be observed (Oldroyd et al. 2001). Genetic data show that a single worker subfamily produces anarchic drones, indicating a high heritability of this trait. Honeybee colonies containing these selfish workers tolerate a few anarchistic workers without an obvious reduction of colony fitness (Montague and Oldroyd 1998; Barron et al. 2001). Anarchistic workers are less inhibited by queen pheromone secretion than wild type workers (Hoover et al. 2005). However, in contrast to socially parasitic workers of the Cape honeybee, anarchistic workers do not dominantly reproduce under queenless conditions (Montague and Oldroyd 1998; Beekman and Oldroyd 2003). In addition, these workers are very rare, lay male eggs and do not

show queen-like mandibular pheromone production (Oldroyd et al. 1999). Anarchistic workers are natal nestmates and therefore do not represent social parasitism. The anarchy syndrome, which is characterized as a failure of policing, is thought to be a behavioural mutation carried by paternal genes (Montague and Oldroyd 1998). This may also be the case for socially parasitic workers of *A. m. capensis*. However, parasitic *A. m. capensis* workers were independently found several times in colonies of other honeybee subspecies (Onions 1912; Lundie, 1954; Johannsmeier, 1983; Koeniger and Würkner 1992; Allsopp and Crewe, 1993; Woyke 1995). It appears unlikely that the full set of traits needed to complete the reproductive cycle of socially parasitic workers (Neumann and Hepburn, 2002; Neumann and Moritz, 2002) arose from independent mutations in the various reported cases (Onions 1912; Lundie, 1954; Johannsmeier, 1983; Koeniger and Würkner 1992; Allsopp and Crewe, 1993; Woyke 1995). In light of our data we therefore conclude that socially parasitic behaviour of workers is not a mutant cheater event (Dietemann et al. 2006b).

9.2 A socially parasitic lineage of *A. m. capensis* workers

In the first part of my thesis I could show that socially parasitic behaviour of honeybee workers is not only a man made phenomenon mediated by professional beekeeping practise but that it is an inherent part of the natural behaviour of *A. m. capensis* workers. The “dwindling colony syndrome” are the symptoms of infestation resulting from an invasion by parasitic *A. m. capensis* workers into *A. m. scutellata* populations (Allsopp 1992; Allsopp and Crewe 1993; Hepburn and Allsopp 1994; Neumann and Hepburn 2002; Moritz et al. 2005). This incision set an ideal case study system, which I here used to investigate proximate mechanisms underlying worker social parasitism under queenright conditions.

Honeybee workers normally do not reproduce under queenright conditions. The queen pheromones and the brood suppress the activation of the workers’ ovaries as well as the production of queen-like pheromones (Moritz et al. 2000, 2004). However, parasitic *A. m. capensis* workers lay eggs that are reared by the workers of queenright *A. mellifera scutellata* host colonies’. In order to analyse the mechanisms by which socially parasitic *A. m. capensis* workers establish themselves within the queenright host colony, I have experimentally infested *A. m. scutellata* host colonies with parasitic workers of *A. m. capensis*. These workers were monitored for their activation status of their ovaries and the production of queen-like mandibular pheromones during the course of infestation. Furthermore, parasitic brood which appears first was taken, incubated, marked as adults and reintroduced into their respective mother colony to follow their reproductive fate. These analysis showed that 94.4% of the initially infesting parasites had fully activated ovaries and a queen-like mandibular pheromone production referring to the pseudoqueens status. Not only all of the studied *A. m. scutellata* host workers are pheromonally inhibited by the parasitic workers but also only 96.9% of the clonal parasitic offspring do not develop into pseudoqueens. The majority of parasitic workers choose the biochemical pathway of the queen substance (Plettner et al. 1996, 1998) which is the major component of the queen mandibular pheromone (QMP), but the final synthesis from the precursor 9-hydroxy-2-(*E*)-decenoic acid (9-HDA) to the end product 9-keto-2-(*E*)-decenoic acid (9-ODA) was significantly reduced in non-reproducing workers, resulting in a significant correlation between 9-ODA production and ovary activation.

In *Apis mellifera capensis*, the correlation between ovary activation and 9-ODA was confirmed in various studies (Hemmling et al. 1979; Velthuis and van der Kerk 1988; Hepburn 1992; Simon et al. 2005). However, in non-African *A. mellifera* subspecies reproductive workers do not always show a covariance between ovary activation and QMP secretions (Plettner et al. 1993). The production of queen-like pheromones by workers can be detrimental because it provides cues for their recognition and their attacks by non-reproducing nestmates (Visscher and Dukas 1995). In order to avoid the attack of their non-reproducing nestmate workers, this could be a reason why the anarchistic lineage does not produce a queen-like mandibular blend (Oldroyd et al. 1999), but socially parasitic workers *A. m. capensis* can obviously avoid this aggression despite the secretion of queen-like signals. In many species of social insects fertility correlates with chemical cues such as cuticular hydrocarbon profiles (e.g. Peeters 1999; Liebig et al. 2000; Cuvilier-Hot et al. 2001, 2004; Dietemann et al. 2003). The correlation between 9-ODA and ovary activation, and the 9-ODA mediated colony level decisions during the course of infestation (refrain of host and offspring workers' ovary activation Härtel et al. 2006d, prevention of successful re-queening, Martin et al. 2002b) suggests that the queen substance secretion of socially parasitic workers has the character of a fertility signal (Härtel et al., in prep). Fertility signals are supposed to play a major role in the regulation of reproduction in social insects (Endler et al. 2006) and may add another dimension to the understanding of how queen pheromones operate (Dor et al. 2005). It turns out that the socially parasitic workers of *A. m. capensis* utilize a queen-like mandibular pheromone secretion to gain a prominent reproductive position within the *A. m. scutellata* host colony. Under colony conditions the data of ovary dissection and QMP analyses revealed that the first infesting parasites are not inhibited by the pheromone secretions of *A. m. scutellata* host queen (Hoover et al. 2003) and brood (Arnold et al. 1994; Mohammedi et al. 1998). Thus, these laying *A. m. capensis* workers are less responsive to the inhibitory queen pheromonal signals. The observed reproductive inhibition of the clonal parasitic offspring generation indicates a stronger collective inhibitory signal of the parental parasitic pseudoqueens compared to the host queens' signal. The extreme differences in the phenotype of clonal parasitic workers show that genetic variance is not solely necessary to initiate the self-organising process of reproduction and sterility among honeybee workers. The experiments demonstrate that the outstanding secretion of the queen substance 9-ODA by the socially parasitic pseudoqueens is a powerful tool to achieve reproductive dominance in *A. m. scutellata* host colonies. The study showed that the first invading parasitic workers can monopolize reproduction in the host colony and break the reproductive division of labour by overcoming the inhibitory pheromone secretion of host queen and brood, which appears crucial for the success of *A. m. capensis* worker social parasites.

A previous study of (Martin et al. 2002a) showed that eggs, which were laid by socially parasitic workers can evade policing of host workers. In combination with observations that the hatched larvae are preferentially fed by the host workers (Beekman et al. 2000; Allsopp et al. 2003), the reproduction of the parasite within the foreign colony is very effective.

The loss of the host queen is an integral part of the infestation cycle by socially parasitic workers (Martin et al. 2002a; Neumann and Hepburn 2002). It was suggested that a queen-worker conflict for reproduction during the infestation with socially parasitic worker must arise (Moritz et al. 2003). Moritz et al. (2003) found that lethal fights between parasitic workers and the queen (similar to queen –queen fights)

could resolve this conflict, resulting in the rare death of the queen or more likely in the death of parasitic workers. Normally, the queen-worker conflict is resolved due to inhibitory pheromone secretions by the queen honeybee and worker policing. Given that parasitic workers are not inhibited by the queen pheromones' the conflict between resident host queens and parasitic workers may also result in a pheromonal competition. The host queen pheromonal signal could decrease which would result in her rejection. This would be a further explanation of the observed queen loss in infested *A. m. scutellata* colonies. In paired arena assays we investigated the manner in which parasitic workers pheromonally compete with host queens of different age and subspecies. The pheromonal interaction between the *A. m. capensis* pseudoqueens and the *A. m. scutellata* host queens show that *A. m. scutellata* queens do not prevent the production of queen-like mandibular gland compounds by the parasites. There are no differences in the pheromonal ratio of queen-like components between *A. m. scutellata* queens and parasitic workers. Nevertheless, the total amount of produced 9-ODA is still higher in *A. m. scutellata* queens. The pheromonal competition experiments demonstrate that a single native *A. m. capensis* worker taken from the endemic range is able to pheromonally dominate a virgin *A. m. scutellata* queen. On one occasion, an invasive parasitic worker produced more 9-ODA than the native *A. m. capensis* queen it was paired with. This suggests that the social parasites could also infest queenright colonies in population of their own subspecies (see above). In contrast to earlier studies (Moritz et al. 2003) we have never observed physical conflicts between the paired bees indicating that physical interactions are rather rare. The study underlines under the condition of a reductionist approach that overcoming of the inhibitory queen pheromones' effect is essential for the establishment of socially parasitic workers. Below a certain threshold of pheromones produced by the weakened queen, the parasite might be preferred over her and accepted as reproductive by the host workers. This may also result in the rejection of the host queen by her own *A. m. scutellata* workers. Thus, the loss of the host queen might be the consequence of a progressive weakening of the host queen resulting from a combination of physical fights (Moritz et al. 2003) and from pheromonal competition with parasitic workers (Dietemann et al. 2006a).

Pheromonal competition governs reproductive dominance hierarchies among *A. m. capensis* workers, with the most dominant worker suppressing the 9-ODA production of subordinate ones (Moritz et al. 2000, 2004, Simon et al. 2005). In order to answer the question if the socially parasitic lineage is adapted by the rapid selection for reproductive dominance we compared the queen-like pheromone production of parasitic with non-parasitic workers of different origin in arena tests. We expected the following increasing order of reproductive dominance among honeybee workers: (1) *A. m. scutellata*, (2) naturally occurring hybrids (*A. m. scutellata* × *A. m. capensis*) from the introgression zone, (3) native *A. m. capensis* queen offspring, (4) native *A. m. capensis* worker offspring and (5) parasitic *A. m. capensis* lineage found in infested *A. m. scutellata* colonies. Our data show that socially parasitic workers pheromonally dominate all other worker groups that we have analyzed. The observed order of pheromonal dominance corresponds to the one predicted on theoretical grounds. Given the importance of queen-like pheromones in acquiring reproductive status, our data underline that parasitic workers are selected for their superior ability to produce these mandibular gland signals. Since thelytoky and other traits for worker reproductive dominance are genetically linked (Lattorff et al. in review) and less

disturbed by recombination (Baudry et al. 2004), eventually a worker genotype with strong reproductive dominance must have a selective advantage. Thus, our data confirm the theoretical prediction of rapid selection for reproductive dominance among workers of the translocated *A. m. capensis* population. The selection of socially parasitic workers of a particular genotype (Kryger 2001a,b) is thus a sympatric process in the range of *A. m. scutellata*.

The socially parasitic workers are spatially separated from their *A. m. capensis* source population but the questions of an already established sympatric reproductive barrier to gene flow to their intraspecific *A. m. scutellata* hosts remains open. A sympatric barrier to gene flow would point into the direction of a speciation process within the social parasites. Gene flow from the sexually reproducing host population to the parasitic worker population is not possible, because honeybee workers cannot mate. Also gene flow from the parasite to the social host population is constrained. Since thelytoky is determined by a single recessive gene (Lattorff et al. 2005), parasitic workers lay exclusively female offspring. The only possible way for gene flow from the parasite to the host population is the production of queens from parasitic *A. m. capensis* offspring. Here we evaluated with genetic tools, if these gene flow constraints between the intraspecific host and social parasite have an impact at the population level. In order to analyze reproductive isolation between *A. m. capensis* social parasites and the *A. m. scutellata* host populations, we collected both host and parasite workers from infested host colonies in all South African provinces, where infestations of *A. m. scutellata* colonies by parasitic *A. m. capensis* workers occurred. Independent of the sampling locality, all parasitic workers (N=257) have the genetic signature of a clone founded by a single ancestral worker genotype. The invasive range of the parasitic lineage could be expanded to 275,000 km², which is larger than the endemic range of *A. m. capensis* in the Cape region of South Africa (Hepburn and Radloff 1998). It could be shown that gene flow between the *A. m. scutellata* host population and its intraspecific social parasite is rare, indicating a strong sympatric reproductive barrier. The genetic data show that this barrier to gene flow appears to be a stronger effect than assortative mating (Feder et al. 1994; Via 1999; Malausa et al. 2005). The reproductive separation is most likely achieved by mechanisms underlying the reproductive division of labour in honeybee colonies. The caste determination within the honeybee colony supplies the first barrier to gene flow because honeybee workers cannot mate. Nevertheless, gene flow from the parasite to the host is possible since thelytokously produced female parasitic larvae may also develop into queens. However, the strong collective mandibular signal of the initial infesting parasitic pseudoqueens appears to be important in the limitation to gene flow, because the queen substance primes not only the physiology of recipient workers (Kaatz et al. 1992), it has furthermore an inhibitory influence on queen rearing (Pettis et al. 1997). Thus, 9-ODA is not only important in the division of labor in the honeybee colony (Seeley 1985), it might also mediate the inhibition of parasitic queen rearing which is a feature in the reproductive barrier to gene flow between host and social parasite. This barrier is pre-zygotic, affecting the queen rearing process in infested host colonies prior to the possibility of interbreeding between parasitic female sexuals and host males. The large amount of parasitic brood found in the colonies at the beginning of infestation (Martin et al. 2002b, von der Heide 2004) also inhibits the thelytokous production of parasitic queens. Thus, queen-like pheromone secretion and parasitic brood production show a synergistic effect which results in the strong restrictions to gene flow. The results of the social parasite population confirm earlier

studies which indicate that only a single clonal lineage is infesting the *A. m. scutellata* population (Kryger 2001a,b; Baudry et al. 2004). The most frequent and widespread multilocus parasite genotype refers most likely to the founding parasitic worker. The data suggest that thelytokous worker reproduction is a feasible route to sympatric reproductive isolation. Given that the genetic separation of host and parasite results in sympatric speciation, this thesis supplies a new evolutionary pathway for Emery's rule.

The vast majority of *A. m. scutellata* colonies are wild (Hepburn and Radloff 1998) and transmission is crucial for the socially parasitic life cycle of honeybee workers (Neumann et al. 2001; Neumann and Hepburn 2002). Nevertheless, infested colonies in apiaries produce a lot of parasitic workers (Martin et al. 2002b) and it is certain that these workers can infest new host colonies either as a small swarm and subsequent mergers or as a single worker (individual vs. colonial transmission pathway, Neumann and Hepburn 2002). It has been shown in several occasions (Allsopp and Crewe, 1993; Greeff 1997, Dietemann et al. 2006c) that the infestation by the Cape honeybee *A. m. capensis* parasites caused dramatic impact on the managed population of *A. m. scutellata*. This raised concerns that wild *A. m. scutellata* populations might also be affected, which is however unknown. Swarms provide samples from the wild and managed population, thereby allowing to quantify infestation rates. Here I addressed the question whether socially parasitic *A. m. capensis* workers can also be found in wild populations. For that purpose, I have surveyed swarms in beekeeping areas and in nature reserves. The survey showed that infestations of swarms occur in beekeeping regions. In contrast, I was unable to find any infestations of wild swarms in nature reserves, suggesting that infestations are rare in wild populations. Furthermore, I also showed with DNA genotyping that black tergites are not a reliable indicator for socially parasitic *A. m. capensis* workers. But, the elimination of colonies with black tergite workers supplies a method to avoid false negative diagnosis (Härtel et al. 2006b). Population ecological modelling showed that unless colony densities in the wild were high, it would be unlikely that parasitic workers could massively spread into the wild population. *Vice versa* the high host colony density in apiaries increases the probability of new infestations (Moritz 2002). Unfortunately, data about natural infestations, with the transmissions of socially parasitic workers from unmanipulated *A. m. scutellata* colonies to other unmanipulated colonies are lacking. Since parasitic transmission is an important parameter, future research should address this topic in more detail.

This thesis provides new insights into social parasitism by laying workers in the eusocial insects in general and in honeybees in particular. When parasites belong to different subspecies, species or genera than their host, identifying the similarities and differences in behaviour or chemical signals between the protagonists can shed light on the mechanisms underlying the evolution of social parasitism by workers and of reproductive division of labour in the eusocial insects. In this respect, I have shown that socially parasitic workers of *A. mellifera* use the same pheromonal cues as the host queens do. The parasitic workers are less responsive to the inhibitory pheromone secretion of the host queen than the host workers. Moreover, worker parasites are less policed by the host workers. The host queen is either engaged in lethal fights or pheromonally out competed. As a consequence, the social parasites achieve dominant positions within the reproductive hierarchy of the host colony. The queen-like pheromone production is correlated to the ovary status in *A. m. capensis* workers, indicating a fertility signal character. This signal is accepted even from

unrelated host colonies. Thus, pheromonally mediated reproductive dominance is independent of relatedness, and can be established between clone mates, full sisters, cousins, or random unrelated nestmates. Furthermore, I have given an example of the establishment of a reproductive barrier to gene flow through mechanisms underlying the reproductive division of labor in eusocial insect colonies in contrast to the known assortative mating among selfish reproductives (Härtel et al. 2006c).

9.3 Future perspective of the socially parasitic lineage

The invasion of socially parasitic *A. m. capensis* workers into *A. m. scutellata* populations sets a fascinating host-social parasite system. The two protagonists are the sexually reproducing *A. m. scutellata* hosts and the thelytokously reproducing *A. m. capensis* social parasite. While the host population has the advantage of Mendelian segregation and genetic recombination, the social parasite consists of numerous clonal individuals. Since parasitism is thought to be the major force which drives the evolution of sex (Maynard-Smith 1978) it is widely believed that sexual reproduction is advantageous over clonal reproduction. Here we have a study system, which allows us to investigate sexual host reproduction versus parasitic clonal reproduction within an intraspecific host-social parasite system. Natural selection for aggression towards workers with activated ovaries (Crewe 1984; Neumann et al. 2006) would favour *A. m. scutellata* colonies which can prevent take over by socially parasitic workers because such colonies can be expected to have a higher fitness by producing more drones and surviving swarms (Moritz and Southwick 1992). If there is a genetic basis for this behaviour, the respective allele frequency will increase within *A. m. scutellata* populations. As a result, the epidemic spread of *A. m. capensis* social parasites should be reduced. Nevertheless, as long as South African beekeepers catch their swarms within populations, which are not in contact with the parasite, natural selection cannot work. Besides the considerable man-mediated transmission of the social parasites by beekeepers, this is maybe an additional reason why the “dwindling colony syndrome” is still a major problem for South African apiculture. On the other hand the clonal parasitic lineage may adapt through beneficial mutation events. On a longer run this better adapted genotype would be dominant over the former genotype and maybe the beginning of a co-evolutionary arms race between host and social parasite. Now, we are only witnessing the very first steps of the evolution of a host-social parasite system (Neumann and Moritz 2002). The socially parasitic lineage would only be stable in evolutionary time scales if the parasites have a sufficient natural transmission rate and do not lead to the swift extinction of the host. The current observed high virulence would at first result in a massive reduction of the *A. m. scutellata* host density and on a longer run, to the extinction of this obligate and genetically separated socially parasitic worker lineage.

10. Zusammenfassung

Das Ziel meiner Arbeit war die Analyse eines durch legende Arbeiterinnen der Kaphonigbiene *Apis mellifera capensis* verursachten Sozialparasitismus. Bislang war intraspezifischer Sozialparasitismus durch legende Arbeiterinnen entweder wenig beachtet oder, wie im Falle von *A. m. capensis*, als ein durch Imkerei hervorgerufenen Artefakt betrachtet worden. Im Gegensatz hierzu konnte ich im ersten Teil meiner Arbeit zeigen, dass dieser Sozialparasitismus tatsächlich nicht nur ein durch menschliche Einflüsse hervorgerufenes Phänomen ist, sondern einen natürlichen Teil des Verhaltensrepertoires der Honigbiene darstellt. Im zweiten Teil der Arbeit habe ich unter Zuhilfenahme von genetischen und gaschromatographischen Techniken die komplexen proximalen Mechanismen untersucht, mit denen *A. m. capensis* Arbeiterinnen den sozialparasitischen Status innerhalb der *A. m. scutellata* Wirtskolonie etablieren. Hierdurch ergänzt meine Arbeit die eher deskriptiven Veröffentlichungen zum intraspezifischen Sozialparasitismus bei Hummeln und Zwerghonigbienen substantiell. Weiterhin können die Ergebnisse dieser Arbeit helfen, den Sozialparasitismus von legenden *A. m. capensis* Arbeiterinnen, der in Südafrika zu erheblichen Völkerverlusten führt, besser zu verstehen und zu behandeln.

10.1 Honigbienen Arbeiterinnen als Sozialparasiten

In Kolonien eusozialer Insekten wurde intraspezifischer Sozialparasitismus als Fortpflanzungsmöglichkeit für Arbeiterinnen bisher wenig beachtet. Dieses Verhalten folgt einer alternativen Fortpflanzungsstrategie in der direkte Fitness in einer fremden Wirtskolonie anstelle von inklusiver Fitness im Heimatnest erreicht wird. Erst kürzlich wurden einige wenige Beispiele von Sozialparasitismus durch legende Arbeiterinnen beschrieben. So reproduzieren sich beispielsweise nestfremde Arbeiterinnen der Erdhummel *Bombus terrestris* nach dem „Competition Point“, einer Phase im Koloniezyklus in der die reproduktive Dominanz der Königin nachlässt und ihre Arbeiterinnen ebenfalls mit der Fortpflanzung beginnen (Lopez-Vaamonde et al. 2004). Bei der Zwerghonigbiene, *Apis florea*, pflanzen sich parasitische Arbeiterinnen in weisellosen Kolonien fort (Nanork et al. 2005). Auch die Arbeiterinnen der Kaphonigbiene, *A. m. capensis*, wurden schon öfter als Sozialparasiten anderer Honigbienen-Unterarten beschrieben (Onions 1912; Lundie 1954; Johannsmeier 1983; Koeniger und Würkner 1992; Allsopp und Crewe 1993; Woyke 1995). Dieser Sozialparasitismus führt zu tiefgreifenden Veränderungen in der Struktur der Wirtskolonie. Bisher wurde diese komplexe Verhaltensweise als ein durch menschliche Eingriffe geschaffenes Phänomen betrachtet, welches durch Wanderimkerei hervorgerufen wird.

In meiner Arbeit habe ich nun die Frage behandelt, ob Arbeiterinnen der Kaphonigbiene auch innerhalb ihrer eigenen Unterart sozialparasitisches Verhalten zeigen. Um die Mutterschaft der sich reproduzierenden Arbeiterinnen zu untersuchen, habe ich in diesem Zusammenhang die Nachkommenschaft von sechs weisellosen *A. m. capensis* Kolonien genotypisiert. Diese genetischen Analysen haben gezeigt, dass in weisellosen Völkern die Reproduktion durch nestfremde Arbeiterinnen dominiert werden kann. Sozialparasitisches Verhalten von legenden Arbeiterinnen kann somit als ein integraler Teil der Biologie der Kaphonigbiene angenommen

werden. Doch obwohl die Nachkommenschaft der sich parasitisch fortpflanzenden Arbeiterinnen über die Hälfte der Gesamtnachkommenschaft einer weisellosen Kolonie ausmachen kann, ist die Häufigkeit parasitischer Nachkommen auf der Populationsebene eher gering. Meine Ergebnisse (Härtel et al. 2006a) stehen im Einklang mit den kürzlich entdeckten sozialparasitischen Arbeiterinnen der Erdhummel und Zwerghonigbiene. In allen Fällen wurden parasitische Arbeiterinnen unter Koloniebedingungen gefunden, die durch einen reproduktiven Wettstreit zwischen einzelnen Arbeiterinnen gekennzeichnet sind (Page und Robinson 1994; Moritz et al. 1996; Martin et al. 2004). Dennoch, die Kopplung von Sozialparasitismus an Koloniebedingungen mit nachlassenden, ehemals auf Gruppenselektion beruhenden Interessen und intrakolonialen Konflikten schließt nicht zwangsläufig aus, dass in weiselrichtigen Kolonien von *A. m. capensis* Sozialparasitismus durch legende Arbeiterinnen ebenfalls vorkommen kann. Tatsächlich deutet das „dwindling colony syndrome“ (Allsopp und Crewe 1993) darauf hin, dass weiselrichtige Kolonien von *A. m. scutellata* ebenfalls hochgradig anfällig für die Infektion durch *A. m. capensis* sind. Folglich kann man höchstwahrscheinlich sozialparasitische Arbeiterinnen auch in weiselrichtigen *A. mellifera* Kolonien anderer Unterarten finden.

Meine Daten über sozialparasitisches Verhalten von Arbeiterinnen bereiteten die Basis für die Studie von Pirk et al. (in prep.), welche die lang etablierte Ansicht von „worker policing“ (Woyciechowski und Lomnicki 1987; Ratnieks 1988) in Frage stellt „...our most important example of kin selection in action“ (Beekman und Oldroyd 2005). Pirk et al. konnten zeigen, dass der Einfluss von Nestgenosserkennung auf Oophagie höher ist als der Einfluss der Kaste. Dies deutet darauf hin, dass „worker policing“ wahrscheinlich in erster Linie eine Verhaltensantwort auf parasitische Arbeiterinnenreproduktion ist und nicht nur eine Strategie, um einen Königinnen-Arbeiterinnen Konflikt über die Reproduktion im Bienenvolk zu lösen. Wenn fremde Arbeiterinnen dem „worker policing“ zu einem gewissen Grad entgehen können (Martin et al. 2002a), dann ist die Selektion auf der Gruppenebene (Kolonie) nicht in Übereinstimmung mit der Selektion auf der Individualebene. In anderen Worten: die Vorzüge individueller Reproduktion werden durch die Herabsetzung der Koloniefitness aufgehoben. In Einklang mit der theoretischen Annahme, dass Thelytokie egoistische Arbeiterinnenreproduktion verstärkt (Greeff 1996), sollten die Interessen der Gruppe gut an den selektiven Druck durch Nestgenossinnen- oder sozialparasitische Arbeiterinnenreproduktion in *A. m. capensis* angepasst sein. In der Tat zeigen die Daten über „worker policing“, dass als Verhaltensantwort auf vermehrte Arbeiterinnenreproduktion begriffen werden kann, die erwartete Varianz. Sich erfolgreich reproduzierende Arbeiterinnen wurde gefunden (Petty, 1922; Moritz et al. 1999; Beekman et al. 2002; Neumann et al. 2003), dennoch konnte „worker policing“ auch in der Abwesenheit von auf Verwandtenselektion basierenden Gründen beobachtet werden (Beekman et al. 2002; Pirk et al. 2003). Weiterhin wurde von Aggressionsverhalten gegenüber Arbeiterinnen mit entwickelten Ovarien berichtet (Crewe 1984; Anderson, 1968). Andererseits findet aber erfolgreiche Arbeiterinnenreproduktion in weiselrichtigen *A. m. capensis* Kolonien statt (Petty 1922). Dementsprechend ist Aggression gegenüber legenden Arbeiterinnen kein obligates Verhaltensmuster in der Kaphonigbiene. Die hier auf der Kolonieebene beschriebenen Verhaltensunterschiede gegenüber sich entwickelnden Arbeiterinnen deuten auf verschiedenartige Bedingungen für Sozialparasitismus in *A. m. capensis* hin. Einige weiselrichtige Kaphonigbienenvölker die kein „worker policing“ ausüben, scheinen für die Infektion mit sozialparasitischen Arbeiterinnen anfällig zu sein. Bisher

gibt es aber keine Berichte über umfangreiche Koloniezusammenbrüche, die durch parasitische *A. m. capensis* Arbeiterinnen hervorgerufen wurden. Diese stabile Situation gibt Grund zu der Annahme, dass sozialparasitisches Verhalten von Arbeiterinnen nur sehr selten auf Populationsebene beobachtet werden kann.

Das Anarchiesyndrom von Honigbienenarbeiterinnen beschreibt einen ähnlichen, aber sehr viel selteneren Fall von egoistischer Arbeiterinnenreproduktion (Oldroyd et al. 1994). Anarchistische Arbeiterinnen pflanzen sich unter weiselrichtigen Koloniebedingungen fort, da die von ihnen gelegten Eier dem „worker policing“ entkommen können (Oldroyd und Ratnieks 2000). Weiterhin kann in diesen anarchistischen Kolonien eine verminderte oder veränderte Zusammensetzung von Brutpheromonen beobachtet werden (Oldroyd et al. 2001), welche normalerweise die reproduktive Arbeiterinnenentwicklung hemmen. Wie genetische Daten zeigten, produziert eine einzige Arbeiterinnensubfamilie anarchistische Drohnen im Volk (Montague und Oldroyd 1998; Barron et al. 2001). Demzufolge kann eine hohe Heritabilität dieses Merkmals angenommen werden. Honigbienenkolonien, die Arbeiterinnen mit diesem egoistischen Verhalten beherbergen, tolerieren sie jedoch ohne offensichtliche Verminderung ihrer Fitness. Anarchistische Arbeiterinnen werden durch die Königinnenpheromone weniger inhibiert als Wildtyparbeiterinnen (Hoover et al. 2005). Dennoch reproduzieren sich, im Gegensatz zu sozialparasitischen Arbeiterinnen der Kaphonigbiene, die anarchischen Arbeiterinnen nicht dominant unter weisellosen Bedingungen (Montague und Oldroyd 1998; Beekman und Oldroyd 2003). Zudem sind diese Arbeiterinnen sehr selten, legen ausschließlich männliche Eier und zeigen keine Produktion von königinnenähnlichen Mandibeldrüsen-Pheromonen (Oldroyd et al. 1999). Anarchistische Arbeiterinnen sind natürliche Nestgenossinnen und repräsentieren somit keinen wirklichen Sozialparasitismus. Das Anarchie-Syndrom welches als Fehler im „worker policing“ beschrieben worden ist, wird als Verhaltensmutation angesehen, die über paternale Gene vererbt wird (Montague und Oldroyd 1998). Andererseits wurden parasitische *A. m. capensis* Arbeiterinnen unabhängig voneinander in Kolonien verschiedener *A. mellifera* Unterarten gefunden (Onions 1912; Lundie, 1954; Johannsmeier, 1983; Koeniger und Würkner 1992; Allsopp und Crewe, 1993; Woyke 1995). Es wird als unwahrscheinlich angesehen, dass das Verhalten, welches zu einer sozialparasitischen Lebensstrategie benötigt wird, aus vielen unabhängigen, aber in die gleiche Richtung weisenden Mutationen erfolgt ist. Angesichts dieser Tatsache und im Vergleich mit unseren Daten ziehen wir den Schluss, dass sozialparasitisches Verhalten von sich reproduzierenden Arbeiterinnen kein „mutant cheater event“ ist (Dietemann et al. 2006b).

10.2 Eine sozialparasitische Linie von *A. m. capensis* Honigbienen

Wie ich im ersten Teil meiner Arbeit zeigen konnte, handelt es sich beim sozialparasitischen Verhalten von Honigbienenarbeiterinnen nicht nur um ein durch Menschen verursachtes Phänomen, sondern vielmehr ist es ein integraler Bestandteil des natürlichen Verhaltens von *A. m. capensis* Arbeiterinnen. Das „dwindling colony syndrome“ beschreibt die Infektionssymptome, die durch die Invasion von parasitischen *A. m. capensis* Arbeiterinnen in *A. m. scutellata* Populationen zu beobachten sind (Allsopp 1992; Allsopp und Crewe 1993; Hepburn und Allsopp 1994; Neumann und Hepburn 2002; Moritz et al. 2005). Diese Invasion sozialparasitischer Arbeiterinnen ist ein ideales Modellsystem, welches ich hier für die Untersuchung von

proximaten Mechanismen von Sozialparasitismus durch Arbeiterinnen unter weiselrichtigen Bedingungen nutzen konnte.

Honigbienenarbeiterinnen reproduzieren sich normalerweise nicht unter weiselrichtigen Bedingungen. Die Königinnenpheromone und die von der Brut produzierten Pheromone unterdrücken die Aktivierung der Ovarien als auch die Produktion von königinnenähnlichen Pheromonen in Arbeiterinnen (Moritz et al. 2000, 2004). Ungeachtet dessen legen parasitische *A. m. capensis* Arbeiterinnen Eier, die von den *A. m. scutellata* Arbeiterinnen der Wirtskolonie versorgt werden. Um zu untersuchen, welche Mechanismen von *A. m. capensis* Arbeiterinnen im Prozess der Etablierung des sozialparasitischen Status genutzt werden, habe ich *A. m. scutellata* Kolonien experimentell mit *A. m. capensis* infiziert. Die Arbeiterinnen wurden auf den Status ihrer Ovarien und die Produktion von Königinnenpheromonen hin untersucht. Weiterhin wurde die primär erzeugte parasitische Brut entnommen, inkubiert, markiert und zurück in die Wirtskolonie eingebracht, um ihr reproduktives Schicksal zu verfolgen. Diese Analysen zeigten, dass 94,4% der experimentell eingebrachten Bienen aktivierte Ovarien hatten und ein königinnenähnliches Pheromonbouquet produzierten. Zusammengenommen unterstreichen diese Daten den Pseudoköniginnen-Status der parentalen Parasitengeneration. Weiterhin wurden nicht nur die *A. m. scutellata* Wirtsarbeiterinnen durch die Pheromone inhibiert, sondern auch 96,9 % der klonalen parasitischen Nachkommen verblieben steril und entwickelten sich nicht zu parasitischen Pseudoköniginnen. Trotzdem schlugen die Mehrheit der parasitischen Arbeiterinnen den biochemischen Weg der Königinnensubstanz ein (Plettner et al. 1996, 1998), welche die Hauptkomponente des Königinnen Mandibular Pheromons (QMP) darstellt. Die finale Synthese vom 9-hydroxy-2-(*E*)-decenoic acid (9-HDA) zum Endprodukt 9-keto-2-(*E*)-decenoic acid (9-ODA) war allerdings signifikant reduziert in den nicht-reproduzierenden parasitischen Arbeiterinnen, welches eine starken Korrelation zwischen 9-ODA-Produktion und Ovarienaktivierung zur Folge hat. Für *A. m. capensis* konnte diese Korrelation in mehreren Studien bestätigt werden (Hemmling et al. 1979; Velthuis und van der Kerk 1988; Hepburn 1992; Simon et al. 2005). Im Gegensatz zur gefundenen Korrelation zeigen reproduktive Arbeiterinnen von europäischen *A. mellifera* Unterarten nicht immer eine Kovarianz zwischen Ovarienaktivierung und 9-ODA Produktion (Plettner et al. 1993). Die Produktion von königinnenähnlichen Pheromonen durch Arbeiterinnen kann nachteilig für diese werden, da es die Identifikation und den Angriff durch nicht entwickelte Arbeiterinnen ermöglicht (Visscher und Dukas 1995). Um diesen Angriff zu vermeiden, haben z. B. anarchistische Arbeiterinnen wahrscheinlich kein königinnenähnliches Mandibeldrüsensekret (Oldroyd et al. 1999). Der Erfolg, den sozialparasitische *A. m. capensis* Arbeiterinnen haben, deutet allerdings darauf hin, dass diese nicht nachhaltig angegriffen werden.

Die Fortpflanzungsfähigkeit korreliert bei vielen Arten von sozialen Insekten mit chemischen Substanzen wie zum Beispiel mit kutikularen Kohlenwasserstoffen (Peeters 1999; Liebig et al. 2000; Cuvilier-Hot et al. 2001, 2004; Dietemann et al. 2003). Die in dieser Untersuchung gefundene Korrelation zwischen 9-ODA und Ovarienaktivierung und die durch 9-ODA vermittelten Entscheidungen auf der Kolonieebene (unterdrücken von Wirts- und Nachkommenarbeiterinnen, Härtel et al. 2006d; die Verhinderung von erfolgreichen Nachschaffungszellen, Martin et al. 2002b) geben Hinweise darauf, dass die Sekretion der Königinnensubstanz von parasitischen Arbeiterinnen den Charakter eines Fruchtbarkeitssignals haben könnte (Härtel et al. in prep). Neuerdings wird angenommen, dass Fruchtbarkeitssignale eine

wichtige Rolle bei der Regulation der Reproduktion in sozialen Insektenstaaten spielen (Endler et al. 2006). Diese neue Sichtweise trägt weiter zum Verständnis der Funktion von Königinnenpheromonen bei (Dor et al. 2005). Sozialparasitische Arbeiterinnen von *A. m. capensis* erreichen über die Sekretion eines königinnenähnlichen Mandibularpheromons eine prominente reproduktive Position innerhalb der *A. m. scutellata* Kolonie. Die Analyse der Ovarien und Pheromonproduktion ergab, dass die Parasiten der ersten Generation nicht durch die von der Wirtskönigin (Hoover et al. 2003) und der Brut (Arnold et al. 1994; Mohammedi et al. 1998) sekretierten Pheromone unterdrückt werden. Somit reagieren die reproduzierenden Arbeiterinnen weniger auf die direkt und indirekt von der Königin hervorgerufene, hemmende Pheromonproduktion. Die beobachtete Inhibition der Reproduktion von parasitischen Nachkommen veranschaulicht pheromonale Unterschiede während der parasitischen Übernahme. Die Pseudoköniginnen erzeugen vermutlich ein deutlich stärkeres kollektives Pheromonsignal als es die *A. m. scutellata* Wirtskönigin vermag. Die resultierenden extremen Differenzen im Phänotyp der klonalen parasitischen Arbeiterinnen lassen den Schluss zu, dass genetische Varianz nicht unbedingt notwendig ist, um den Selbstorganisationsprozess in reproduktive und sterile Arbeiterinnen zu initiieren. Zusammenfassend betrachtet ist die herausragende Sekretion der Königinnensubstanz durch die sozialparasitischen Pseudoköniginnen ein wirkungsvolles Werkzeug für die Erlangung der reproduktiven Dominanz innerhalb einer *A. m. scutellata* Kolonie. Diese Studie konnte herausarbeiten, dass die zuerst in die Wirtskolonie eindringenden Arbeiterinnen die Reproduktion monopolisieren. Des Weiteren ist die Überwindung des inhibitorischen Pheromonsignals der Wirtskönigin und damit das Aufbrechen der reproduktiven Arbeitsteilung der Schlüssel für den Erfolg der *A. m. capensis* Arbeiterinnen als Sozialparasiten. Damit wird eine vorhergehende Untersuchung (Martin et al. 2002a) ergänzt, nach der durch sozialparasitische Arbeiterinnen gelegte Eier im Wirtsvolk nicht mittels „worker policing“ entfernt werden. Die Reproduktion der Parasiten ist sehr effektiv, da die schlüpfenden Larven auch noch bevorzugt von den Wirtsarbeiterinnen gefüttert werden (Beekman et al. 2000; Allsopp et al. 2003).

Der Verlust der *A. m. scutellata* Wirtskönigin ist ein integraler Bestandteil des Infektionszyklus parasitischer Arbeiterinnen (Martin et al. 2002a; Neumann und Hepburn 2002). Moritz et al. (2003) postulierten das Auftreten eines Königinnen-Arbeiterinnen Konflikt zwischen Wirts- und Pseudoköniginnen. Es wurde von tödlichen Duellen zwischen parasitischen Arbeiterinnen und der Wirtskönigin (ähnlich Königin-Königin-Kämpfen) berichtet (Moritz et al. 2003). Meistens führen diese Duelle zum Tod der parasitischen Arbeiterin, können aber gelegentlich auch den Tod der Wirtskönigin zur Folge haben. Normalerweise werden in Honigbienenenvölkern Königin-Arbeiterin Konflikte über pheromonale Kommunikation gelöst. Wenn parasitische Arbeiterinnen nicht auf die pheromonalen Signale der Königin reagieren, dann kann ein pheromonaler Wettbewerb zwischen den Kontrahenten entstehen. Dieser Wettbewerb könnte zu einer Schwächung der Königin führen, die schließlich durch ihre Arbeiterinnen abgestoßen werden kann. Sollte es so sein, dann wäre eine alternative Erklärung für den beobachteten Königinnenverlust in infizierten *A. m. scutellata* Kolonien gegeben. In Arenaversuchen haben wir untersucht, auf welche Weise parasitische Arbeiterinnen auf Pheromonebene mit Königinnen unterschiedlichen Alters und verschiedener Unterart konkurrieren können. Die Ergebnisse dieses Versuches zeigen, dass die *A. m. scutellata* Königinnen die

Produktion von königinnenähnlichen Mandibeldrüsenpheromonen durch die parasitischen Pseudoköniginnen nicht unterbinden können. Es konnten keine Unterschiede zwischen den *A. m. scutellata* Königinnen und den parasitischen Arbeiterinnen in den Pheromonverhältnissen der mandibularen Königinnenkomponenten gefunden werden. Dennoch ist die Gesamtmenge von 9-ODA in Königinnen größer. Die Experimente zeigen darüber hinaus, dass eine einzige *A. m. capensis* Arbeiterin aus dem natürlichen Verbreitungsgebiet in der Kapregion, eine jungfräuliche *A. m. scutellata* Königin pheromonal dominieren kann. In einem Fall produzierte eine sozialparasitische Arbeiterin sogar mehr 9-ODA als die endemische *A. m. capensis* Königin. Sozialparasitische Arbeiterinnen können demnach auch weiselrichtige Kolonien im natürlichen Verbreitungsgebiet infizieren. Wir haben niemals physische Auseinandersetzungen zwischen den gepaarten Bienen beobachten können, derartige Konflikte scheinen folglich eher selten aufzutreten. Diese Studie unterstreicht unter den Bedingungen eines reduktionistischen Ansatzes, dass das Überwinden des inhibitorischen Effekts der Königinnenpheromone ein essentieller Schritt bei der Etablierung der sozialparasitischen Arbeiterinnen ist. Wenn die Pheromonproduktion der Wirtskönigin einen bestimmten Schwellenwert unterschreitet, dann könnten die parasitische Pseudoköniginnen bevorzugt als sich reproduzierende Individuen akzeptiert werden. Der Verlust der Wirtskönigin ist wahrscheinlich eine Folge der Schwächung der Königin, die aus einer Kombination von physischen Kämpfen und Pheromonwettbewerb mit parasitischen Arbeiterinnen resultiert (Dietemann et al. 2006a).

Pheromonaler Wettbewerb bestimmt die reproduktiven Dominanzhierarchien zwischen *A. m. capensis* Arbeiterinnen, bei der die dominanteste Arbeiterin die 9-ODA-Produktion der subordinaten Arbeiterinnen unterdrückt (Moritz et al. 2000, 2004, Simon et al. 2005). Um die Frage beantworten zu können, ob die sozialparasitische Linie sich durch eine schnelle Selektion für reproduktive Dominanz angepasst hat, haben wir die königinnenähnliche Pheromonproduktion von parasitischen und nicht-parasitischen Arbeiterinnen verschiedener Herkünfte untersucht. Wir haben dabei die folgende aufsteigende Dominanzhierarchie zwischen den Honigbienenarbeiterinnen angenommen: 1) *A. m. scutellata*, 2) natürlich vorkommende Hybride (*A. m. scutellata* x *A. m. capensis*) aus der Hybridzone, 3) von Königinnen abstammende *A. m. capensis* Arbeiterinnen, 4) von Arbeiterinnen abstammende *A. m. capensis* Arbeiterinnen und 5) Parasitische *A. m. capensis* Arbeiterinnen aus der invasiven Linie, die in infizierten *A. m. scutellata* Kolonien entnommen wurden. Die sozialparasitischen *A. m. capensis* Arbeiterinnen dominieren pheromonal alle anderen untersuchten Arbeiterinnengruppen. Die von uns beobachtete Arbeiterinnenhierarchie entsprach der vorher theoretisch erarbeiteten. Zieht man die Bedeutung der Königinnenpheromone bei der Etablierung des reproduktiven Status in Betracht, unterstreichen unsere Daten die Annahme, wonach parasitische Arbeiterinnen durch ihre herausragende Fähigkeit zur Produktion von königinnenähnlichen Mandibeldrüsenpheromonen einer positiven Selektion unterliegen. Da Thelytokie und andere Merkmale für Arbeiterinnenreproduktion genetisch gekoppelt sind (Lattorff et al. in review) und weniger durch Rekombination von einander getrennt werden (Baudry et al. 2004), sollte ein Arbeiterinnengenotyp mit starker reproduktiver Dominanz im Vorteil sein. Unsere Daten legen demzufolge eine schnelle Selektion für pheromonvermittelte reproduktive Dominanz zwischen den in das neue Verbreitungsgebiet gebrachten *A. m. capensis* Arbeiterinnen nahe. Die

Selektion von sozialparasitischen Arbeiterinnen eines bestimmten Genotyps (Kryger 2001a,b) innerhalb des Verbreitungsgebietes der Wirtspopulation ist daher ein sympatrischer Prozess.

Die sozialparasitischen *A. m. capensis* Arbeiterinnen sind räumlich von ihrer Ursprungspopulation getrennt. Dennoch bleibt die Frage nach einer eventuell bereits etablierten sympatrischen Reproduktionsbarriere zum intraspezifischen *A. m. scutellata* Wirt offen. Diese würde auf einen Artbildungsprozess in der parasitischen Linie hindeuten. Genfluss von einer sich sexuell fortpflanzenden Population zu den parasitischen Arbeiterinnen ist nicht möglich, weil die Honigbienenarbeiterinnen nicht zur Paarung fähig sind. Der Genfluss vom Parasiten zum Wirt ist nur eingeschränkt möglich, da thelytoke Reproduktion durch ein einziges rezessives Gen determiniert wird (Lattorff et al. 2005), legen parasitische Arbeiterinnen ausschließlich weibliche Eier. Somit ist die Produktion von männlichen Geschlechtstieren durch Arbeiterinnen nicht möglich. Der einzig mögliche Weg für Genfluss vom Parasiten zum Wirt wäre die Aufzucht von Königinnen aus parasitischen Larven. Wir untersuchten hier mit Hilfe von genetischen Werkzeugen, ob diese genannten Einschränkungen des intraspezifischen Genflusses einen Einfluss auf Populationsebene haben. Um die reproduktive Isolation zwischen *A. m. capensis* und der *A. m. scutellata* Wirtspopulation zu untersuchen, haben wir Arbeiterinnen beider Subspezies von infizierten Wirtskolonien in allen südafrikanischen Provinzen wo diese gemeldet wurden, gesammelt. Unabhängig vom Fundort zeigen alle (N=257) parasitischen Arbeiterinnen die genetische Signatur eines Klons. In meiner Arbeit konnte das invasive Verbreitungsgebiet der parasitischen Linie, die auf nur eine Arbeiterin zurückzuführen ist, auf 275.000 km² erweitert werden. Ihr Verbreitungsgebiet ist damit sogar größer als das endemische Verbreitungsgebiet von *A. m. capensis* in der Kapregion von Südafrika (Hepburn und Radloff 1998). Wir konnten darlegen, dass der Genfluss zwischen der *A. m. scutellata* Wirtspopulation und dem intraspezifischen Parasiten sehr selten ist, was auf eine starke sympatrische Barriere hindeutet. Die genetischen Daten offenbaren, dass diese Barriere einen stärkeren Effekt zu haben scheint, als die assortative Paarung (Feder et al. 1994; Via 1999; Malausa et al. 2005). Die reproduktive Separation wird höchstwahrscheinlich durch Mechanismen erreicht, die der reproduktiven Arbeitsteilung in Honigbienenkolonien zugrunde liegen. Die Kastendetermination innerhalb der Honigbienenkolonie ist die erste Barriere für einen Genfluss, da Honigbienenarbeiterinnen sich nicht paaren können. Dennoch ist Genfluss möglich, da eine durch Thelytokie produzierte Larve theoretisch zu einer Königin herangezogen werden kann. Das starke kollektive Mandibeldrüsenpheromon der initial infizierenden Pseudoköniginnen scheint als eine zweite wichtige Barriere zu wirken, da die produzierte Königinnensubstanz nicht nur einen Einfluss auf die Physiologie der Wirtsarbeiterinnen (Kaatz et al. 1992), also der reproduktiven Arbeitsteilung hat, sondern ebenfalls das Heranziehen von Königinnen beeinflusst (Pettis et al. 1997). Die gefundene Barriere ist praezygotisch, da sie das Aufziehen von Königinnen in infizierten Kolonien betrifft bevor es zu einer Kreuzung von parasitischen Königinnen und Wirtsdrohnen kommen kann. Darüber hinaus verhindert die große Menge an parasitischer Brut, die zu Beginn einer Infektion beobachtet werden kann (Martin et al. 2002b, von der Heide 2004), ebenfalls die thelytoke Produktion von parasitischen Königinnen. Daher arbeiten die von den Parasiten direkt oder indirekt produzierten Pheromone synergistisch gegen einen Genfluss. Das Ergebnis der Untersuchungen der sozialparasitischen Population bestätigt frühere Studien, in denen angedeutet wurde, dass nur eine einzige klonale

Linie die *A. m. scutellata* Populationen infiziert. Der am weitesten verbreitete multilocus Genotyp stellt wahrscheinlich den Gründergenotyp dar. Die genetischen Daten deuten an, dass die thelytoke Reproduktion eine mögliche Art der sympatrischen reproduktiven Isolation darstellt. Zieht man in Betracht, dass die genetische Separation des Wirts und des Parasiten in sympatrischer Speziation erfolgt, dann fügt diese Dissertation einen neuen evolutionsbiologischen Weg zur Emery Regel hinzu.

Für die parasitische Lebensweise von Arbeiterinnen ist die Transmission ein entscheidender Punkt (Neumann et al. 2001; Neumann und Hepburn 2002). Die große Mehrheit der *A. m. scutellata* Wirtskolonien kommt wild vor (Hepburn und Radloff 1998). Da infizierte *A. m. scutellata* Kolonien in Bienenständen jedoch eine große Menge an parasitischen *A. m. capensis* Arbeiterinnen produzieren (Martin et al. 2002b), erscheint es wahrscheinlich, dass sie neue Kolonien durch Schwarmfusion oder als Einzelbienen infizieren können (Kolonie- versus Einzel-Transmission, Neumann und Hepburn 2002). Es konnte in mehreren Untersuchungen gezeigt werden (Allsopp und Crewe, 1993; Greeff 1997, Dietemann et al. 2006c), dass die Infektion durch Kaphonigbienen einen starken Einfluss auf bewirtschafteten Populationen von *A. m. scutellata* haben. Um die Frage zu beantworten, ob die sozialparasitischen *A. m. capensis* Arbeiterinnen ausschließlich in bewirtschafteten *A. m. scutellata* Populationen gefunden werden können oder auch wilde Populationen infizieren, habe ich Schwärme sowohl in Regionen mit reger Imkertätigkeit als auch in Naturschutzgebieten untersucht und verglichen. In den Regionen mit Imkertätigkeit konnten Infektionen festgestellt werden. Schwärmen die in Naturschutzgebieten untersucht wurden waren jedoch frei von Infektionen. Eine Evaluierung der Methode für die Identifizierung der parasitischen Arbeiterinnen mittels ihrer schwarzen Tergite, konnte mit Hilfe von DNA-Genotypisierung keinen kausalen Zusammenhang mit der Unterart *A. m. capensis* herstellen. Folglich ist diese Methode für die Identifizierung von parasitischen Arbeiterinnen nicht zureichend. Dennoch kann durch die Eliminierung von Kolonien, die Bienen mit schwarzen Tergiten beinhalten, eine falsch negative Diagnose verhindert werden (Härtel et al. 2006b). Einer populationsökologischen Modellierung zufolge ist nur unter der Voraussetzung von hohen Koloniedichten in der Wildpopulation eine massive Ausbreitung der Parasiten wahrscheinlich (Moritz 2002). Leider fehlen bisher Daten über natürliche Infektionsraten, d.h. inwiefern parasitische Arbeiterinnen von wilden *A. m. scutellata* Kolonien andere wilde Kolonien infizieren können. Diese Frage sollte in zukünftigen Untersuchungen Beachtung finden.

Obwohl meine Arbeit viele Fragen des intraspezifischen Sozialparasitismus von Arbeiterinnen im speziellen Fall behandelt, kann sie ebenfalls allgemeine Einblicke in das Phänomen Sozialparasitismus bei sozialen Insekten geben. Wenn Parasiten zu unterschiedlichen Unterarten, Arten oder Gattungen wie ihr Wirt gehören, dann können sowohl Ähnlichkeiten als auch Unterschiede im Verhalten oder in der chemischen Kommunikation zwischen den beiden Protagonisten genutzt werden, um deren zum Sozialparasitismus führende evolutive Mechanismen zu identifizieren. Des Weiteren können diese Daten genutzt werden, um Kenntnisse über Mechanismen zu gewinnen, die der reproduktiven Arbeitsteilung in Insektenstaaten zugrunde liegen. Unter diesem Aspekt habe ich gezeigt, dass sozialparasitische Arbeiterinnen von *A. mellifera* die gleichen Pheromonsignale nutzen, wie die Honigbienenköniginnen. Wie bereits gesagt reagieren sozialparasitische Arbeiterinnen weniger auf die inhibitorischen Signale der Wirtskönigin. Außerdem können sie sich dem „worker

„policing“ der Wirtsarbeiterinnen entziehen. Die Wirtskönigin wird entweder in tödliche Kämpfe verwickelt oder im pheromonalen Wettstreit geschwächt. Als Konsequenz erlangen die Sozialparasiten eine dominante Position innerhalb der reproduktiven Hierarchie der Wirtskolonie. Die Produktion des königinnenähnlichen Pheromons kann mit dem Status der Ovarien der *A. m. capensis* Arbeiterinnen korreliert werden, welches andeutet, dass es sich um ein Fertilitätssignal handelt. Dieses Signal wird auch von nicht verwandten Wirtskolonien akzeptiert. Daher ist die durch Pheromone vermittelte reproduktive Dominanz von Verwandtschaft unabhängig und kann auch zwischen klonalen Arbeiterinnen, Schwestern, Cousinsen oder zufälligen, nichtverwandten Nestgenossen etabliert werden. Weiterhin konnte ich ein Beispiel für eine Reproduktionsbarriere aufzeigen, die durch Mechanismen etabliert wird, welche der reproduktiven Arbeitsteilung in eusozialen Insektenkolonien zugrunde liegt. Dies stellt eine Alternative zur herkömmlichen reproduktiven Isolation durch assortative Paarung dar, die in der Regel bei der Evolution von Sozialparasitismus durch Geschlechtstiere angenommen wird.

10.3 Zukunftsperspektiven der sozialparasitischen Linie

Die Invasion von sozialparasitischen Arbeiterinnen der Kaphonigbiene in Populationen der benachbarten Unterart *A. m. scutellata* schaffte die Voraussetzung für ein faszinierendes Wirt/Parasit System. Die zwei Protagonisten sind die sich sexuell fortpflanzende *A. m. scutellata* Wirtspopulation und die über thelytoke Parthenogenese reproduzierenden Sozialparasiten. Während die Wirtspopulation den Vorteil von Mendelscher Aufspaltung und genetischer Rekombination hat, bestehen die Sozialparasiten aus zahllosen klonalen Individuen. Es wird weithin angenommen, dass Parasitismus die zentrale Kraft ist, welche die Evolution von Sex beeinflusst (Maynard-Smith 1978). Sexuelle Fortpflanzung sollte daher theoretisch im Vorteil gegenüber klonaler Reproduktion sein. Somit haben wir hier ein intraspezifisches System, welches uns den Vergleich zwischen sexueller und klonaler Fortpflanzung erlaubt. Natürliche Selektion innerhalb der *A. m. scutellata* Populationen würde Kolonien begünstigen, die Aggressionsverhalten gegen sich entwickelnde sozialparasitische Arbeiterinnen erkennen lassen (Crewe 1984; Neumann et al. 2006). Diese Kolonien könnten eine größere Fitness erreichen, da sie die Infektionen vermeiden und demzufolge eine größere Anzahl von Drohnen und überlebensfähigen Schwärmen erzeugen können (Moritz und Southwick 1992). Sollte dieses Verhalten eine genetische Grundlage haben, dann könnten die dazugehörigen Allelfrequenzen innerhalb der *A. m. scutellata* ansteigen. Die epidemieartige Ausbreitung der parasitischen Linie sollte dadurch deutlich eingeschränkt werden. Nichtsdestotrotz, solange südafrikanische Imker ihre Schwärme in Populationen fangen, die nicht in Kontakt mit dem Parasiten stehen, kann die natürliche Selektion nicht wirken. Neben der durch die Imkerei weitgehend unterstützten Transmission der Sozialparasiten ist dies wahrscheinlich ein weiterer Grund warum das „dwindling colony syndrome“ immer noch ein beträchtliches Problem für die südafrikanische Imkerei darstellt. Andererseits könnte die klonale Arbeiterinnenlinie durch vorteilhafte Mutationen an die neuen Bedingungen adaptieren. Ein besser adaptierter Genotyp könnte sich gegen den bisherigen durchsetzen und ein „co-evolutionary arms race“ zwischen den Sozialparasiten und der Wirtspopulation beginnen. Momentan können wir nur die ersten Schritte einer Evolution eines Wirt-Parasiten Systems bezeugen (Neumann

und Moritz 2002). Die sozialparasitische Linie kann sich nur dann über evolutionsbiologische Zeiträume halten, wenn sie ihre Wirtspopulation nicht vollständig auslöscht. Die zur Zeit beobachtete hohe Virulenz würde zunächst die Wirtspopulation massiv reduzieren und auf längere Sicht zur Extinktion dieser obligaten und genetisch isolierten sozialparasitischen Linie führen.

10.4 Literatur

- Allsopp MH (1992) The capensis calamity. *S Afr Bee J* **64**: 52-55.
- Allsopp MH, Crewe RM (1993) The Cape honeybee as a Trojan horse rather than the hordes of Jenghiz Khan. *Am Bee J* **133**: 121-123.
- Allsopp MH, Calis JNM, Boot WJ (2003) Differential feeding of worker larvae affects caste characters in the Cape honeybee, *Apis mellifera capensis*. *Behav Ecol Sociobiol* **54**: 555-561.
- Beekman M, Calis JNM, Boot WJ (2000) Parasitic honeybees get royal treatment. *Nature* **404**:723.
- Arnold G, LeConte Y, Trouiller J, Hervet H, Chappe B, Masson C (1994) Inhibition of worker honeybee ovaries development by a mixture of fatty-acid esters from larvae. *Comptes Rendus De L Academie Des Sciences Serie III* **317**: 511-515.
- Anderson RH (1968). The effect of queen loss on colonies of *Apis mellifera capensis*. *S Afr J Agric Sci* **11**: 383-388.
- Barron AB, Oldroyd BP, Ratnieks FL (2001) Worker reproduction in honey-bees (*Apis*) and the anarchic syndrome: a review. *Behav Ecol and Sociobiol* **50**: 199-208.
- Baudry E, Kryger P, Allsopp M, Koeniger N, Vautrin D, Mougél F, Cornuet J-M, Solignac M (2004) Whole-genome scan in thelytokous-laying workers of the Cape honeybee (*A. m. capensis*): Central fusion, reduced recombination rates and centromere mapping using half tetrad analysis. *Genetics* **167**: 243-252.
- Beekman M, Good G, Allsopp MH, Radloff S, Pirk CWW, Ratnieks FLW (2002) A non-policing honey bee colony (*Apis mellifera capensis*). *Naturwissenschaften* **89**: 479-482.
- Beekman M, Oldroyd BP (2003) Different policing rates of eggs laid by queenright and queenless anarchistic honey-bee workers (*Apis mellifera* L.) *Behav Ecol and Sociobiol* **54**: 480-484.
- Beekman M, Oldroyd BP (2005) Honeybee workers use cues other than egg viability for policing. *Biol Lett* **1**: 129-132.
- Crewe RM (1984) Differences in behaviour and morphology between *capensis* and *adansonii*. *S Afr Bee J* **56**: 16-21.
- Cuvillier-Hot V, Cobb M, Malosse C, Peeters C (2001) Sex, age and ovarian activity affect cuticular hydrocarbons in *Diacamma ceylonense*, a queenless ant. *J Insect Physiol* **47**: 485-493.
- Cuvillier-Hot V, Lenoir A, Peeters C (2004) Reproductive monopoly enforced by sterile police workers in a queenless ant. *Behav Ecol* **15**: 970-975.
- Dietemann V, Peeters C, Liebig J, Thivet V, Hölldobler B (2003) Cuticular hydrocarbons mediate recognition of queens and reproductive workers in the ant *Myrmecia gulosa*. *Proc Natl Acad Sci USA* **100**: 10341-10346.
- Dietemann V, Pflugfelder J, Härtel S, Neumann P, Crewe RM (2006a) Social parasitism by honeybee workers (*Apis mellifera capensis* Esch.): evidence for

- pheromonal resistance to host queens signals. *Behaviour Ecology and Sociobiology*: in press.
- Dietemann V, Neumann P, Härtel S, Crewe RM (2006b) Pheromonal dominance and the selection of a socially parasitic honeybee worker lineage (*Apis mellifera capensis* Esch.). *J Evol Biol*: in review.
- Dietemann V, Lubbe A, Crewe RM (2006c) Human factors facilitating the spread of a parasitic honeybee in South Africa. *J Econ Entomol* **99**: 7-13.
- Dor R, Katzav-Gozansky T, Hefetz A (2005) Dufour's gland pheromone as a reliable fertility signal among honeybee (*Apis mellifera*) workers. *Behav Ecol and Sociobiol* **58**: 270-276.
- Dugatkin L A (1997) *Cooperation among animals: an evolutionary perspective*. Oxford: Oxford University Press.
- Dugatkin L A (2004) *Principles of animal behaviour*. New York: W. W. Norton.
- Endler A, Liebig J, Hölldobler B (2006) Queen fertility, egg marking and colony size in the ant *Camponotus floridanus*. *Behav Ecol and Sociobiol* **59**: 490-499.
- Feder JL, Opp S, Wlazlo B, Reynolds K, Go W, Spisak S (1994) Host Fidelity is an Effective Premating Barrier Between Sympatric Races of the Apple Maggot Fly. *Proc Natl Acad Sci* **91**: 7990-7994.
- Greeff JM (1996) Effects of thelytokous worker reproduction on kin-selection and conflict in the Cape honeybee, *Apis mellifera capensis*. *Phil Trans R Soc Lond [B]* **351**: 617-625.
- Greeff JM (1997) The Cape honeybee and her way north: an evolutionary perspective. *S. Afr. J. Sci.* **93**: 306-308.
- Härtel S, Neumann P, Raasen FS, Moritz RFA, Hepburn, HR (2006a) Social parasitism by Cape honeybee workers in colonies of their own subspecies (*Apis mellifera capensis* Esch.). *Insectes Soc* **53**: in press.
- Härtel S, Neumann P, Kryger P, von der Heide C, Moltzer G-J, Crewe RM, van Praagh JP, Moritz RFA (2006b) Infestation levels of *Apis mellifera scutellata* swarms by socially parasitic Cape honeybee workers (*A. m. capensis* Esch.). *Apidologie* **37**: in press.
- Härtel S, Neumann P, Kryger P, Moritz RFA, Crewe RM (2006c) Emery's rule in Cape honeybees (*Apis mellifera capensis*). *Evolution*: submission to.
- Härtel S, Neumann P, Wossler TC, Moltzer G-J, Moritz RFA, Crewe RM (2006d) Dominance hierarchies among clonal socially parasitic workers (*Apis mellifera capensis* Esch.). *J comp Physiol A*: submission to.
- Hemmling C, Koeniger N, Ruttner F (1979) Quantitative Bestimmung der 9-Oxodecensäure im Lebenszyklus der Kaphonigbiene (*Apis mellifera capensis* Escholtz) *Apidologie* **10**: 227-240.
- Hepburn HR (1992) Pheromonal and ovarial development covary in Cape worker honeybees (*Apis mellifera capensis*). *Naturwissenschaften* **79**:523-524.
- Hepburn HR, Allsopp MH, (1994) Reproductive conflict between honeybees: usurpation of *Apis mellifera scutellata* colonies by *Apis mellifera capensis*. *S Afr J Sci* **90**: 247-249.
- Hepburn HR, Radloff SE (1998) *Honeybees of Africa*. Springer Verlag, Berlin Heidelberg New York.
- Hoover SER, Keeling CI, Winston ML, Slessor KN (2003) The effect of queen pheromones on worker honey bee ovary development. *Naturwissenschaften* **90**: 477-480.

- Hoover SER, Keeling CI, Winston ML, Oldroyd BP (2005) Retinue attraction and ovary activation: responses of wild type and anarchistic honey bees (*Apis mellifera*) to queen and brood pheromones. *Behav Ecol and Sociobiol* **59**: 278-284.
- Johannsmeier M.F. 1983. Experiences with the Cape bee in the Transvaal. *S Afr Bee J* **55**: 130-138
- Kaatz HH, Hildebrandt H, Engels, W (1992) Primer effect of queen pheromone on juvenile hormone biosynthesis in adult worker honey bees. *J Comp Physiol B* **162**: 588-592.
- Koeniger G. and Würkner W. 1992. Die Kap Honigbienen (*Apis mellifera capensis*) Natürliche Verbreitung und die Schwierigkeiten der Haltung unter unseren klimatischen Bedingungen. *Die Biene* **128**: 583-588.
- Kryger P (2001a) The Capensis pseudo-clone, a social parasite of African honey bees. In Proceedings, 2001 Berlin Meeting of the European Section of IUSSI, p. 208, IUSSI, Berlin.
- Kryger P (2001b) The pseudo-clone of *Apis mellifera capensis* – an obligate social parasite in honeybees. In: Proceedings, XXXVII International Apicultural Congress, p.33, Durban South Africa.
- Lattorff HMG, Moritz RFA, Fuchs S (2005) A single locus determines thelytokous parthenogenesis of laying honeybee workers (*Apis mellifera capensis*). *Heredity* **94**: 533-537.
- Liebig J, Peeters C, Oldham NJ, Markstädter C, Hölldobler B (2000) Are variations in cuticular hydro-carbons of queens and workers a reliable signal of fertility in the ant *Harpegnathos saltator*? *Proc Natl Acad Sci USA* **97**: 4124-4131.
- Lopez-Vaamonde C, Koning JW, Brown RM, Jordan WC, Bourke AFG (2004) Social parasitism by male-producing reproductive workers in a eusocial insect. *Nature* **430**: 557-560.
- Lundie A. E. 1954. Laying workers produce worker bees. *S Afr Bee J* **29**: 10-11.
- Malausa T, Bethenod MT, Bontemps A, Bourguet D, Cornuet J-M, Ponsard S (2005) Assortative Mating in Sympatric Host Races of the European Corn Borer. *Science* **308**: 258-260.
- Martin CG, Oldroyd BP, Beekman M (2004) Differential reproductive success among subfamilies in queenless honeybee (*Apis mellifera* L.) colonies. *Behav Ecol and Sociobiol* **56**: 42-49.
- Martin SJ, Beekman M, Wossler TC, Ratnieks FLW (2002a) Parasitic Cape honeybee workers, *Apis mellifera capensis* evade policing. *Nature* **415**: 163-165.
- Martin SJ, Wossler TC, Kryger P (2002b) Usurpation of African *Apis mellifera scutellata* colonies by parasitic *Apis mellifera capensis* workers. *Apidologie* **33**: 215–232.
- Mohammedi A, Paris A, Causer D, Le Conte Y (1998) Effect of aliphatic esters on ovary development of queenless bees. *Naturwissenschaften* **85**: 455-458.
- Montague CE, Oldroyd BP (1998) The evolution of worker sterility in honey bees: An investigation into a behavioral mutant causing failure of worker policing. *Evolution* **52**: 1408-1515.
- Moritz RFA (2002) Population dynamics of the Cape bee phenomenon: The impact of parasitic laying worker clones in apiaries and natural populations. *Apidologie* **33**: 233-244.
- Moritz RFA, Southwick EE (1992) *Bees as superorganisms. An evolutionary reality.* Springer Verlag, Berlin, Heidelberg, New York.

- Moritz RFA, Kryger P, Allsopp MH (1996) Competition for royalty in bees. *Nature* **384**: 31.
- Moritz RFA, Kryger P, Allsopp M (1999) Lack of worker policing in the Cape Honeybee (*Apis mellifera capensis*). *Behaviour* **136**: 1079-1092.
- Moritz RFA, Simon UE, Crewe RM (2000) Pheromonal contest between honeybee workers. *Naturwissenschaften* **87**: 395-397.
- Moritz RFA, Pflugfelder J, Crewe RM (2003) Lethal fighting between honeybee queens and parasitic workers (*Apis mellifera*). *Naturwissenschaften* **90**: 378-381.
- Moritz RFA, Lattorff HMG, Crewe RM (2004) Honeybee workers (*Apis mellifera capensis*) compete for producing queen-like pheromone signals. *Proc Soc Lond B Suppl* **271**: S98-S100.
- Moritz RFA, Härtel S, Neumann P (2005) Global invasions of the western honeybee (*Apis mellifera* L.) and the consequences for biodiversity. *Écoscience* **12**: 289-301.
- Nanork P, Paar J, Chapman NC, Wongsiri S, Oldroyd BP (2005) Asian honeybees parasitize the future dead. *Nature* **437**: 829.
- Neumann P, Hepburn HR (2002) Behavioural basis for social parasitism of Cape honeybees (*Apis mellifera capensis* Esch.). *Apidologie* **33**: 165-192.
- Neumann P, Moritz RFA (2002) The Cape honeybee phenomenon: the evolution of a social parasite in real time? *Behav Ecol and Sociobiol* **52**: 271-281.
- Neumann P, Pirk CWW, Hepburn HR, Moritz RFA (2003) Spatial differences in worker policing facilitate social parasitism by Cape honeybee workers (*Apis mellifera capensis* Esch.) in queenright host colonies. *Insectes Soc* **50**: 109-113.
- Neumann P, Radloff SE, Moritz RFA, Hepburn HR, Reece SL (2001) Social parasitism by honeybee workers (*Apis mellifera capensis* Escholtz): host finding and resistance of hybrid host colonies. *Behav Ecol* **12**: 419-428.
- Neumann P, Raassen FS, Härtel S, Wendt M, Hepburn HR, Pirk CWW (2006) Social analogue of immune memory in honeybee colonies. *Naturwissenschaften*: in revision.
- Oldroyd BP, Ratnieks FLW (2000) Evolution of worker sterility in honey-bees (*Apis mellifera*): how anarchistic workers evade policing by laying eggs that have low removal rates. *Behav Ecol and Sociobiol* **47**: 268-273.
- Oldroyd BP, Halling L, Rinderer TE (1999) Development and behaviour of anarchistic honeybees. *Proc Roy Soc Lond B* **266**: 1875-1878.
- Oldroyd BP, Wossler TC, Ratnieks FLW (2001) Regulation of ovary activation in worker honey-bees (*Apis mellifera*): larval signal production and adult response thresholds differ between anarchistic and wild-type bees. *Behav Ecol and Sociobiol* **50**: 366-370.
- Oldroyd BP, Smolenski AJ, Cornuet J-M, Crozier RH (1994) Anarchy in the beehive: a failure of worker policing in *Apis mellifera*. *Nature* **371**: 479.
- Onions GW (1912) South African "fertile-worker bees". *S Afr Agric J* **1**: 720-728.
- Page RE, Robinson GE (1994) Reproductive competition in queen-less honeybee colonies (*Apis mellifera* L.). *Behav Ecol and Sociobiol* **35**: 99-107.
- Pirk CWW, Neumann P, Ratnieks FLW (2003) Cape honeybees, *Apis mellifera capensis*, police worker laid eggs despite the absence of relatedness benefits. *Behav Ecol* **14**: 347-352.
- Peeters C, Monnin T, Malosse C (1999) Cuticular hydrocarbons correlated with reproductive status in a queenless ant. *Proc R Soc B* **266**: 1323-1327.
- Petty FW (1922) Workers laying in comb of extracting supers, Elsenberg Apiary. *J Dep Agr Union S Afr* **4**: 122-124.

- Pettis JS, Higo HA, Pankiw T, Winston ML (1997) Queen rearing suppression in the honey bee – evidence for a fecundity signal. *Insect Soc* **44**: 311-322.
- Plettner E, Slessor KN, Winston ML (1998) Biosynthesis of mandibular acids in honey bees (*Apis mellifera*). De novo synthesis, route of fatty acid hydroxylation and caste-selective β -oxidation. *Insect Biochem and Mol Biol* **28**: 31-42.
- Plettner E, Slessor KN, Winston ML, Oliver JE (1996) Caste-selective pheromone biosynthesis in honeybees. *Science* **271**:1851-1853
- Plettner E, Slessor KN, Winston ML, Robinson GE, Page RE (1993) Mandibular gland components and ovarian development as measures of caste differentiation in the honey bee (*Apis mellifera* L.). *J Insect Physiol* **39**: 235-240.
- Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *Am Nat* **132**: 217-236.
- Seeley TD (1985) *Honeybee ecology: a study of adaptation in social life*. Princeton University Press, Princeton.
- Simon U, Moritz RFA, Crewe RM (2005) Reproductive dominance among honeybee workers in experimental groups of *Apis mellifera capensis*. *Apidologie* **36**: 413-419.
- Velthuis HHW, van der Kerk A (1988) Age, environment, and genes in relation to the mandibular gland secretion of pure and hybrid *Apis mellifera capensis* worker bees. In: Needham GR, Page RE, Delfinado-Baker M, Bowman CE (eds.) *Africanized honeybees and bee mites*. Ellis Horwood, Chichester, pp 80-86.
- Via S (1999) Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice. *Evolution* **53**: 1446-1457.
- Visscher PK, Dukas R (1995) Honey bees recognise development of nestmates' ovaries. *Anim Behav* **49**: 542-544.
- von der Heide C (2004) Infestation levels of natural honeybee populations (*Apis mellifera scutellata*) with socially parasitic Cape honeybee workers (*A. m. capensis* Esch.) and consequences for pollination success. MSc thesis Universität Hannover, Germany.
- Whitfield J (2002) The police state. *Nature* **416**: 782–784.
- Woyciechowski M, Lomnicki A (1987) Multiple mating of queens and sterility of workers among eusocial Hymenoptera. *J Theor Biol* **128**: 317-327.
- Woyke J. 1995. Invasion of Capensis bee, In: Proc. First Int. Electronic Conf. Cape Bee problem in South Africa, 5-30 June 1995 (Magnuson P., Ed.), Pretoria, PPRI, p. 35.

11 Appendix

11.1 Declaration on the contributions to the manuscripts/papers on which this thesis is based:

1. Härtel S, Neumann P, Raassen FS, Moritz RFA, Hepburn, HR (2006a) Social parasitism by Cape honeybee workers in colonies of their own subspecies (*Apis mellifera capensis* Esch.). *Insectes Soc* **53**: 183-193.

Project idea, behavioural assays, DNA analysis, statistical data analysis, manuscript writing and preparation

2. Härtel S, Neumann P, Kryger P, von der Heide C, Moltzer G-J, Crewe RM, van Praagh JP, Moritz RFA (2006b) Infestation levels of *Apis mellifera scutellata* swarms by socially parasitic Cape honeybee workers (*Apis mellifera capensis* Esch.). *Apidologie* **37**: 462-470.

Field work, genetic analysis, statistical data analysis, manuscript writing and preparation

3. Härtel S, Neumann P, Kryger P, Moritz RFA, Crewe RM (2006c) Emery's rule in Cape honeybees (*Apis mellifera capensis*). *Evolution*: to be submitted.

Data collection, DNA analysis, population genetic analyses, manuscript writing and preparation

4. Härtel S, Neumann P, Wossler TC, Moltzer G-J, Moritz RFA, Crewe RM (2006d) Dominance hierarchies among clonal socially parasitic workers (*Apis mellifera capensis* Esch.). *J comp Physiol A* : to be submitted.

Project idea, field work, data collection, genetic analyses, statistical data analysis, manuscript writing and preparation

5. Dietemann V, Pflugfelder J, **Härtel S**, Neumann P, Crewe RM (2006a) Social parasitism by honeybee workers (*Apis mellifera capensis* Esch.): evidence for pheromonal resistance to host queens signals. *Behaviour Ecology and Sociobiology* : **60**: 785-793.

Queen breeding, bio assay, data collection, manuscript writing

6. Dietemann V, Neumann P, **Härtel S**, Crewe RM (2006b) Pheromonal dominance and the selection of a socially parasitic honeybee worker lineage (*Apis mellifera capensis* Esch.). *J. Evol. Biol.* : in press.

bio assay, data collection, manuscript writing

7. Neumann P, Raassen FS, **Härtel S**, Wendt M, Hepburn HR, Pirk CWW (2006) Social analogue of immune memory in honeybee colonies. *Biol letters*: to be submitted.

Field work, data collection, manuscript writing

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11.3 Curriculum vitae

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Personal Details

Date and place of birth: October 1st 1968 in Berlin, Germany
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Education

09/1975-07/1981 Elementary school, Berlin
 09/1981-06/1989 Secondary school “Broendby Oberschule“, Berlin
 07/1989-08/1991 Various travels and volunteers
 09/1991-02/1994 Trainee at the KRONE AG, Berlin
 02/1994-09/1994 Position as industrial sales representative at KRONE AG, Berlin
 10/1994-12/1999 Study of Biology
 Martin-Luther-University of Halle-Wittenberg
 Major subjects: Zoology, Genetics, Biochemistry and Botany
 11/2000 MSc thesis: Title: “Mapping of QTLs for the post capping period of drones *Apis mellifera* L.”
 Supervisor: Prof. Robin F.A. Moritz (Molecular Ecology)
 grade: sehr gut (A)
 12/2000-09/2001 Martin-Luther-University of Halle-Wittenberg
 Research assistant in a Sachsen-Anhalt funded project “Molecular analysis of the sex determining pathway of the honeybee *Apis mellifera*”
 Supervisor: Dr. M. Beye (Honeybee Sex Determination Group)
 Since 10/2001- Martin-Luther-University of Halle-Wittenberg
 PhD project: “Social parasitism in honeybees (*Apis mellifera capensis* Esch)”.
 Supervisor: Dr. P. Neumann (Evolutionary Ecology)
 PhD thesis:
 Title: “Evolution of intraspecific social parasitism in honeybee workers (*Apis mellifera capensis* Esch).”

Professional Career

| Position | Period |
|---|---------------------------|
| Visiting researcher, Rhodes University, Grahamstown, South Africa, Field work for the PhD project, Principal Collaborators: Prof. Randall Hepburn and Prof. S.E. Radloff | 02.01-10.02.2004 |
| Visiting researcher, University of Pretoria, South Africa Field work for the PhD project, Principal Collaborators: Prof. Robin Crewe, Dr. V. Dietemann | 03.01.-19.03.2003 |
| Visiting researcher, Rhodes University, Grahamstown, South Africa, Field work for the PhD project, Principal Collaborators: Prof. Randall Hepburn and Prof. S.E. Radloff | 2.01.-25.04.2002 |
| Research and teaching assistant at the Institut für Zoologie, MLU Halle-Wittenberg in Emmy Noether Project of the DFG to PN, Main Supervisors: PD Dr. P. Neumann and Prof. Robin F. A. Moritz | Since 7.10.2001 |
| Visiting researcher, University of California Davis, USA Honeybee developmental genetic studies, Principal Collaborator: Prof. Robert Page | 03.05.-12.06.2001 |
| Research and teaching assistant in a Sachsen-Anhalt funded project, Sex determination pathway of the honeybee, Principal investigator: Dr. M. Beye | 18.12.2000- 30.09.2001 |
| Visiting student, Rhodes University Grahamstown, South Africa; course on African honeybees in the group of Prof. Randall Hepburn | 02.03.-24.03.2000 |

11.4 Publications

peer reviewed

- Beye M, **Härtel S**, Hagen A, Hasselmann M, Omholt SW (2002) Specific developmental gene silencing in the honey bee using a homeobox motif. *Insect Molecular Biology* **11**: 527-532.
- Neumann P, **Härtel S** (2004) Removal of small hive beetle (*Aethina tumida* Murray) eggs and larvae by African honeybee colonies (*Apis mellifera scutellata* Lepeletier). *Apidologie* **35**: 31-36.
- Moritz RFA, **Härtel S**, Neumann P (2005) Global invasions of the western honeybee (*Apis mellifera* L.) and the consequences for biodiversity. *Écoscience* **12**: 289-301.
- ***Härtel S**, Neumann P, Raassen FS, Moritz RFA, Hepburn HR (2006) Intraspecific social parasitism by workers among Cape honeybees colonies (*Apis mellifera capensis* Esch.) *Insectes sociaux* **53**: 183-193.
- ***Härtel S**, Neumann P, Kryger P, von der Heide C, Moltzer G.-J, Crewe RM, van Praagh J, Moritz RFA (2006) Levels of infestation of wild *Apis mellifera scutellata* populations by socially parasitic Cape honeybee workers (*A. m. capensis* Esch.) *Apidologie* **37**: 462-470.
- ***Härtel S**, Neumann P, Kryger P, Moritz RFA, Crewe RM (2006) Emery's rule in the honeybee (*Apis mellifera capensis*). *Evolution*: to be submitted
- ***Härtel S**, Wossler TC, Neumann P, Moltzer G-J, Moritz RFA, Crewe RM (2006) Dominance hierarchies among clonal socially parasitic workers (*Apis mellifera capensis* Esch). *J comp Physiol A*: to be submitted
- *Neumann P, Raassen FS, **Härtel S**, Wendt M, Hepburn HR, Pirk CWW (2006) Social analogue of immune memory in honeybee colonies. *Biol Letters*: to be submitted
- *Dietemann V, Pflugfelder J, **Härtel S**, Neumann P, Crewe RM (2006) Social parasitism by honeybee workers (*Apis mellifera capensis* Esch.): evidence for pheromonal resistance to host queens signals. *Behaviour Ecology and Sociobiology* **60**: 785-793.
- *Dietemann V, Neumann P, **Härtel S**, Pirk C.W.W., Crewe RM (2007) Pheromonal dominance and the selection of a socially parasitic honeybee worker lineage (*Apis mellifera capensis* Esch.). *J Evol Biol*: in press

manuscripts in preparation

- Härtel S**, Neumann P, Wossler TC, von der Heide C, Crewe RM (2006) Socially parasitic honeybee workers pheromonally gain reproductive isolation from their conspecific host. *Behaviour Ecology and Sociobiology*: to be submitted 2006
- Härtel S**, Neumann P, Hepburn HR (2006) Behaviour of socially parasitic Cape honeybee workers in colonies of their intraspecific host *A. m. scutellata*. *Animal behaviour*: in prep.

non-peer reviewed

- Neumann P, **Härtel S**, Kryger P, Moritz RFA, Crewe RM (2005) Sympatric barrier to gene flow based on reproductive division of labor in honeybees. In: Proceedings of the 11th German-American Frontiers of Science Symposium, Irvine, California, USA, in press.
- Härtel S**, Neumann P, Kryger P, Wossler TC, Moritz RFA, Crewe RM (2005) Worker-based pathway to Emery's rule in honeybees (*Apis mellifera capensis* Esch.). In Kaatz HH, Becher M, Moritz RFA (Eds.) Proceedings of the 19th Congr. IUSSI (German section), Halle, Germany, p. 114.
- Härtel S**, Neumann P, Raassen FS, Moritz RFA, Hepburn, HR (2005) Intraspecific social parasitism in the Cape honeybee (*Apis mellifera capensis* Esch.) In Kaatz HH, Becher M, Moritz, RFA (Eds.) Proceedings of the 19th Congr. IUSSI (German section), Halle, Germany, p. 152.
- Härtel S**, Neumann P, Pflugfelder J, Dietemann V, Moritz RFA, Crewe RM (2005) Emery's rule in honeybee workers (*Apis mellifera capensis* Esch.). In: Proceedings of the graduate meeting "Evolutionary Chemical Ecology" of the DZG Studiengruppe Evolutionsbiologie, Würzburg, Germany, p. 13.
- Härtel S**, Wossler TC, Neumann P, Moltzer G-J, Moritz RFA, Crewe RM (2004) Socially parasitic Cape honeybee workers suppress the development of their offspring. In: Proceedings of the 1st European Conference of Apidology, Udine, Italy, p. 63.
- Spiewok S, **Härtel S**, Neumann P (2004) Plasticity in oviposition behaviour of small hive beetles, *Aethina tumida*. In: Proceedings of the 1st European Conference of Apidology, Udine, Italy, p. 87.
- Dietemann V, Neumann P, **Härtel S**, Pflugfelder J, Crewe RM (2004) Levels of selection and the evolution of the socially parasitic Cape honeybee workers in South Africa. In: Proceedings of the 1st European Conference of Apidology, Udine, Italy, p. 58-59.
- Härtel S**, Neumann P (2004) Evolution in real time: local adaptation of socially parasitic honeybee workers (*Apis mellifera capensis* Esch.). In: Proceedings of the graduate meeting "Co-evolution" of the Studiengruppe Evolutionsbiologie, Münster, Germany, p. 21.
- Neumann P, **Härtel S**, Hepburn HR (2003) Small hive beetle ovipositors vs. honeybee tongues: co-evolutionary arms race between *Aethina tumida* Murray (Coleoptera: Nitidulidae) and *Apis mellifera* L. In: Proceedings of the 18th Congr. IUSSI (German section), Regensburg, Germany, p. 34.
- Härtel S**, Neumann, P, Dietemann V, Crewe RM, Moritz RFA (2003) Evolutionary processes facilitate the invasive success of social parasitic honeybees (*Apis mellifera capensis* Esch.). In: Proceedings of the 33rd Annual Conference of the Ecological Society (GFOE), Halle (Saale), Germany, p. 143.
- Härtel S**, Neumann P, Dietemann V, Crewe, RM (2003) Social parasitism and the evolution of pheromonal dominance in Cape honeybee workers, *Apis mellifera capensis* (Esch.). In: Proceedings of the 18th Congr. IUSSI (German section), Regensburg, Germany, p. 22.
- Moltzer G-J, **Härtel S**, Neumann P, Hepburn HR (2002) Dwindling of *A. m. scutellata* (Lepeletier) field colonies infested by social parasitic Cape honeybee workers (*Apis mellifera capensis* Esch.). In: Proceedings of 95. Annual Meeting of the Deutsche

Zoologische Gesellschaft Halle (Saale), Germany, p. 83.; *Zoology*: 105 Suppl. V (95.1 Abstracts).

Härtel S, Neumann P, Moltzer G-J, Hepburn, HR (2002) Behavior of social parasitic Cape honeybee workers (*Apis mellifera capensis* Esch.) in host colonies (*A. m. scutellata* Lepeletier). In: Proceedings of 95. Annual Meeting of the Deutsche Zoologische Gesellschaft Halle (Saale), Germany, p. 81.; *Zoology*:105 Suppl. V (95.1 Abstracts).

Härtel S, Neumann P (2002) Parasitic Cape honeybee clones: an example for an invasive subspecies. In: Proceedings of the ESF workshop "Biological invasions in terrestrial ecosystems: an evolutionary perspective", Halle (Saale), Germany, p. 8.

Publications denoted by * indicate the papers/manuscripts, on which the thesis is based.

11.5 Erklärung

Hiermit erkläre ich, dass diese Arbeit von mir bisher weder der Mathematisch-Naturwissenschaftlich-Technischen Fakultät der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion eingereicht wurde. Ich erkläre, dass ich mich bisher noch nicht um den Doktorgrad beworben habe. Ferner erkläre ich, dass ich diese Arbeit selbständig und nur unter Zuhilfenahme der angegebenen Hilfsmittel und Literatur angefertigt habe.

Halle (Saale), den 2006

.....
Stephan Härtel