

**Molecular genetic analysis of reproductive dominance
hierarchies in the honeybee colony (*Apis mellifera* L.)**

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1 Introduction

1.1 Conflict in social insects

Social insects are often seen as harmonious units with the workers sacrificing their lives for the good of the colony (e.g. Seeley 1995). Actually conflict is widespread in social insect colonies as is the case for any other level biological organisation (Hurst *et al.* 1996; Hurst & Werren 2001). Conflict may occur between genes, cells and organisms, generally at every level of the major transitions of life (Maynard Smith & Szathmary 1997). Conflict exists due to different genetic interests of the involved counterparts, where every partner tries to selfishly enhance their own fitness, often at the expense of others. In animal societies potential conflict is high, because groups of individuals are composed of genetically distinct units. This holds true for all kinds of societies, except when build up of clonal organisms. The highly advanced societies of insects (ants, bees, wasps and termites) are characterized by a well-developed division of labour (Wilson 1971). Most obviously reproduction is shared between colony members. Usually a single (or a few) queen(s) monopolize reproduction whereas the functionally sterile worker caste engages in brood rearing, nest building and maintenance as well as in foraging (Wilson 1971). The evolution of a sterile worker caste has been a puzzling issue for evolutionary biologists for a long time. Already Darwin (1859) recognised this as the major difficulty of his theory of natural selection. It has taken more than one hundred years until William Hamilton provided a solution to the problem with the introduction of the “inclusive fitness theory” (1964). Individuals may increase their fitness by reproducing, but they may also increase their fitness by an increased reproductive output of related individuals sharing genes identity by descent. Thus, workers helping her sister or mother queen, may gain fitness. Due to the relatedness asymmetries created by the haplo-diploid system of Hymenoptera, fitness gain by helping is enhanced. Generally an increase is possible, when the benefit of helping exceeds the cost caused by the reduction of the own reproduction. This is known as Hamilton’s rule, which has been formulated as:

$$B * r > C \tag{1}$$

with B = benefit, r = relatedness and C = cost.

1.2 Worker reproduction

Worker reproduction is completely absent only in a few ant species missing entirely reproductive organs, e.g. in the genera *Solenopsis*, *Pheidole*, *Tetramorium*, and *Eciton* (Wilson 1971; Oster & Wilson 1978; Fletcher & Ross 1985). In case of workers possessing reproductive organs, they are only able to lay male destined eggs via parthenogenesis (arrhenotoky), which is the usual way of male production in the Hymenoptera. Since workers always are unmated they lack the semen to fertilise eggs (Bourke 1988). Due to relatedness benefits workers compete with the queen over male production, since they are more closely related to their own sons ($r=0.5$) and

to nephews (sons of their sisters, $r=0.375$) than to their brothers (sons of the queen, $r=0.25$). This consideration holds true for monogynous colonies with monandrous queens. With multiple mating the relatedness to their nephews will change and decreases towards $r=0.125$. Hence workers should always favour their own sons, but under multiple mating they should favour their brothers over their nephews. This has been noticed several times (Starr 1984, Woyciechowski & Lomnicki 1987) before the theory of “worker policing” has been formulated (Ratnieks 1988). Experimental evidence of worker policing in the honeybee, by testing the egg-removal rates of queen-laid and worker-laid eggs, confirmed the theory (Ratnieks & Visscher 1989). In recent years evidence has accumulated by testing the theoretical predictions with other eusocial insect species. In the common yellow jacket, where both monandrous and polyandrous colonies exist, it could be shown that worker policing only occurs in colonies headed by multiply mated queens (Foster & Ratnieks 2000). However, also doubts about the ultimate reason for egg-removal in honeybees have been expressed. Pirk and co-workers (2004) showed that egg removal might be explained by differences in viability of worker- and queen-laid eggs. Additionally a meta-analysis performed on 50 species of ants, bees and wasps showed, that the relatedness pattern alone cannot explain the occurrence of worker policing (Hammond & Keller 2004).

The development of the workers’ ovaries often is suppressed in the presence of the queen. Chemical cues are the primary source for suppression of ovary activation (Winston & Slessor 1992; Arnold *et al.* 1994). Hence, under queenless conditions the suppressing signal disappears and workers can develop their ovaries and start egg laying.

1.3 Worker reproduction in the honeybee (*Apis mellifera* L.)

The Western honeybee, *Apis mellifera* L., is highly polyandrous, with an average of 17 matings of the queen during her nuptial flight(s) (Neumann & Moritz 2000; Palmer & Oldroyd 2000). Intracolony relatedness in the honeybee is exceptional low ($r=0.25$). The large number of subfamilies present in the colony sets the stage for several potential conflicts. There is severe conflict among queens, since colonies usually rear up to ten queens, but only one is going to take over the colony. After emergence, rival sister queens fight to death with only one queen surviving to head the new colony (Winston 1987). Conflict between queens and workers over male production may exist, but seems to be resolved by worker policing (Ratnieks 1988; Ratnieks & Visscher 1989; but see 1.2 for alternative explanations).

Between subfamilies conflict may occur over selecting larvae for queen rearing. One would expect nepotism to occur, but during the course of evolution it should spread rapidly in populations. Conflict between subfamilies as well as individual workers may occur over reproduction. Loss of the queen is rapidly recognised by the workers, which start competing with their fellow workers for reproductive dominance. Several traits related to reproduction are used in competitive displays. Aggression is rising in queenless colonies, but so far no reports have been given whether potential laying workers fight physically with each other. It seems more likely that aggressive encounters are initiated by older workers (Evers & Seeley 1986; Visscher & Dukas 1995). Potential reproductive workers compete with each other via chemicals secreted by the mandibular glands. The secretion of these glands contains a

blend of fatty acids, which is dominated by 10-hydroxy-2(E)-decenoic acid (10-HDA) and 10-hydroxy-decanoic acid (10-HDAA). In queens this blend is dominated by the so-called queen substance, 9-oxo-2(E)-decenoic acid (9-ODA), which acts as a primer pheromone suppressing the workers' ovarian development (Winston & Slessor 1992). The main compounds are synthesized in a caste specific manner (Plettner *et al.* 1996). Starting with the identical substance in both castes subsequent processing is divided into two caste-specific pathways resulting in the caste-specific blend. However, both castes are capable of using both pathways (Plettner *et al.* 1998). Reproductive workers preferentially shift their biosynthesis of mandibular gland fatty acids towards the typical queen pathway resulting in the predominance of 9-ODA in the secretions of laying workers (Crewe & Velthuis 1980). In paired workers under queenless conditions one might find a dominant and a subordinate worker based on the amount of secreted 9-ODA. The amount of 9-ODA of the subordinate is positively correlated to the amount of the dominant worker indicating some form of pheromonal competition (Moritz *et al.* 2000). However, the temporal process of this competition is unknown so far.

Traits related to worker reproduction are highly heritable, with the heritabilities (h^2) ranging from 0.27 (ovary development) up to 0.89 (9-ODA production) (Moritz & Hillesheim 1985). Thus the strong genetic differences detected suggest that these are reflected as differences between subfamilies. Indeed, the reproductive success of different subfamilies under queenless conditions differs to a great extent. Strong intracolony selection takes place with a few subfamilies dominating all the others as is expressed in the highly skewed subfamily composition detected after 3 and 9 weeks after queen loss (Moritz *et al.* 1996).

A rare mutant phenomenon of worker reproduction has been observed in so-called "anarchistic" honeybee colonies (Oldroyd *et al.* 1994). These colonies are characterized by the occurrence of drone brood above the queen excluder, which only can be derived from laying workers. Indeed, microsatellite analysis showed that a single subfamily contributes to male production under queenright conditions (Oldroyd *et al.* 1994). Anarchistic workers are able to develop their ovaries in the presence of the queen and additionally they lay eggs that are not removed by their sister workers. These workers differ from laying workers in queenless colonies since they never produce a queen-like pheromone bouquet (Oldroyd *et al.* 1999) and are not superior egg-layers under queenless conditions (Monatgue & Oldroyd 1998).

1.4 The phenomenon of the Cape honeybee (*Apis mellifera capensis* Esch.)

Amongst the subspecies of the Western honeybee (*A. mellifera* L.) the Cape honeybee (*A. m. capensis* Esch.) is unique. The distribution range of this subspecies is restricted to the Fynbos biome of South Africa (Crewe & Hepburn 1991). The workers of this subspecies display some extraordinary traits with respect to worker reproduction. These workers have a very high capability to reproduce. Reproduction occurs even under queen right conditions (Moritz *et al.* 1999). Since "anarchistic bees" do show also this trait this is not the only peculiarity of Cape honeybee workers. The offspring produced by laying workers of the Cape honeybee is nearly exclusive female

(Onions 1912; Anderson 1963). Since workers are always unmated and therefore lack sperm to fertilize eggs, they reproduce parthenogenetically. This form of parthenogenesis (with all female offspring) is known as thelytoky (White 1984). However, queens of this subspecies do reproduce sexually as queens of all other subspecies do.

The cytogenetic mechanism for restoring diploidy has been studied in detail (Verma & Ruttner 1983). After meiosis the two central haploid nuclei fuse to form the diploid zygote. This mechanism is called automixis with central fusion. Generally this mechanism increases homozygosity of loci located between chiasmata and telomeres. In honeybees an increase in homozygosity may be lethal because of the sex determining system, where the zygosity state at a single nuclear locus determines the sex of the zygote (Whiting 1943; Beye *et al.* 2003). However, in laying workers of the Cape honeybee crossing-over events are to a great extent reduced or even absent (Moritz & Haberl 1994; Baudry *et al.* 2004). A consequence of the reduced recombination rate is that laying worker offspring is genotypically a clone of her mother. Nevertheless, the evolutionary consequences of worker thelytoky with respect to colony organisation are poorly understood.

Thelytokous worker reproduction in the Cape honeybee may increase conflict within the colony. The genetic composition of the colony is dramatically altered when thelytokous worker reproduction occurs and relatedness between colony members (especially between workers and nieces) changes. Worker policing behaviour should be relaxed (Greeff 1996) but may occur at low frequencies, because colony efficiency may be increased by policing worker laid eggs (Pirk *et al.* 2003). Moreover, selection on queenlike pheromone production by workers and a very rapid ovary development is evolutionarily favoured. Since the genetic value of worker produced diploid females is much higher than for worker produced haploid males, selection should favour queen-like traits in thelytokous workers (Greeff 1996). The evolutionary model by Greeff (1996) is just the extension of a prediction made by Hamilton (1964), who states that thelytokous worker reproduction may open another road for selfish selection.

The ultimate outcome of selfish selection for reproductive traits is seen in the northern part of South Africa, where Cape honeybee workers occur as social parasites. They reproduce exclusively via thelytokous parthenogenesis in their host colonies of the northern subspecies *A. m. scutellata* (Neumann & Moritz 2002), since about 400 colonies have been introduced to the northern range by migratory beekeepers. Yearly thousands of colonies die due to the "dwindling colony syndrome" caused by reproductive *A. m. capensis* workers (Greeff 1997). Exclusive parthenogenetic reproduction reproductively isolates parasite from host eventually promoting sympatric speciation of a new parasite species (Neumann & Moritz 2002). This idea is strongly supported by population genetic data. DNA microsatellite analysis has shown that the parasite spreading in an area of about 275,000 km² consists of a single clonal lineage most likely descending from a single parasitic worker bee (Baudry *et al.* 2004; Härtel *et al.* 2005). Moreover, nearly no hybridisation between host and parasite is detected (0.71 % compared to about 5 % in *Rhagoletis* flies (Feder *et al.* 1994), serving as the classical example of sympatric speciation). Usually, the two subspecies usually interbreed in the hybrid zone separating the endemic ranges of the two subspecies (Härtel *et al.* 2005).

1.5 Sociogenetics & sociogenomics of the honeybee

The honeybee is not one of the traditional model organisms in genetics, but the methods, resources and data on genetics and genomics are emerging (Page *et al.* 2002). In the new field of sociogenetics and sociogenomics the honeybee serves as the number one model system. Several advantages are connected with the honeybee system. Basically the general interest focuses on the sociality and related traits of honeybees as the division of labour. Moreover, the haplo-diploid genetic system enhances several genetic studies. With haploid males derived from a single queen individual meiosis' can be followed thereby greatly facilitating linkage map construction. Neutral molecular markers like RAPD markers have been used to construct linkage maps (Hunt & Page 1995) as well as for mapping several quantitative trait loci (QTL). Specific interest has been focused on social characteristics, which are not provided by the typical model organisms like *Drosophila melanogaster* or *Caenorhabditis elegans*. Major QTLs were mapped for pollen hoarding (Hunt *et al.* 1995, Page *et al.* 2000, Ruepell *et al.* 2004), defensive behaviour (Hunt *et al.* 1998; Arechavaleta-Velasco *et al.* 2003), learning (Chandra *et al.* 2001), body size (Hunt *et al.* 1998), alarm pheromone levels (Hunt *et al.* 1999) and hygienic behaviour (Lapidge *et al.* 2002). Due to the shortcomings of the RAPD technology extensive effort has been invested to develop more than 500 microsatellite markers covering the whole genome (Solignac *et al.* 2003). These markers have been genetically mapped resulting in a linkage map arranged into 24 linkage groups spanning 4381 cM (Baudry *et al.* 2004; Solignac *et al.* 2004). The physical genome size has been estimated as 180 Mb (Jordan & Brosemer 1974), which translates into an average recombination rate of ~41 kb/cM, a value that is 10 fold higher than in *Drosophila melanogaster* (Merriam *et al.* 1991). This high recombination rate is the highest ever reported for any animal species (Gadau *et al.* 2000) and may account for the excess of 8 linkage groups, when compared to the number of chromosomes of $n=16$ (Nachtsheim 1913). The high recombination rate requires the usage of a large number of molecular markers for mapping studies (either single genes or QTL), but turns into an advantage once a significantly linked marker is found, because a small genetic distance translates into a small physical distance.

So far the only system where a gene for a binary trait has been mapped is the sex determining system of the honeybee (Beye *et al.* 1996). The haplo-diploid system of the Hymenoptera with males being haploid and females being diploid is rather the outcome than the sex determining system itself. It was first shown by Whiting (1943) in the minute wasp *Bracon hebetor* that the allelic condition of a series of multiple alleles at a single nuclear locus determines sex. Individuals, which are heterozygous, develop into females whereas individuals that are either homo- or hemizygous develop into males. In the honeybee it was shown that this system also is the primary sex determining mechanism using inbred crosses (Mackensen 1951). In honeybees diploid males never occur, because they are cannibalised by the workers at the larval stage (Woyke 1963). Using inbred crosses the sex determining locus was genetically mapped on chromosome 8 (Beye *et al.* 1996). Using a fine mapping strategy within this region (Hasselmann *et al.*

2001) the gene (*csd* = *complementary sex determiner*) could be identified at the molecular level (Beye *et al.* 2003).

Since linkage or association between neutral molecular markers and a certain phenotype can only reveal the most downstream genetic polymorphisms, which are the raw material for selection, the biochemical and physiological cascades that are upstream of that polymorphisms can not be ruled out. For this purpose the differential expression of genes between alternative phenotypes has to be tested. A cDNA library developed from the brain of 400 worker honeybees representing about 6000 different genes has been used to establish a cDNA microarray (Whitfield *et al.* 2002). The expression pattern of individual workers, belonging to two broad behavioural categories, nurses and foragers, predict the individual behaviour (Whitfield *et al.* 2003). Large differences between queenright and queenless workers were revealed by testing the differential gene expression (Grozinger *et al.* 2003).

The available genomic resources finally have led to the sequencing of the honeybee genome (Baylor College of Medicine). The immediate publishing of the genome sequence and the raw annotation of the genome serves as a helpful instrument in genomic studies.

1.6 Aims of the work

The Cape honeybee, *Apis mellifera capensis*, will serve as a model system for the investigation of reproductive conflicts between workers bees. The role of 9-ODA for individual conflict over reproductive dominance will be determined. Since queens use this substance for the reason of queen control over worker reproduction and laying workers might produce this substance in large quantities, it might also be involved in reproductive competition between workers. In Cape honeybees workers nearly exclusively reproduce via thelytoky whereas workers of other subspecies reproduce by arrhenotokous parthenogenesis. Utilizing the haplo-diploid system of honeybees the genetic basis for thelytokous parthenogenesis will be analysed. Evolutionary theory predicts strong selection for reproductive traits of workers in colonies with thelytokous worker reproduction. In comparative analyses of thelytokous and arrhenotokous workers an empirical test for the theory will be given. Combining genetic, molecular biological and physiological methods, the interplay between genetics and behaviour will be revealed, which might strongly interfere with colony organisation when thelytokous worker reproduction occurs.

1.7 References

- Anderson RH, 1963 The laying worker in the Cape honeybee, *Apis mellifera capensis*. *J Apic Res* **2**: 85-92.
- Arechavaleta-Velasco ME, Hunt GJ, Emore C, 2003 Quantitative trait loci that influence the expression of guarding and stinging behaviors of individual honey bees. *Behav Genet* **33**: 357-364.
- Arnold G, LeConte Y, Trouiller J, Hervet H, Chappe B *et al.*, 1994 Inhibition of worker honeybee ovaries development by a mixture of fatty-acid esters from larvae. *CR Acad Sci III-Vie* **317**: 511-515.
- Baudry E, Kryger P, Allsopp MH, Koeniger N, Vautrin D *et al.*, 2004 Whole-genome scan in thelytokous laying workers of the Cape honeybee (*Apis*

- mellifera capensis*): Central fusion, reduced recombination rates, and centromere mapping using half-tetrad analysis. *Genetics* **167**: 243-252.
- Beye M, Moritz RFA, Crozier RH, Crozier YC, 1996 Mapping the sex locus of the honeybee (*Apis mellifera*). *Naturwissenschaften* **83**: 424-426.
- Beye M, Hasselmann M, Fondrk MK, Page RE, Omholt SW, 2003 The gene *csd* is the primary signal for sexual development in the honeybee and encodes an SR-type protein. *Cell* **114**: 419-429.
- Bourke AFG, 1988 Worker reproduction in the higher eusocial Hymenoptera. *Q Rev Biol* **63**: 291-311.
- Chandra SBC, Hunt GJ, Cobey S, Smith BH, 2001 Quantitative trait loci associated with reversal learning and latent inhibition in honeybees (*Apis mellifera*). *Behav Genet* **31**: 275-285.
- Crewe RM, Hepburn HR, 1991 Portrait of the Cape honeybee, *Apis mellifera capensis*. *Apidologie* **22**: 567-580.
- Crewe RM, Velthuis HHW, 1980 False queens: a consequence of mandibular gland signals in worker honey bees. *Naturwissenschaften* **67**: 467-469.
- Darwin C, 1859 *On the origin of species*. John Murray, Albemarle Street, London.
- Evers CA, Seeley TD, 1986 Kin discrimination and aggression in honey bee colonies with laying workers. *Anim Behav* **34**: 924-925.
- Feder JL, Opp SB, Wlazlo B, Reynolds K, Go W *et al.*, 1994 Host fidelity is an effective premating barrier between sympatric races of the apple maggot fly. *Proc Natl Acad Sci USA* **91**: 7990-7994.
- Fletcher DJC, Ross KG, 1985 Regulation of reproduction in eusocial Hymenoptera. *Annu Rev Entomol* **30**: 319-343.
- Foster KR, Ratnieks FLW, 2000 Facultative worker policing in a wasp. *Nature* **407**: 692-693.
- Gadau J, Page RE, Werren JH, Schmid-Hempel P, 2000 Genome organization and social evolution in Hymenoptera. *Naturwissenschaften* **87**: 87-89.
- Greeff JM, 1996 Effects of thelytokous worker reproduction on kin-selection and conflict in the Cape honeybee, *Apis mellifera capensis*. *Philos T Roy Soc B* **351**: 617-625.
- Greeff JM, 1997 The Cape honeybee and her way north: an evolutionary perspective. *S Afr J Sci* **93**: 306-308.
- Grozinger CM, Sharabash NM, Whitfield CW, Robinson GE, 2003 Pheromone-mediated gene expression in the honey bee brain. *Proc Nat Acad Sci USA* **100**: 14519-14525.
- Hamilton WD, 1964 The genetical evolution of social behaviour, I & II. *J Theor Biol* **7**: 1-52.
- Hammond RL, Keller L (2004) Conflict over male parentage in social insects. *PLoS Biol* **2**: 1472-1482.
- Härtel S, Neumann P, Kryger P, Moritz RFA, Crewe RM, 2005 Emery's rule in the Cape honeybee (*Apis mellifera capensis* Esch.). *submitted*.
- Hasselmann M, Fondrk MK, Page RE, Beye M, 2001 Fine scale mapping in the sex locus region of the honey bee (*Apis mellifera*). *Ins Mol Biol* **10**: 605-608.
- Hunt GJ, Page RE, 1995 Linkage map of the honey bee, *Apis mellifera*, based on RAPD markers. *Genetics* **139**: 1371-1382.
- Hunt GJ, Page RE, Fondrk MK, Dullum CJ, 1995 Major quantitative trait loci affecting honey bee foraging behavior. *Genetics* **141**: 1537-1545.

- Hunt GJ, Guzmán-Novoa E, Fondrk MK, Page RE, 1998 Quantitative trait loci for honey bee stinging behavior and body size. *Genetics* **148**: 1203-1213.
- Hunt GJ, Collins AM, Rivera R, Page RE, Guzmán-Novoa E, 1999 Quantitative trait loci influencing honeybee alarm pheromone levels. *J Hered* **90**: 585-589.
- Hurst GDD, Werren JH, 2001 The role of selfish genetic elements in eukaryotic evolution. *Nature Rev Genet* **2**: 597-606.
- Hurst LD, Atlan A, Bengtsson BO, 1996 Genetic conflicts. *Quart Rev Biol* **71**: 317-364.
- Jordan RA, Brosemer RW, 1974 Characterisation of DNA from three bee species. *J Ins Physiol* **20**: 2513-2520.
- Lapidge KL, Oldroyd BP, Spivak M, 2002 Seven suggestive quantitative trait loci influence hygienic behavior of honey bees. *Naturwissenschaften* **89**: 565-568.
- Mackensen O, 1951 Viability and sex determination in the honey-bee (*Apis mellifera* L.). *Genetics* **36**: 500-509.
- Maynard Smith J, Szathmary E, 1997 *The Major Transitions in Evolution*. Oxford University Press, Oxford. 360 pp.
- Merriam J, Ashburner M, Hartl DL, Kafatos FC, 1991 Toward mapping and cloning the genome of *Drosophila*. *Science* **254**: 221-225.
- 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**: 1408-1415.
- Moritz RFA, Haberl 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, Hillesheim E, 1985 Inheritance of dominance in honeybees (*Apis mellifera capensis* Esch.). *Behav Ecol Sociobiol* **17**: 87-89.
- Moritz RFA, Kryger P, Allsopp MH, 1996 Competition for royalty in bees. *Nature* **384**: 31.
- Moritz RFA, Kryger P, Allsopp MH, 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 (*Apis mellifera capensis*). *Naturwissenschaften* **87**: 395-397.
- Nachtsheim H, 1913 Cytologische Studien über die Geschlechtsbestimmung bei der Honigbiene (*Apis mellifica* L.). *Arch Zellforsch* **11**: 169-241.
- Neumann P, Moritz RFA, 2000 Testing genetic variance hypotheses for the evolution of polyandry in the honeybee (*Apis mellifera* L.). *Insectes Soc* **47**: 271-279.
- Neumann P, Moritz RFA, 2002 The Cape honeybee phenomenon: the sympatric evolution of a social parasite in real time? *Behav Ecol Sociobiol* **52**: 271-281.
- 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.
- Oster GF, Wilson EO, 1978 *Caste and Ecology in Social Insects*. Princeton University Press, Princeton. 352 pp.

- Page RE, Gadau J, Beye M, 2002 The emergence of Hymenopteran genetics. *Genetics* **160**: 375-379.
- Page RE, Fondrk MK, Hunt GJ, Guzman-Novoa E, Humphries MA *et al.*, 2000 Genetic dissection of honeybee (*Apis mellifera* L.) foraging behavior. *J Hered* **91**: 474-479.
- Palmer KA, Oldroyd BP, 2000 Evolution of multiple mating in the genus *Apis*. *Apidologie* **31**: 235-248.
- Pflugfelder J, Koeniger N, 2003 Fight between virgin queens (*Apis mellifera*) is initiated by contact to the dorsal abdominal surface. *Apidologie* **34**: 249-256.
- 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. *P Natl Acad Sci USA* **101**: 8649-8651.
- 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, 1998 Biosynthesis of mandibular acids in honey bees (*Apis mellifera*): *de novo* synthesis, route of fatty acid hydroxylation and caste selective β -oxidation. *Ins Biochem Mol Biol* **28**: 31-42.
- Ratnieks FLW, 1988 Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *Am Nat* **132**: 217-236.
- Ratnieks FLW, Visscher PK, 1989 Worker policing in the honey bee. *Nature* **342**: 796-797.
- Rueppell O, Pankiw T, Nielsen DI, Fondrk MK, Beye M *et al.*, 2004 The genetic architecture of the behavioral ontogeny of foraging in honeybee workers. *Genetics* **167**: 1767-1779.
- Seeley TD, 1995 *The Wisdom of the Hive. The Social Physiology of Honey Bee Colonies*. Harvard University Press, Cambridge. 368 pp.
- Solignac M, Vautrin D, Loiseau A, Mougel F, Baudry E *et al.*, 2003 Five hundred and fifty microsatellite markers for the study of the honeybee (*Apis mellifera* L.) genome. *Mol Ecol Notes* **3**: 307-311.
- Solignac M, Vautrin D, Baudry E, Mougel F, Loiseau A *et al.*, 2004 A microsatellite-based linkage map of the honeybee, *Apis mellifera* L. *Genetics* **167**: 253-262.
- Starr CK, 1984 Sperm competition, kinship, and sociality in the aculeate Hymenoptera. In Smith RL (ed) *Sperm Competition and the Evolution of Animal Mating Systems*. Academic Press, Orlando. pp 427-464.
- 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, Dukas R, 1995 Honey bees recognize development of nestmates' ovaries. *Anim Behav* **49**: 542-544.
- White MJD, 1984. Chromosomal mechanisms in animal reproduction. *Bull Zool* **51**: 1-23.
- Whitfield CW, Band MR, Bonaldo MF, Kumar CG, Liu L *et al.*, 2002 Annotated expressed sequence tags and cDNA microarrays for studies of brain and behavior in the honey bee. *Genome Res* **12**: 555-566.
- Whitfield CW, Cziko AM, Robinson GE, 2003 Gene expression profiles in the brain predict behavior in individual honey bees. *Science* **302**: 296-299.

- Whiting PW, 1943 Multiple alleles in complementary sex determination of *Habrobracon*. *Genetics* **28**: 365–382.
- Wilson EO, 1971 *The Insect Societies*. Harvard University Press, Cambridge. 548 pp.
- Winston ML, 1987 *Biology of the Honeybee*. Harvard University Press, Cambridge. 281 pp.
- Winston ML, Slessor KN, 1992 The essence of royalty: honey bee queen pheromone. *Am Sci* **80**: 374-385.
- Woyciechowski M, Lomnicki A, 1987 Multiple mating of queens and the sterility of workers among eusocial Hymenoptera. *J Theor Biol* **128**: 317-327.
- Woyke J, 1963 What happens to diploid drone larvae in a honeybee colony? *J Apic Res* **2**: 73–76.

**Honeybee workers (*Apis mellifera capensis*) compete
for producing queen-like pheromone signals**

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Proceedings of the Royal Society London B **271**: S98-S100 (2004)

Abstract

Physical fights are the usual means of establishing dominance hierarchies in animal societies. This form of dominance behaviour is most strongly expressed in honeybee queens who engage in fights to the death to establish themselves in the colony. Workers can also compete for reproductive dominance resulting in the establishment of stable hierarchies. They do not engage each other physically, but use pheromones that mimic those produced by queens. The dynamics of pheromone production in paired workers suggests that they engage in a pheromonal contest. Because queen pheromones suppress ovary activation, the contest results in the sterility of the loser.

Keywords: *Apis mellifera capensis*; dominance; queen pheromone; laying workers

**A single locus determines thelytokous
parthenogenesis of laying honeybee workers (*Apis
mellifera capensis*)**

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Heredity **94**: 533-537 (2005)

Abstract

The evolution and maintenance of parthenogenetic species are a puzzling issue for evolutionary biology. Although the genetic mechanisms that act to restore diploidy are well examined, the underlying genes that switch from sexual reproduction to parthenogenesis have not been analysed. There are several species that are polymorphic for the sexual and parthenogenetic reproduction, which most likely have a genetic basis. We use the South African honeybee subspecies *Apis mellifera capensis* to analyse the genetic control of thelytokous worker reproduction. Due to the caste system of honeybees it is possible to establish classical backcrosses using sexually reproducing queens and drones of both arrhenotokous and thelytokous subspecies, and to test the parthenogenesis type in the resulting workers. We found Mendelian segregation for thelytoky of laying workers, which appears to be controlled by a single major gene (*th*). The segregation pattern indicates a recessive allele causing thelytoky. We found no evidence for maternal transmission of bacterial endosymbionts controlling parthenogenesis. Thelytokous parthenogenesis of honeybee workers appears to be a classical qualitative trait, because we never observed any form of mixed parthenogenesis type (amphitoky).

Keywords: *Apis mellifera capensis*, automixis, double backcross, evolution of sex, parthenogenesis, thelytoky

A selfish gene drives selection for selfish honeybee workers

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Abstract

Selfish genes typically enhance their propagation from one generation to the next at the expense of other genes¹. One mechanism for these selfish genes to spread is to manipulate meiotic and sexual processes by inhibiting the gametes carrying the alternative allele². Such a selfish gene has recently been identified in laying workers of the Cape honeybee (*Apis mellifera capensis*)³. Workers that are homozygous for the allele *thelytoky* at the *th* – locus, produce female offspring parthenogenetically (=thelytoky), through central fusion of meiotic nuclei, with no chromosomal recombination and suppressed crossing over, thereby maintaining homozygosity for this allele. Evolutionary theory^{4,5} predicts selection for selfish worker reproduction in species carrying such a gene. Thus it is not surprising to find that thelytokous workers of *A. m. capensis* have recently evolved into social parasites⁶. These parasitic workers pheromonally mimic the queen, suppress reproduction in other workers, and make host colonies rear their offspring. Here we show that the *th* - gene, which selfishly prevents recombination with other genes and maintains the parasitic lineage as a genetic clone⁷, also controls selfish behaviour in parasitic workers.

Keywords: *Apis mellifera capensis*, reproductive dominance, selfish gene, social parasite, thelytoky

**Fine mapping of the pleiotropic *thelytoky* gene in the
honeybee, *Apis mellifera* L.**

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Abstract

Reproduction in social insect societies is highly skewed. Queens monopolize reproduction whereas workers appear to be functionally sterile. Under certain conditions workers may start reproducing parthenogenetically by laying unfertilized male eggs (arrhenotoky). However, an exception to this rule provides the Cape honeybee, *Apis mellifera capensis*. Workers of this subspecies are strictly thelytokous. The type of parthenogenesis of workers is determined by a single gene, *thelytoky*. Here we report the genetic mapping of that gene. The genome of the honeybee was covered with 546 microsatellite markers, which revealed a single linkage group (13) carrying the gene. Fine mapping was achieved using newly developed microsatellite markers aided by the availability of the genome sequence of the honeybee. We identified a target region comprising 11.4 cM corresponding to 163 kb which includes nine putative genes.

Keywords: *Apis mellifera*, pleiotropy, gene mapping, reproductive dominance, selfish gene, thelytoky

6 Summary

The work presented addresses both proximate and ultimate regulatory mechanisms determining reproductive hierarchies in worker honeybees. The Cape honeybee, *Apis mellifera capensis*, is used as a model system because its laying workers are characterised by a particularly swift and explicit expression of queenlike phenotypes. *A. m. capensis* workers lay unfertilized eggs through thelytokous parthenogenesis and pheromonally mimic the queen when competing with each other for gaining reproductive dominance. They shift their mandibular gland secretions towards a more queenlike blend with 9-ODA as the predominant compound. Dominant workers use 9-ODA to establish and maintain their dominant position in the social hierarchy among workers (Crewe *et al.* 2004; Moritz *et al.* 2004).

A strong influence of genetic factors was found for both, the mode of parthenogenesis and the composition of the mandibular gland secretions. Genetic analysis of a double backcross (Lattorff *et al.* 2004; Solignac *et al.* 2004) revealed a single recessive gene, *thelytoky* (*th*), determining the type of parthenogenesis of worker honeybees. Moreover, it could be shown that the type of parthenogenesis is a strictly qualitative trait, since no intermediate parthenogenesis forms (amphitoky) were detected (Lattorff *et al.* 2005a).

The *thelytoky* gene not only influences the type of parthenogenesis of worker honeybees, it also affects the 9-ODA production as well as the ovary activation in individual workers. Hence, the *thelytoky* gene has strong pleiotropic effects (Lattorff *et al.* 2005b) and plays a central role in the social organization of the colony.

The *thelytoky* locus was mapped to chromosome 13 using 546 microsatellite markers. Within the target region a fine mapping was carried out using additional markers developed directly from the published genome sequence. The *thelytoky* gene was mapped to an interval of about 11 cM flanked by two microsatellite markers (AT012 and HB011). Within that region nine genes were identified, which were predicted by automatic genome annotation. A strong candidate for the *thelytoky* gene is a transcription factor belonging to the family of the LBP-9 (*M. musculus*), *grainy head* (*grh*, *D. melanogaster*) and *geminin* (*gem*, *D. melanogaster*) genes (Lattorff *et al.* 2005c).

6.1 Genetic and molecular analysis of the pleiotropic *thelytoky* gene

The genetic basis of the parthenogenesis type of honeybee workers was clarified in the course of this work (Lattorff *et al.* 2005a). Due to the haplo-diploid system of honeybees, genetic analyses are facilitated. Backcrosses are easily established. Here the system was exploited to establish a double-backcross by inseminating a segregating F1 hybrid queen with the semen of two differing drones. Instead of using two backcrosses separately two backcrosses can be analysed within the offspring of the same queen, thus utilising the same meiosis, which may facilitate the construction of linkage maps.

Due to the high recombination rate during the queen's meiosis a huge number of markers is required to accurately saturate the genome (Gadau *et al.* 2000). The density of potential microsatellite markers is very high within the genome of the honeybee, which led to the development of over 1000

markers (Solignac *et al.* 2003, Solignac personal communication). 546 of these markers were used for mapping the *thelytoky* gene (Lattorff *et al.* 2005c). For a subsequent fine mapping, additional markers were developed aided by the published genome sequence. The immediately published results of the genome sequencing efforts provided help during the search for candidate genes within the genomic target region. Nine putative genes were identified. One of them is a transcription factor, and hence an excellent candidate to act as a pleiotropic gene, as it was shown for the *thelytoky* gene (Lattorff *et al.* 2005b).

The high recombination rate of the honeybee requires a large number of markers for mapping, but once a region is identified, the physical size of the potential region is rather small. Thus a manageable number of candidate genes can be identified, which enhances further investigations. This advantage has been instrumental in mapping the sex-locus of the honeybee (Beye *et al.* 1996) and its final molecular identification (Beye *et al.* 2003).

6.2 The pleiotropic *thelytoky* gene in an evolutionary context

The discovery of a single gene that pleiotropically influences a complex behaviour like worker reproduction and thus strongly interferes with colony organisation in the honeybee is not a singular case in the animal kingdom. For example, in the amoeboid slime mold, *Dictyostelium discoideum*, a single gene, *dimA*, with pleiotropic effects was recently discovered (Foster *et al.* 2004). Cells of *D. discoideum* aggregate under poor dietary conditions to form a fruiting body, which consists of a non-reproductive stalk and the reproductive spore. The gene *dimA* is required to receive the signalling molecule DIF-1 that causes differentiation into prestalk cells. Thus a lack of that gene allows cells to cheat and preferentially enter the prespore cells. In mixtures of mutant (*dimA*⁻) and wild-type cells the *dimA*⁻ cells are excluded from the spore demonstrating the pleiotropic effects of that gene (Foster *et al.* 2004).

Single genes may also have impact on behaviour and organisation of more complex societies like those of social insects. In the red fire ant *Solenopsis invicta*, the biallelic *Gp-9* gene encodes a signal involved in the sociogenetic control of colony organisation. Depending on the allelic state at this gene either mono- or polygynous colonies develop. Workers homozygous (*BB*) for this gene, in absence of *Bb* workers, do not tolerate multiple queens and hence *BB* queens always head monogynous colonies. The *Bb* workers tolerate multiple queens, but only when they bear the *b* allele. Heterozygote workers detect *BB* queens and kill them resulting in polygynous colonies always headed by *Bb* queens (Keller & Ross 1998; Ross & Keller 1998; Krieger & Ross 2002). Mono- and polygynous colonies differ largely in their phenotype, because queen number influences several characteristics of social insect colonies like aggressiveness, colony foundation and reproductive output of colonies (Bourke & Franks 1995).

The example from the red fire ants shows that indeed genetic factors may interact in the caste determination cascade. Recent studies, mainly on ants, show that genetic caste determination is more common than once believed (Fraser *et al.* 2002; Helms Cahan *et al.* 2002; Julien *et al.* 2002; Volny & Gordon 2002; Helms Cahan & Keller 2003; Helms Cahan *et al.* 2004). Classical theory accounted only for nutritional differences as the main factors

influencing caste fate. The best-studied example is the honeybee, where young larvae are selected by nursing workers and given a differential nutritional treatment than worker destined larvae (Winston 1987). However, genetic differences between individual larvae and between larvae of different subfamilies may account for the observed unequal probability getting reared as a queen. Here genetic differences may predispose certain individuals getting reared as a queen (Moritz *et al.* 2005).

The narrow time window of totipotent larvae (1st larval stage) in the honeybee shows a high level of inflexibility (Weaver 1957; Woyke 1971; Weiss 1974). Morphological differentiation is irreversible in either direction. But workers may mimic queens with respect to physiological and behavioural properties. Thus there seems phenotypic plasticity for caste even in highly advanced eusocial insects. Here workers can be compared with primitively eusocial species, where morphological differentiation is missing. In these species, e.g. *Ropalidia spec.* (Gadagkar 1991) or *Polistes spec.* (Jeanne 1991), individuals form dominance hierarchies by behavioural and physiological interactions among group members. In the Cape honeybee a single pleiotropic gene accounting for thelytokous parthenogenesis as well as other fitness traits has driven selection towards queen-like workers. Cape honeybee workers may perfectly resemble the queen. Interestingly, in the Cape honeybee worker destined larvae have gained the ability to manipulate nursing workers thereby enhancing their caste fate towards a more queenlike appearance as adults (Beekman *et al.* 2000; Calis *et al.* 2002; Allsopp *et al.* 2003).

Ultimately, the strong selection pressures present in endemic Cape honeybee populations due to the strong intracolony conflict and the unique ability of thelytokous worker reproduction have led to the evolution of a self-replicating selfish social parasite (Neumann & Moritz 2002). Due to the activity of migratory beekeepers highly dominant workers have had the chance of entering hives of *A. m. scutellata*, and reproducing themselves via thelytoky. Selection has favoured a single clonal lineage now distributed over the large range of *A. m. scutellata* in northern part of South Africa (Härtel *et al.* 2005). In the socially parasitic honeybees in South Africa the selfish *thelytoky* gene has found the ultimate outcome. The strong selection acting on the thelytoky gene may have promoted the adaptation for a social parasitic life style. Finally the effect of this single gene has led to the loss of sociality within the social parasitic population. Socially parasitic bees are morphological similar to workers, but with respect to physiology and behaviour they are queens (Martin *et al.* 2002; Sole *et al.* 2002; Martin & Jones 2004). This has also been shown clearly in interactions with queens, where they may attack queens but are also attacked by queens. These fights are similar to the virgin queen fights (Moritz *et al.* 2003). The evolution of the social parasitic Cape honeybees may be one of the rarely occurrences where “*nature has rewound the tape*” (Gadagkar 1997). This formulation in the strict sense is not true, since these workers may have lost their sociality, but did not return to a solitary life, comparable to the ancestors of honeybees, instead they took a line of a dependent (parasitic) life style. Thus, one of the major transitions of life (Maynard Smith & Szathmary 1997) was reset, but not completely to its former state.

The question may arise why this selfish gene has not spread in all populations (or subspecies) of the honeybee. The complementary allele,

called the wild-type (+) allele, of this locus is fixed in all other populations. This allele is associated with arrhenotokous worker reproduction (production of haploid males via parthenogenesis), low production of queen pheromones and slower ovary activation. Generally, the +allele is not associated with a queenlike phenotype. Under these circumstances the +allele acts as a non-selfish gene or formulated with other words – it acts as an altruistic gene. The inclusive fitness theory (Hamilton 1964) provides an excellent model explaining the spread of altruistic alleles in populations. Thus, there are factors governing the spread of altruistic alleles and may counteract selfish genes. Empirically this has been shown for artificially produced colonies composed of dominant or subordinate workers, respectively. Colonies consisting entirely of dominant workers have a low fitness, because the worker force simply does not engage in tasks other than egg laying (Hillesheim *et al.* 1989). This example clearly shows that colony level selection may act to decrease the number of selfish elements.

A selfish gene, the *thelytoky* gene of the honeybee, has been shown to have a strong impact on the selection pressures within colonies, which also effect colony organisation. Selfish elements may drive evolution strongly to non-expected outcomes (Hurst *et al.* 1996) as seen in the Cape honeybee with the evolution of a social parasite thereby causing the complete loss of social organisation (Neumann & Moritz 2002).

6.3 References

- 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.
- Beye M, Moritz RFA, Crozier RH, Crozier YC, 1996 Mapping the sex locus of the honeybee (*Apis mellifera*). *Naturwissenschaften* **83**: 424-426.
- Beye M, Hasselmann M, Fondrk MK, Page RE, Omholt SW, 2003 The gene *csd* is the primary signal for sexual development in the honeybee and encodes an SR-type protein. *Cell* **114**: 419-429.
- Bourke AFG, Franks NR, 1995 *Social Evolution in Ants*. Princeton University Press, Princeton, 529 pp.
- 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, Moritz RFA, Lattorff HMG, 2004 Trapping pheromonal components with silicone rubber tubes: fatty acid secretions in honeybees (*Apis mellifera*). *Chemoecology* **14**: 77-79.
- Foster KR, Shaulsky G, Strassmann JE, Queller DC, Thompson CRL, 2004 Pleiotropy as a mechanism to stabilize cooperation. *Nature* **431**: 693-696.
- Fraser VS, Kaufmann B, Oldroyd BP, Crozier RH, 2000 Genetic influence on caste in the ant *Camponotus consobrinus*. *Behav Ecol Sociobiol* **47**: 188-194.
- Gadagkar R, 1991 *Belonogaster*, *Mischocyttarus*, *Parapolybia*, and the Independent-founding *Ropalidia*. In Ross KG, Matthews RW (eds) *The Social Biology of Wasps*. Comstock Publishing Ass., Ithaca. pp 149-190.

- Gadagkar R, 1997 Social evolution – has nature ever rewound the tape? *Curr Sci* **72**: 950-956.
- Gadau J, Page RE, Werren JH, Schmid-Hempel P, 2000 Genome organization and social evolution in Hymenoptera. *Naturwissenschaften* **87**: 87-89.
- Härtel S, Neumann P, Kryger P, Moritz RFA, Crewe RM, 2005 Emery's rule in the Cape honeybee (*Apis mellifera capensis* Esch.). *submitted*.
- Helms Cahan S, Keller L, 2003 Complex hybrid origin of genetic caste determination in harvester ants. *Nature* **424**: 306-309.
- Helms Cahan S, Parker JD, Rissing SW, Johnson RA, Polony TS, *et al.* 2002 Extreme genetic differences between queens and workers in hybridizing *Pogonomyrmex* harvester ants. *Proc Roy Soc Lond B* **269**: 1871–1877.
- Helms Cahan S, Glennis E, Julian GE, Rissing SW, Schwander T, Parker JD, Keller L, 2004 Loss of phenotypic plasticity generates genotype-caste association in harvester ants. *Curr Biol* **14**: 2277-2282.
- Jeanne RL, 1991 The Swarm-founding Polistinae. In Ross KG, Matthews RW (eds) *The Social Biology of Wasps*. Comstock Publishing Ass., Ithaca. pp 191-231.
- Julian GE, Jennifer H. Fewell JH, Gadau J, Johnson RA, Larrabee D, 2002 Genetic determination of the queen caste in an ant hybrid zone. *Proc Natl Acad Sci USA* **99**: 8157–8160.
- Keller L, Ross KG, 1998 Selfish genes: a green beard in the red fire ant. *Nature* **394**: 573-575.
- Krieger MJB, Ross KG, 2002 Identification of a major gene regulating complex social behavior. *Science* **295**: 328-332.
- Lattorff HMG, Fuchs S, Solignac M, Moritz RFA, 2004 Using DNA fingerprinting to facilitate back crossing experiments in the honeybee (*Apis mellifera*). *Apidologie* **35**: 547-548.
- Lattorff HMG, Moritz RFA, Fuchs S, 2005a A single gene determines thelytokous parthenogenesis in honeybees (*Apis mellifera capensis*). *Heredity* **94**: 533-537.
- Lattorff HMG, Moritz RFA, Crewe RM, 2005b A selfish gene drives selection for selfish workers. *Nature* (in review).
- Lattorff HMG, Moritz RFA, Solignac M, 2005c Fine mapping of the pleiotropic *thelytoky* gene in the honeybee (*Apis mellifera*). *Genetics* (to be submitted).
- Martin SJ, Jones GR, 2004 Conservation of Bio synthetic pheromone pathways in honeybees *Apis*. *Naturwissenschaften* **91**: 232-236.
- Martin SJ, Jones GR, Châline N, Middleton H, Ratnieks FLW, 2002 Reassessing the role of the honeybee (*Apis mellifera*) Dufour's gland in egg marking. *Naturwissenschaften* **89**: 528 – 532.
- Maynard Smith J, Szathmary E, 1997 *The Major Transitions in Evolution*. Oxford University Press, Oxford. 360 pp.
- Moritz RFA, Lattorff HMG, Crewe RM, 2004 Honeybee (*Apis mellifera capensis*) compete for producing queen-like pheromone signals. *Proc Roy Soc Lond B* **271**: S98-S100.
- Moritz RFA, Pflugfelder J, Crewe RM, 2003 Lethal fighting between honeybee queens and parasitic workers (*Apis mellifera*). *Naturwissenschaften* **90**: 378-381.
- Neumann P, Moritz RFA, 2002 The Cape honeybee phenomenon: the sympatric evolution of a social parasite in real time? *Behav Ecol Sociobiol* **52**: 271-281.

- Ross KG, Keller L, 1998 Genetic control of social organization in an ant. *Proc Natl Acad Sci USA* **95**: 14232-14237.
- Sole CL, Kryger P, Hefetz A, Katzav-Gozansky T, Crewe RM, 2002 Mimicry of queen Dufour's gland secretions by workers of *Apis mellifera scutellata* and *A. m. capensis*. *Naturwissenschaften* **89**: 561 – 564.
- Solignac M, Vautrin D, Baudry E, Mougel F, Loiseau A *et al.*, 2004 A microsatellite-based linkage map of the honeybee, *Apis mellifera* L. *Genetics* **167**: 253-262.
- Volny VP, Gordon DM, 2002 Genetic basis for queen–worker dimorphism in a social insect. *Proc Natl Acad Sci USA* **99**: 6108–6111.
- Weaver N, 1957 Effects of larval age on dimorphic differentiation of the female honey bee. *Ann Entomol Soc Am* **50**: 283-294.
- Weiss K, 1974 Zur Frage des Königinnengewichtes in Abhängigkeit von Umlarvalter und Larvenversorgung. *Apidologie* **5**: 127-147.
- Winston ML, 1987 *Biology of the Honeybee*. Harvard University Press, Cambridge. 281 pp.
- Woyke J, 1971 Correlations between the age at which honeybee brood was grafted, characteristics of the resultant queens, and results of insemination. *J Apic Res* **10**: 45-55.

7 Zusammenfassung

In dieser Arbeit werden proximate und ultimate Regulationsmechanismen reproduktiver Dominanzhierarchien von Honigbienen Arbeiterinnen untersucht. Die Kap Honigbiene, *Apis mellifera capensis*, wird in dieser Arbeit als Modell Organismus genutzt, weil die legenden Arbeiterinnen einen sehr königinnenähnlichen Phänotyp aufweisen. Arbeiterinnen der Kap Honigbiene legen unbefruchtete Eier durch thelytoky Parthenogenese und imitieren die Königin in Bezug auf die Pheromonproduktion, wenn sie miteinander um die reproduktive Dominanz konkurrieren. Im Verlauf eines solchen Konkurrenzkampf können sie die Mandibeldrüsensekrete zu einer mehr königinnenähnlichen Zusammensetzung mit 9-ODA als der vorherrschenden Komponente verändern. Dominante Arbeiterinnen nutzen das produzierte 9-ODA zur Etablierung und Sicherung ihrer Dominanzposition (Crewe *et al.* 2004; Moritz *et al.* 2004).

Ein sehr starker Einfluss genetischer Faktoren konnte sowohl für die Parthenogeneseform als auch für die Mandibeldrüsensekrete aufgezeigt werden. Die genetische Analyse einer Doppel-Rückkreuzung (Lattorff *et al.* 2004; Salignac *et al.* 2004) zeigte, das ein einzelnes rezessives Gen, *thelytoky* (*th*), den Typ der Parthenogenese der Arbeiterinnen bestimmt. Weiterhin konnte gezeigt werden, das der Parthenogenesetyp ein qualitatives Merkmal ist, da keine intermediären Formen der Parthenogenese (Amphitokie) gefunden wurden (Lattorff *et al.* 2005a).

Das *thelytoky* Gen beeinflusst jedoch nicht nur den Typ der Parthenogenese, sondern auch die 9-ODA Produktion und die Ovarienentwicklung bei einzelnen Arbeiterinnen. Das *thelytoky* Gen zeigt also einen hohen Grad an Pleiotropie (Lattorff *et al.* 2005b) und spielt eine zentrale Rolle in der sozialen Organisation des Bienenvolks.

Das *thelytoky* Gen konnte mit Hilfe von 546 Mikrosatelliten DNA Markern auf dem Chromosom 13 kartiert werden. Innerhalb der potentiellen Zielregion konnte das Gen mit zusätzlichen Markern, welche mit Hilfe der publizierten Genomsequenz direkt entwickelt wurden, feinkartiert werden. Das *thelytoky* Gen konnte in einem Intervall von 11 cM, welches von zwei Markern (AT012 und HB011) flankiert wird, kartiert werden. Innerhalb dieser Region wurden 9 Gene detektiert, welche durch die automatisierte Genomannotierung vorausgesagt wurden. Ein Kandidatengen ist ein Transkriptionsfaktor, welcher zur Familie von LBP-9 (*M. musculus*), *grainy head* (*grh*, *D. melanogaster*) und *gemin* (*gem*, *D. melanogaster*) gehört (Lattorff *et al.* 2005c).

7.1 Genetische und molekulare Analyse des pleiotropen *thelytoky* Gens

Die genetische Grundlage des Parthenogenesetyps der Honigbienen Arbeiterinnen wurde innerhalb dieser Arbeit (Lattorff *et al.* 2005a) analysiert. Auf Grund des haplo-diploiden Systems der Honigbiene werden genetische Analysen deutlich erleichtert. Rückkreuzungen sind einfach zu etablieren. Das halop-diploide System wurde genutzt, um eine Doppelrückkreuzung herzustellen, indem man eine segregierende F1 Hybrid-Königin mit dem Samen von zwei unterschiedlichen Drohnen besamt. Anstatt zwei separate Rückkreuzungen zu verwenden, können zwei Rückkreuzungen innerhalb des

Nachwuchses der gleichen Königin analysiert werden und dabei gleiche Meiose zur Etablierung einer genetischen Karte verwendet. Wegen der hohen Rekombinationsrate der Königin ist eine sehr große Anzahl von genetischen Markern erforderlich, um das Genom vollständig abzudecken (Gadau *et al.* 2000). Die Dichte der potentiellen Mikrosatelliten Marker im Genom der Honigbiene ist sehr hoch. Dies hat zur Entwicklung von mehr als 1000 Markern geführt (Solignac *et al.* 2003; Solignac personal communication). Diese Mikrosatelliten Marker wurden für die Kartierung des *thelytoky* Gens (Lattorff *et al.* 2005c) benutzt. Für eine Feinkartierung konnten mit Hilfe der veröffentlichten Genomsequenz zusätzliche Marker entwickelt werden. Die umgehend veröffentlichte Genomsequenz der Honigbiene hat auch die Identifizierung von Kandidatengen erleichtert. Neun Gene wurden identifiziert. Bei einem handelt es sich um einen Transkriptionsfaktor, welcher ein hochrangiger Kandidat ist, um als pleiotropes Gen zu agieren, wie es für das *thelytoky* Gen (Lattorff *et al.* 2005b) gezeigt wurde. Die hohe Rekombinationsrate der Honigbiene erfordert eine sehr große Anzahl von Markern, die aber, sobald eine Region kartiert wurde, eine geringe physikalische Größe des entsprechenden Bereichs bedeutet. So kann eine handliche Anzahl von Kandidatengen gefunden werden, die weitere Untersuchungen erheblich erleichtern. Dieser Vorteil ist bei der Kartierung des sex-locus der Honigbiene (Beye *et al.* 1996) und der folgenden molekularen Charakterisierung (Beye *et al.* 2003) hilfreich gewesen.

7.2 Das pleiotrope *thelytoky* Gen in einem evolutionären Kontext

Die Entdeckung, dass ein einzelnes Gen mit pleiotropen Effekten, komplexe Verhaltensweisen, wie die Arbeiterinnenreproduktion und damit auch die Organisation des Honigbienenvolks beeinflusst, erscheint erstaunlich und unerwartet. Es handelt sich dabei aber nicht um einen Einzelfall. Bei dem amöboiden Schleimpilz *Dictyostelium discoideum* konnte für ein einzelnes Gen, *dimA*, identifiziert werden, welches pleiotrope Effekte aufweist (Foster *et al.* 2004). Zellen von *D. discoideum* bilden bei schlechten Nahrungsbedingungen Aggregationen, um Fruchtkörper auszubilden, welche aus einem nicht-reproduktiven Stiel und aus einer reproduktiven Spore bestehen. Das Gen *dimA* wird für den Empfang des Signalmoleküls DIF-1 benötigt, welches die Differenzierung in die Stielvorläuferzellen einleitet. Das Fehlen dieses Gens würde den entsprechenden Zellen den Betrug und damit das vorrangige Eindringen in die Sporenvorläuferzellen ermöglichen. In Gemischen von Mutanten (*dimA*⁻) und Wildtyp Zellen werden die *dimA*⁻ Zellen jedoch aus der Spore ausgeschlossen, womit der pleiotrope Effekt des Gens belegt wird (Foster *et al.* 2004).

Einzelne Gene können auch in komplexeren Gesellschaften, wie jenen der sozialen Insekten, einen erheblichen Einfluss auf Verhalten und Organisation der Sozietät ausüben. Bei der roten Feuerameise *Solenopsis invicta* codiert das biallelische Gen *Gp-9* ein Signal, welches in die soziogenetische Kontrolle der Organisation des Volkes involviert ist. In Abhängigkeit des Genotyps an diesem Gen entwickeln sich entweder mono- oder polygyne Völker. Homozygote Arbeiterinnen (*BB*) tolerieren bei Abwesenheit von *Bb* Arbeiterinnen nur eine Königin, weshalb monogyne Völker immer eine *BB* Königin haben. Die heterozygoten *Bb* Arbeiterinnen

tolerieren mehrere Königinnen, allerdings nur wenn diese das *b* Allel tragen. Heterozygote Arbeiterinnen können *BB* Königinnen detektieren und töten diese, was dazu führt, dass polygyne Völker immer eine *Bb* Königin aufweisen (Keller & Ross 1998; Ross & Keller 1998; Krieger & Ross 2002). Die monogynen und polygynen Völker unterscheiden sich bezüglich ihres Phänotyps sehr stark. Die Anzahl an Königinnen beeinflusst vielfältige Charakteristika der Völker, wie z.B. Aggressivität, Volksgründung und den reproduktiven Erfolg der Völker (Bourke & Franks 1995).

Das Beispiel der roten Feuerameisen zeigt eindeutig, dass genetische Faktoren mit dem Kastensystem interagieren können. Neuere Studien, insbesondere an Ameisen, zeigen, dass die genetische Kastendetermination häufiger vorkommt, als einstmals angenommen wurde (Fraser *et al.* 2000; Helms Cahan *et al.* 2002; Julien *et al.* 2002; Volny & Gordon 2002; Helms Cahan & Keller 2003; Helms Cahan *et al.* 2004). Laut der klassischen Theorie sind ausschließlich Umweltfaktoren, wie die Ernährung, ausschlaggebend für die Kastendetermination. Das wohl am besten studierte Objekt ist die Honigbiene, bei der junge Larven durch die Pflegebiene auserwählt werden um anschließend eine andersartige Fütterung zu erhalten als die Larven, die sich zu Arbeiterinnen entwickeln (Winston 1987). Jedoch spielen genetische Unterschiede zwischen Individuen und zwischen Larven unterschiedlicher Subfamilien eine entscheidende Rolle, welche für die beobachteten Unterschiede in der Wahrscheinlichkeit als Königin aufgezogen zu werden verantwortlich sind. Genetische Unterschiede prädisponieren bestimmte Individuen zur Königin aufgezogen zu werden (Moritz *et al.* 2005).

Totipotente Larven der Honigbienen existieren nur in einem sehr schmalen zeitlichen Fenster (1. Larvenstadium), wodurch eine Unflexibilität des Kastensystems verdeutlicht wird (Weaver 1957; Woyke 1971; Weiss 1974). Morphologische Unterschiede sind in jeder Richtung irreversibel. Aber Arbeiterinnen können Königinnen bezüglich Physiologie und Verhalten nachahmen. Phänotypische Plastizität der Kasten scheint also bei den hochentwickelten eusozialen Insekten vorzukommen. Arbeiterinnen stehen auf einem Level vergleichbar mit primitiv eusozialen Arten, bei denen keine morphologischen Kastenunterschiede vorkommen. Bei diesen Arten, z.B. *Ropalidia spec.* (Gadagkar 1991) oder *Polistes spec.* (Jeanne 1991), bilden die Individuen Dominanzhierarchien durch Verhaltens- und Pheromoninteraktionen aus. Bei der Kap Honigbiene verschiebt ein einzelnes pleiotropes Gen, welches für die thelytoke Parthenogenese sowie andere Fitnessmerkmale verantwortlich ist, die als Arbeiterinnen determinierten Individuen in einen königinnenähnlichen Phänotyp. Kap Honigbienen Arbeiterinnen können die Königin sehr stark imitieren. Interessanterweise haben die Larven der Kap Bienen bereits Merkmale entwickelt, welche es ihnen erlauben, die Pflegebiene zu manipulieren und dadurch ihre Kastenbestimmung auf einen königinnenähnlichen Weg zu bringen (Beekman *et al.* 2000; Calis *et al.* 2002; Allsopp *et al.* 2003).

Der starke Selektionsdruck, der durch den intrakolonialen Konflikt verursacht wird, hat zur Evolution eines sich selbst-replizierenden egoistischen Sozialparasiten geführt (Neumann & Moritz 2002). Durch die Wanderbienenhaltung haben Imker die Kap Bienen mit den Völkern der benachbarten Rasse in Kontakt gebracht und es so den dominanten Arbeiterinnen ermöglicht sich in den Wirtsvölkern erfolgreich zu

reproduzieren. Die starke Selektion hat einen einzelnen klonalen Genotyp hervorgebracht, welcher nun über weite Teile des *A. m. scutellata* Verbreitungsgebiets im Norden von Südafrika vorherrscht (Härtel *et al.* 2005). In den sozial parasitischen Honigbienen Südafrikas hat das egoistische *thelytoky* Gen sein ultimates Ziel erreicht. Die starke Selektion, die auf dem *thelytoky* Gen lastet, hat die Anpassung an die sozial parasitische Lebensweise gefördert. Letztendlich hat der Effekt dieses Gens zum Verlust der Sozialität geführt. Sozial parasitische Bienen sind morphologisch Arbeiterinnen ähnlich, sind aber in bezug auf Physiologie und Verhalten Königinnen (Martin *et al.* 2002; Sole *et al.* 2002; Martin & Jones 2004). Sehr offensichtlich wird dies bei Interaktionen mit Königinnen, bei denen sie attackiert werden als auch die Königinnen angreifen. Diese Kämpfe sind den Königinnenkämpfen nach dem Schlupf sehr ähnlich (Moritz *et al.* 2003). Die Evolution zur sozial parasitischen Lebensweise innerhalb der Kap Honigbienen kann als eine der Situationen gewertet werden, bei der "nature has rewound the tape" (Gadagkar 1997). Jedoch ist diese Formulierung in seiner strikten Auslegung nicht ganz exakt, da diese Arbeiterinnen zwar nicht mehr sozial leben, aber andererseits nicht zu dem solitären Leben der Vorfahren der Honigbienen zurückgekehrt sind. Anstelle dessen haben sie eine parasitische Lebensweise entwickelt. Eine der Haupttransitionen des Lebens (Maynard Smith & Szathmary 1997) wurde rückgängig gemacht, jedoch nicht vollständig umgekehrt.

Es stellt sich die Frage, warum sich das *thelytoky* Gen nicht in allen Populationen ausgebreitet hat. Das alternative Allel (Wildtyp-Allel, +-allele) dieses Gens ist in allen anderen Unterarten der Honigbienen fixiert. Dieses Allel ist mit arrhenotoker Arbeiterreproduktion (parthenogenetische Erzeugung von haploiden Männchen), einer niedrigen Produktion der Königinnensubstanz und einer langsamen Ovarentwicklung assoziiert. Das +-Allel ist also nicht mit einem königinnenähnlichen Phänotyp verbunden. Unter diesen Umständen kann das +-Allel als ein nicht-egoistisches Gen oder ein altruistisches Gen angesehen werden. Die Theorie der inklusiven Fitness (Hamilton 1964) bietet eine Grundlage, mit der die Ausbreitung von altruistischen Genen in Populationen erklärt werden kann. Es scheint also Faktoren zu geben, welche die Ausbreitung altruistischer Allele fördern und damit egoistischen Genen entgegenwirken können. Experimentell wurde dies mit Völkern bestätigt, welche ausschließlich aus dominanten Arbeiterinnen bestehen. Diese Völker zeigen eine verringerte Fitness, weil die Arbeiterinnen sich nicht an den für sie typischen Aufgaben beteiligen (Hillesheim *et al.* 1989). Dieses Beispiel zeigt, dass entgegengesetzte Selektionskräfte auf der Volksebene agieren, die die Anzahl egoistischer Elemente auf einem geringen Niveau halten.

Ein egoistisches Gen, das *thelytoky* Gen der Honigbiene, hat, wie gezeigt wurde, einen starken Einfluss auf den Selektionsdruck innerhalb des Honigbienenvolks. Egoistische genetische Elemente können zu sehr unerwarteten Resultaten führen (Hurst *et al.* 1996). Bei der Kap Honigbiene kommt dies mit der Evolution einer sozial parasitischen Lebensweise, die zum kompletten Verlust der sozialen Organisation geführt hat, zum Ausdruck (Neumann & Moritz 2002).

7.3 Literatur

- 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.
- Beye M, Moritz RFA, Crozier RH, Crozier YC, 1996 Mapping the sex locus of the honeybee (*Apis mellifera*). *Naturwissenschaften* **83**: 424-426.
- Beye M, Hasselmann M, Fondrk MK, Page RE, Omholt SW, 2003 The gene *csd* is the primary signal for sexual development in the honeybee and encodes an SR-type protein. *Cell* **114**: 419-429.
- Bourke AFG, Franks NR, 1995 *Social Evolution in Ants*. Princeton University Press, Princeton, 529 pp.
- 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, Moritz RFA, Lattorff HMG, 2004 Trapping pheromonal components with silicone rubber tubes: fatty acid secretions in honeybees (*Apis mellifera*). *Chemoecology* **14**: 77-79.
- Foster KR, Shaulsky G, Strassmann JE, Queller DC, Thompson CRL, 2004 Pleiotropy as a mechanism to stabilize cooperation. *Nature* **431**: 693-696.
- Fraser VS, Kaufmann B, Oldroyd BP, Crozier RH, 2000 Genetic influence on caste in the ant *Camponotus consobrinus*. *Behav Ecol Sociobiol* **47**: 188-194.
- Gadagkar R, 1991 *Belonogaster*, *Mischocyttarus*, *Parapolybia*, and the Independent-founding *Ropalidia*. In Ross KG, Matthews RW (eds) *The Social Biology of Wasps*. Comstock Publishing Ass., Ithaca. pp 149-190.
- Gadagkar R, 1997 Social evolution – has nature ever rewound the tape? *Curr Sci* **72**: 950-956.
- Gadau J, Page RE, Werren JH, Schmid-Hempel P, 2000 Genome organization and social evolution in Hymenoptera. *Naturwissenschaften* **87**: 87-89.
- Härtel S, Neumann P, Kryger P, Moritz RFA, Crewe RM, 2005 Emery's rule in the Cape honeybee (*Apis mellifera capensis* Esch.). *submitted*.
- Helms Cahan S, Keller L, 2003 Complex hybrid origin of genetic caste determination in harvester ants. *Nature* **424**: 306-309.
- Helms Cahan S, Parker JD, Rissing SW, Johnson RA, Polony TS, *et al.* 2002 Extreme genetic differences between queens and workers in hybridizing *Pogonomyrmex* harvester ants. *Proc Roy Soc Lond B* **269**: 1871–1877.
- Helms Cahan S, Glennis E, Julian GE, Rissing SW, Schwander T, Parker JD, Keller L, 2004 Loss of phenotypic plasticity generates genotype-caste association in harvester ants. *Curr Biol* **14**: 2277-2282.
- Jeanne RL, 1991 The Swarm-founding Polistinae. In Ross KG, Matthews RW (eds) *The Social Biology of Wasps*. Comstock Publishing Ass., Ithaca. pp 191-231.
- Julian GE, Jennifer H. Fewell JH, Gadau J, Johnson RA, Larrabee D, 2002 Genetic determination of the queen caste in an ant hybrid zone. *Proc Natl Acad Sci USA* **99**: 8157–8160.
- Keller L, Ross KG, 1998 Selfish genes: a green beard in the red fire ant. *Nature* **394**: 573-575.

- Krieger MJB, Ross KG, 2002 Identification of a major gene regulating complex social behavior. *Science* **295**: 328-332.
- Lattorff HMG, Fuchs S, Solignac M, Moritz RFA, 2004 Using DNA fingerprinting to facilitate back crossing experiments in the honeybee (*Apis mellifera*). *Apidologie* **35**: 547-548.
- Lattorff HMG, Moritz RFA, Fuchs S, 2005a A single gene determines thelytokous parthenogenesis in honeybees (*Apis mellifera capensis*). *Heredity* **94**: 533-537.
- Lattorff HMG, Moritz RFA, Crewe RM, 2005b A selfish gene drives selection for selfish workers. *Nature* (in review).
- Lattorff HMG, Moritz RFA, Solignac M, 2005c Fine mapping of the pleiotropic *thelytoky* gene in the honeybee (*Apis mellifera*). *Genetics* (to be submitted).
- Martin SJ, Jones GR, 2004 Conservation of Bio synthetic pheromone pathways in honeybees *Apis*. *Naturwissenschaften* **91**: 232-236.
- Martin SJ, Jones GR, Châline N, Middleton H, Ratnieks FLW, 2002 Reassessing the role of the honeybee (*Apis mellifera*) Dufour's gland in egg marking. *Naturwissenschaften* **89**: 528 – 532.
- Maynard Smith J, Szathmary E, 1997 *The Major Transitions in Evolution*. Oxford University Press, Oxford. 360 pp.
- Moritz RFA, Lattorff HMG, Crewe RM, 2004 Honeybee (*Apis mellifera capensis*) compete for producing queen-like pheromone signals. *Proc Roy Soc Lond B* **271**: S98-S100.
- Moritz RFA, Pflugfelder J, Crewe RM, 2003 Lethal fighting between honeybee queens and parasitic workers (*Apis mellifera*). *Naturwissenschaften* **90**: 378-381.
- Neumann P, Moritz RFA, 2002 The Cape honeybee phenomenon: the sympatric evolution of a social parasite in real time? *Behav Ecol Sociobiol* **52**: 271-281.
- Ross KG, Keller L, 1998 Genetic control of social organization in an ant. *Proc Natl Acad Sci USA* **95**: 14232-14237.
- Sole CL, Kryger P, Hefetz A, Katzav-Gozansky T, Crewe RM, 2002 Mimicry of queen Dufour's gland secretions by workers of *Apis mellifera scutellata* and *A. m. capensis*. *Naturwissenschaften* **89**: 561 – 564.
- Solignac M, Vautrin D, Baudry E, Mougél F, Loiseau A *et al.*, 2004 A microsatellite-based linkage map of the honeybee, *Apis mellifera* L. *Genetics* **167**: 253-262.
- Volny VP, Gordon DM, 2002 Genetic basis for queen–worker dimorphism in a social insect. *Proc Natl Acad Sci USA* **99**: 6108–6111.
- Weaver N, 1957 Effects of larval age on dimorphic differentiation of the female honey bee. *Ann Entomol Soc Am* **50**: 283-294.
- Weiss K, 1974 Zur Frage des Königinnengewichtes in Abhängigkeit von Umlarvalter und Larvenversorgung. *Apidologie* **5**: 127-147.
- Winston ML, 1987 *Biology of the Honeybee*. Harvard University Press, Cambridge. 281 pp.
- Woyke J, 1971 Correlations between the age at which honeybee brood was grafted, characteristics of the resultant queens, and results of insemination. *J Apic Res* **10**: 45-55.

8 Appendix

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

1. Moritz RFA, **Lattorff HMG**, Crewe RM, 2004 Honeybee workers (*Apis mellifera capensis*) compete for producing queen-like pheromone signals. *Proc R Soc Lond B* **271**: S98-S100.

behavioural assays, gaschromatographic analysis, statistical data analysis, preparation of manuscript

2. **Lattorff HMG**, Moritz RFA, Fuchs S, 2005 A single locus determines thelytokous parthenogenesis of laying honeybee workers (*Apis mellifera capensis*). *Heredity* **94**: 533-537.

project idea, genetic experiments, behavioural assays, DNA analysis, statistical data analysis, writing manuscript

3. **Lattorff HMG**, Moritz RFA, Crewe RM, 2005 A selfish gene drives selection for selfish honeybee workers. *Nature* submitted (MS # 2005-04-04430).

project idea, genetic experiments, behavioural assays, DNA analysis, statistical data analysis, writing manuscript

4. **Lattorff HMG**, Moritz RFA, Solignac M, 2005 Fine mapping of the pleiotropic *thelytoky* gene in the honeybee, *Apis mellifera* L. Manuscript for *Genetics*.

project idea, genetic experiments, DNA analysis, statistical data analysis, bioinformatic analysis, writing manuscript

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I thank all my co-authors, who gave me the opportunity for research visits in their labs. I would like to thank Prof. Dr. Robin M. Crewe, who gave me the opportunity for a 2 month research visit at the University of Pretoria, Republic of South Africa. Special thanks also to Dr. Per Kryger and Dr. Theresa C. Wossler of the University of Pretoria, who gave me strong support with field work and GC analysis.

Prof. Dr. Michel Solignac gave me the opportunity to stay for a month in Gif-sur-Yvette, France, to use more than 500 microsatellite markers for mapping purposes. My special thanks also belongs to Dominique Vautrin for helping with lab work and introducing me to preparing gels and handling radioactivity.

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This work is dedicated to my mother, Maria Lattorff, who died in 2002 and could not see her son finishing this work.

8.3 Curriculum vitae

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Name Hans Michael Georg Lattorff
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School carrier

Primary School 1980 – 1989 Polytechnische Oberschule „Maxim Gorki“, Kleinmachnow
High School 1989 – 1990 „Kippenberg“-Gymnasium, Bremen
High School 1990 – 1993 „Wolfgang-Borchart-Gymnasium“, Halstenbek 16.06.1993 Abitur (grade 3.4)

Academic education

10/1993-09/1994 Studies of mechanical engineering at the Technical University Hamburg-Harburg, Germany
10/1994-10/2000 Studies of biology (zoology, genetics, botany, paleontology) at the Martin-Luther-University Halle-Wittenberg, Germany, finished diploma on 27.10.2000 (grade 1.4)

Faculty appointments

07-08/2000 Undergraduate student appointment (Studentische Hilfskraft mit Lehraufgaben)
11/2000-12/2001 Graduate student appointment (PhD candidate) (BioService Halle GmbH, funded by ESF and Government of Sachsen-Anhalt)
01/2002-12/2004 Graduate student appointment (PhD candidate) (Institut für Zoologie, Martin-Luther-Universität Halle-Wittenberg, funded by DFG)

Research visits

02-04/2001 University of Pretoria, Republic of South Africa (RM Crewe & P Kryger)
02-03/2003 University of Illinois at Urbana-Champaign, USA (GE Robinson & CM Grozinger)
09-10/2003 Laboratoire Populations, Génétique et Evolution, CNRS, Gif-sur-Yvette, France (M Solignac)
05/2004 Institut für Bienenkunde Oberursel, Johann-Wolfgang-Goethe-Universität Frankfurt/M., Germany (N Koeniger & S Fuchs)

Professional Affiliations:

International Union for the Study of Social Insects (IUSSI)

Public scientific service

I have reviewed manuscripts for *Apidologie*, *Heredity*, *Journal of Evolutionary Biology* and *Molecular Ecology*.

8.4 Publications

peer reviewed

- Moritz RFA, Scharpenberg H, **Lattorff HMG**, Neumann P, 2003 A technical note for using microsattelite DNA analyses in haploid male DNA pools of social Hymenoptera. *Insectes Sociaux* **50**: 398-400.
- *Moritz RFA, **Lattorff HMG**, Crewe RM, 2004 Honeybee workers (*Apis mellifera capensis*) compete for producing queen-like pheromone signals. *Proceedings of the Royal Society London B* **271**: S98-S100.
- Crewe RM, Moritz RFA, **Lattorff HMG**, 2004 Trapping pheromonal components with silicone rubber tubes: fatty acid secretions in honeybees (*Apis mellifera*). *Chemoecology* **14**: 77-79.
- Lattorff HMG**, Kryger P, Moritz RFA, 2005 Queen rearing of clonal social parasitic honeybees (*A. m. capensis* Esch.) and its host *A. m. scutellata* (Lepelletier) reveals a fitness trade-off between castes. *Insectes Sociaux* **52**: in press.
- ***Lattorff HMG**, Moritz RFA, Fuchs S, 2005 A single locus determines thelytokous parthenogenesis of laying honeybee workers (*Apis mellifera capensis*). *Heredity* **94**: 533-537.
- Schlüns H, Moritz RFA, **Lattorff HMG**, Koeniger G, 2005 Paternity skew in colonies of the genus *Apis* L. *Apidologie* **36**: in press.
- Moritz RFA, **Lattorff HMG**, Neumann P, Kraus FB, Hepburn HR, Radloff SE, 2005 Rare royal families in the honeybees. *Current Biology* (MS # CB-D-05-00185, in review)
- ***Lattorff HMG**, Moritz RFA, Crewe RM, 2005 A selfish gene drives selection for selfish honeybee workers. *Nature* submitted (MS # 2005-04-04430, in review).

manuscripts in preparation

- ***Lattorff HMG**, Moritz RFA, Solignac M, 2005 Fine mapping of the pleiotropic *thelytoky* gene in the honeybee (*Apis mellifera* L.). *Genetics* (to be submitted)

non-peer reviewed

- Lattorff HMG**, Neumann P, Moritz RFA, 2001 Spatial separation of pseudoqueens in queenless Cape honeybee colonies (*Apis mellifera capensis* Escholtz). In: Menzel R, Rademacher E (eds.) *Proceedings of the 2001 Berlin Meeting of the European Sections of IUSSI*, IUSSI, Berlin, p7
- Lattorff HMG**, Moritz RFA, Neumann P, 2002 Clustered brood of honeybee pseudoqueens: self-organization or random pattern? *Zoology* **105** Suppl. V (95.1 Abstracts): 27.
- Lattorff HMG**, Moritz RFA, Crewe RM, 2002 Genes and reproductive dominance in honeybee workers (*Apis mellifera*). In: *XIV International Congress of IUSSI The Golden Jubilee Proceedings*, Hokkaido University, Sapporo, Japan, p65.
- Schlüns H, Moritz RFA, **Lattorff HMG**, Koeniger G, 2002 Distribution of paternities in seven species of honeybees (*Apis* sp.). *Apidologie* **32**: 508-509.

- Lattorff HMG**, Moritz RFA, Crewe RM, 2002 Pheromone dynamics of workers of the Cape honeybee (*Apis mellifera capensis*). *Apidologie* **32**: 511-512.
- Lattorff HMG**, Fuchs S, Solignac M, Moritz RFA, 2004 Using DNA fingerprinting to facilitate double-back crossing experiments in the honeybee (*Apis mellifera*). *Apidologie* **35**: 547-548.
- Lattorff HMG**, Fuchs S, Solignac M, Moritz RFA, 2004 Genetic and molecular analysis of thelytokous parthenogenesis in honeybee workers. In: Bernardinelli I, Milani N (eds.) *Proceedings of the First European Conference of Apidology "EurBee" Udine (Italy)*, 19-24 September 2004, Udine, p29-30.
- Lattorff HMG**, Moritz RFA, Fuchs S, Solignac M, 2005 Genetic factors influencing worker reproduction in the honeybee (*Apis mellifera* L.). In: Kaatz HH, Becher M, Moritz RFA (eds) *Bees, Ants and Termites: Applied and Fundamental Research*. IUSI, Halle (Saale), p130.

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

8.5 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 28. April 2005

.....
Michael Lattorff