

**The genetic control of the social parasitism in the Cape
honey bee, *A. m. capensis* ESCH.**

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Table of Contents

General Introduction	1
1 Evolution of eusociality	1
2 The Western honey bee (<i>Apis mellifera</i>)	2
3 Worker reproduction in <i>A. mellifera</i>	4
4 The special case of <i>A. m. capensis</i> Esch.	6
5 Social parasitism of <i>A. m. capensis</i> workers.....	10
6 Genetic control of thelytoky in <i>A. m. capensis</i>	12
7 Aims of this thesis	14
Chapter I.....	15
Thelytoky in Cape honeybees (<i>Apis mellifera capensis</i>) is controlled by a single recessive locus .	15
Chapter II	34
A single SNP turns a social honey bee (<i>Apis mellifera</i>) worker into a selfish parasite.....	34
Chapter III.....	57
The transcriptomic changes associated with the development of social parasitism in the honeybee <i>Apis mellifera capensis</i>	57
General Discussion	84
1 Worker reproduction in <i>A. m. capensis</i>	84
2 Genetic mechanisms underpinning thelytokous worker reproduction in <i>A. m. capensis</i>	85
3 Evolutionary perspective on the genetic control of the social parasitism in <i>A. m. capensis</i>	87
References	94
General Acknowledgments.....	108
Appendix	109
A – Supplementary material – Chapter I	109
B – Supplementary material – Chapter II	157
C – Supplementary material – Chapter III.....	170
D – Curriculum Vitae	181
E – Publication List	183
F – Eidesstattliche Erklärung.....	184

General Introduction

1 Evolution of eusociality

Eusociality is the most advanced form of animal sociality and is defined by overlapping generations, cooperative brood care and the reproductive division of labour (Wilson 1971). The major distinction of eusocial societies to any other social system is the reproductive altruism of the non-reproductive caste, giving up its own reproductive potential, in favour of the reproductive success of the reproductive caste (Hamilton 1964). The evolution of reproductive altruism in complex societies is one of the major transitions in evolution, contradicting Darwin's theory of natural selection (1859) at first view, as he noted in his book *The Origin of Species* (Chapter VII, p. 289, line 24-32):

"I [...] will confine myself to one special difficulty, which at first appeared to me insuperable, and actually fatal to my whole theory. I allude to the neuters or sterile females in insect communities: for these neuters often differ widely in instinct and in structure from both the male and fertile females, and yet from being sterile they cannot propagate their kind."

Darwin (1859) himself suggested 'selection on the family' instead of selection on the individual to overcome this contradiction to his theory. Based on this, about 100 years later, Hamilton introduced his 'inclusive fitness theory' (1964), which plausibly explains why sociality is an evolutionary stable strategy. Through kin selection, individuals may not only increase their fitness by producing own offspring, but also by helping to raise offspring of individuals sharing their genes by descent, like parents or siblings. According to Hamilton's rule, a gain of inclusive fitness is given if $C < B \cdot r$ (C = reproductive costs of the altruist, B = reproductive gain of the receiver, r = genetic relatedness between altruist and receiver). Especially in monogamous species with a haplo-diploid sex-determination system with diploid females and haploid males, relatedness among offspring is high as they share 75% of their genes. Thus, kin selection may favour sterile individuals that help to raise their siblings, to which they are more closely related ($r=0.75$) than to their own offspring ($r=0.5$).

Eusocial societies evolved independently several times in different taxa of vertebrates and arthropods (Andersson 1984, Wilson and Hölldobler 2005). They are known for two mole rat, some shrimp and many insect species, including beetles, thrips, aphids and termites. Sociality is however, particularly frequent in the order of the Hymenoptera (ants, wasps and bees; Andersson 1984, Wilson and Hölldobler 2005, Hughes et al. 2008, Peters et al. 2017). In Hymenoptera, all species are characterised by haplodiploidy and eusociality evolved independently eight times (Wilson and Hölldobler 2005, Hughes et al. 2008, Peters et al. 2017). Strict monogamy and thus, a high degree of relatedness among all colony members, always represented the ancestral state (Hughes et al. 2008) and most likely facilitated these frequent independent evolutionary events (Boomsma 2007).

The most prominent and best studied eusocial Hymenoptera is the Western honey bee, *Apis mellifera* L. Due to the easy handling of honey bee colonies and the high number of available experimental individuals, *A. mellifera* became a model organism, which is frequently used to acquire knowledge about the proximate and ultimate reasons of sociality.

2 The Western honey bee (*Apis mellifera*)

2.1 The honey bee's sex determination system

The haplo-diploid sex determination system of *A. mellifera* was first mentioned by Johannes Dzierson (1845) who suggested that honey bee males develop from unfertilised eggs, but honey bee females from fertilised eggs. Cytological studies (Petrunkevitch 1901, Nachstheim 1913) confirmed Dzierson's hypothesis, as they could show that honey bee females have two sets of chromosomes (diploid), but that honey bee males possess only one set of chromosomes (haploid). A few decades later, it was found that the honey bee's sex determination system is complementary (Mackensen 1951), a mechanism that was first described in the parasitic wasp *Bracon hebetor* Say (Whiting 1933, 1943). After modern molecular biological techniques became available, the gene *complementary sex determiner* (*csd*) was identified as the primary signal for sexual development in honey bees (Beye et al. 2003), which is characterised by large allelic richness (Adams et al. 1977, Hasselmann and Beye 2004, Lechner et al. 2014). Only individuals heterozygous at *csd* develop into fertile females, but

homozygosity results in diploid infertile males, which are normally lethal as workers remove them from the colony at early developmental stages (Woyke 1963). Fertile males can only develop from unfertilized haploid eggs and are thus hemizygous at *csd* (Beye et al. 2003).

2.2 Social structure in honey bee colonies

In general, honey bee colonies are characterised by strong social hierarchies. Several thousand sterile workers (~10,000-60,000) perform all tasks to maintain the colony like brood care, foraging, nest building, colony maintenance and defence, but reproduction is monopolised by a single polyandrous queen (Adams et al. 1977, Winston 1987). While the sterile workers are usually short-lived (4-6 weeks, Winston 1987), the highly fertile queen, which lays up to 1,500 eggs per day (Winston 1987), is long-lived (1-5 years, Page and Peng 2001). The multiple mating of the queen with up to 45 males (Moritz et al. 1996, Palmer and Oldroyd 2000) leads to low intra-colonial relatedness ($r=0.25$), resulting in a high conflict potential among the colony members.

2.3 Potential conflicts in honey bee colonies

Honey bee workers can determine the age, sex and ploidy of larvae and they are able to distinguish between worker and queen laid brood (Woyke 1965, Free and Winder 1983, Ratnieks and Visscher 1989) due to the egg marking pheromones produced by the queen (Jay 1970, Ratnieks 1995), which are absent on worker laid eggs (Ratnieks 1995). Furthermore, kin recognition of brood is known (Noonan and Kolmes 1989), leading to potential competition over queen rearing among subfamilies, as one would expect that workers favour their own subfamilies to nurse new queens. However, queen rearing is neither nepotistic (Breed et al. 1984, Woyciechowski 1990) nor random (Estoup et al. 1994, Moritz et al. 1996), but new queens are mostly reared from rare ‘royal’ subfamilies (Tilley and Oldroyd 1997, Moritz et al. 2005, Lattorff and Moritz 2016, Tarpy et al. 2016, Withrow and Tarpy 2018). Also among the hatching queens, there is strong competition. After emergence, they engage in lethal fights with each other and only the last surviving queen can take over the colony (Winston 1987, Gilley 2001). Moreover, conflict over reproduction may occur between and within castes.

3 Worker reproduction in *A. mellifera*

Honey bee workers possess reproductive organs, but as they are never mated (Bourke 1988), they are usually only capable of producing unfertilised haploid male destined eggs via parthenogenesis (arrhenotoky, Ruttner and Hesse 1981, Winston 1987, Page and Erickson 1988). Accordingly, honey bee workers are more closely related to their sons ($r=0.5$) than to their brothers ($r=0.25$; sons of the queen) and nephews (0.125 ; sons of their sisters) (Ratnieks 1988).

3.1 Worker policing

Worker policing (Woyciechowski and Lomnicki 1987, Ratnieks 1988, Ratnieks and Reeve 1992), which means that workers remove eggs laid by other workers (Ratnieks & Visscher 1989) and show aggression towards nest mates with activated ovaries (Anderson 1968, Evers and Seeley 1986, Visscher and Dukas 1995, Calis et al. 2005), seems to resolve the conflict over male production. Alternatively, the reduced viability of worker laid eggs in comparison to queen laid eggs might explain the increased removal rates of worker produced brood (Velthuis et al. 2002, Pirk et al. 2004). Overall, more than 99% of worker laid eggs are eaten by other workers and cannot develop into viable adults (Visscher 1989, 1996).

3.2 Pheromonal control

In addition to worker policing, in queenright colonies, worker reproduction is effectively controlled by pheromones of the brood (Kropakova and Haslbachova 1971, Jay 1972, Arnold et al. 1994) and the queen (Free 1987, Wossler and Crewe 1999a, Holman 2018). In the case of the queen, particularly, pheromones produced in the Dufour's (Katzav-Gozansky et al. 2001, 2002), tergal (Espelie et al. 1990, Smith et al. 1993, Wossler and Crewe 1999b) and mandibular glands (Butler 1959, Butler et al. 1962, Butler and Fairey 1963, Slessor et al. 1988, Plettner et al. 1993, Winston and Slessor 1998, Hoover et al. 2003, 2005) are known to control worker reproduction. The pheromonal bouquet of the mandibular glands has six major components: 9-ODA (9-oxo-2(E)-decenoic acid), 9-HDA (9-hydroxy-2(E)-decenoic acid), 10-HDA (10-hydroxy-2(E)-decenoic acid), 10-HDAA (10-hydroxy-decanoic acid), HOB (methyl *p*-hydroxybenzoate) and HVA (4-hydroxy-3-methoxyphenylethanol)

(Slessor et al. 1988, 1990, Plettner et al. 1993, Winston and Slessor 1998). The composition of these fatty acids and aromatic compounds is highly caste-specific (Plettner et al. 1993, 1995, 1996, 1997). Starting with the same initial substance, the subsequent pheromone production is split into two caste-specific routes of synthesis. However, both castes can switch between the two pathways (Plettner et al. 1998). In workers, the mandibular gland pheromones are usually dominated by 10-HDA and 10-HDAA (Plettner et al. 1993, 1995, 1996, 1997, Yusuf et al. 2015). The major component of the queen's mandibular gland pheromones (QMP) is the so-called 'queen substance' 9-ODA, which is known to play a key role in the inhibition of queen rearing (Winston et al. 1989, 1990, Melathopoulos et al. 1996, Pettis et al. 1995, 1997) and worker ovary development (Butler et al. 1962, Butler 1963, Hoover et al. 2003, 2005, Strauss et al. 2008). Genetically, under queenright conditions, the Notch signalling pathway, which is induced by the queen pheromones, inhibits oogenesis (Duncan et al. 2016). As a result of the pheromone mediated control, in queenright colonies, the vast majority of workers (>99.9%) has undeveloped ovaries and refrains from reproduction (Visscher 1989, Ratnieks 1993, Visscher 1996, Kocher and Grozinger 2011).

3.3 Queenless conditions

If the queen is lost, her pheromonal control breaks down and workers can activate their ovaries, which is genetically induced by the epidermal growth factor receptor (EGFR) signalling pathway (Formesyn et al. 2014). In queenless colonies, about 10 % of workers activate their ovaries and start laying large quantities of eggs (Sakagami 1958, Velthuis 1970, Page and Erickson 1988). However, not all workers contribute equally to the male production, but their reproductive success strongly depends on their subfamily membership (Page and Erickson 1988, Page and Robinson 1994, Moritz et al. 1996, Martin et al. 2004, Goudie et al. 2012, Oldroyd et al. 2014). Indeed, patrigenes (genes inherited from the father) influence worker reproduction (Galbraith et al. 2016) and traits related to fertility are genetically determined (Moritz and Hillesheim 1985, Jordan et al. 2008a, Goudie et al. 2012, Oldroyd et al. 2014), such as ovary size (Jordan et al. 2008a, Goudie et al. 2012, Oldroyd et al. 2014), presence of a spermatheca (Jordan et al. 2008a), ovary development, trophallactic dominance and queen-like pheromone production (amount of 9-ODA, Moritz and Hillesheim 1985).

3.4 The anarchy syndrome

A rare mutant phenotype among *A. mellifera* workers is anarchistic behaviour (Oldroyd et al. 1994). Anarchistic honey bees act like sterile workers, contributing to all worker responsibilities in the hive and producing worker-like mandibular gland pheromones, but in addition, they lay eggs in the presence of the queen, avoiding worker policing (Oldroyd et al. 1994, 1999, Oldroyd and Ratnieks 2000). As a result, a large proportion of the male brood in anarchistic colonies is laid by workers, which all are members of a single subfamily (Oldroyd et al. 1994, Montague and Oldroyd 1998). As indicated by the parentage skew, the ‘anarchy syndrome’ seems to be genetically determined (Oldroyd et al. 1994, Montague and Oldroyd 1998, Châline et al. 2002). Indeed, the gene *Anarchy*, encoding a peroxisomal membrane protein, was identified to play a key role (Ronai et al. 2016). Knocking down *Anarchy* using RNA interference (RNAi) significantly increased the expression of the gene *Buffy* in ovaries (Ronai et al. 2016), which is known to be involved in programmed cell death (Tanner et al. 2011, Dallacqua and Bitondi 2014) and thus oogenesis. Only under queen-right conditions anarchistic workers produce a large proportion of male eggs in the colony. If the queen is lost, their egg production is no longer above the average (Montague and Oldroyd 1998).

4 The special case of *A. m. capensis* Esch.

Among all honey bee subspecies, the Cape honey bee, *A. m. capensis*, is special. *A. m. capensis* workers possess many unique traits associated with reproduction (Hepburn and Radloff 1998), endowing them with a high reproductive potential and making them more queenlike. They are characterised by a high number of ovarioles (Ruttner 1977, Hepburn and Crewe 1991, Allsopp et al. 2003), are likely to have a spermatheca (Onions 1914, Hepburn and Crewe 1991, Phiancharoen et al. 2010, Goudie et al. 2012), can quickly activate their ovaries (Anderson 1963, Crewe and Velthuis 1980, Ruttner and Hesse 1981, Moritz and Hillesheim 1985, Crewe et al. 1990) and are able to produce queenlike pheromones in their Dufour’s (Martin and Jones 2004), tergal (Wössler and Crewe 1999b, Okosun et al. 2015) and mandibular glands (Hemmling et al. 1979, Plettner et al. 1993, Crewe and Velthuis 1980, Moritz et al. 2000). As a consequence, they have many capabilities that are usually

only reserved for queens. They can inhibit ovary development and queenlike pheromone production in other workers (Hillesheim et al. 1989, Velthuis et al. 1965, Moritz et al. 2000), avoid worker policing and elicit retinue behaviour (Velthuis et al. 1965, 1990, Moritz et al. 2000, 2004). Due to these queenlike traits, they have been termed ‘false queens’ (Sakagami 1958) or ‘pseudoqueens’ (Ruttner 1977, Velthuis et al. 1990). Reproductive dominant pseudoqueens do not engage in worker tasks like brood rearing or foraging, but only engage in reproduction (Hillesheim et al. 1989).

Furthermore, the Cape honey bee is the only known honey bee subspecies, in which workers are able to produce diploid female offspring (de Villiers 1883, Onions 1912). Like workers of all other honey bee subspecies, *A. m. capensis* workers are not mated and reproduce parthenogenetically (Winston 1987). The egg’s diploidy is restored by a central fusion of the meiotic products (automixis, Verma and Ruttner 1983, Cole-Clark et al. 2017). This extraordinary trait is called thelytoky (Onions 1912, Anderson 1963). Because recombination rarely occurs or is even completely absent during thelytokous parthenogenesis (Moritz and Haberl 1994, Baudry et al. 2004), the offspring is genetically identical to the mother (clonal). Thus, relatedness between mother and offspring is high ($r=1$), changing the intra-colonial relationships as the degree of relatedness between workers and their nieces (offspring of sister workers) does not differ from that between workers and their sisters (offspring of the queen) ($r=0.25$). Accordingly, worker policing does no longer correlate with a selective advantage and should be reduced (Hamilton 1972, Greeff 1996). Even though most *A. m. capensis* workers have inactivated ovaries in the presence of a queen due to the royal pheromonal control (Hoover et al. 2003, Härtel et al. 2006b), worker laid offspring can be observed in queenright *A. m. capensis* colonies (Moritz et al. 1999, Beekman et al. 2002). Nevertheless, worker policing is still present, which was assumed to boost the colony’s efficiency (e.g. removal of non-viable eggs, Beekman et al. 2002, Pirk et al. 2003).

Serious conflict among colony members arises under queenless conditions, as selfish thelytokous reproduction dramatically enhances a worker’s inclusive fitness (Greeff 1996). To become a laying worker after queen loss, *A. m. capensis* workers initiate a pheromonal arms race for the production of the strongest queenlike mandibular gland signal. At the end, only the most dominant workers that produce the strongest pheromones are able to suppress ovary activation and queenlike mandibular

gland secretion in the other workers and establish themselves as reproductive dominant pseudoqueens in the hive (Fig. 1; Moritz et al. 1996, 2000, 2002, 2004, Simon et al. 2005). Microsatellite genotyping revealed that these dominant workers belong to a few subfamilies only, indicating strong selection for reproductive dominance (Moritz et al. 1996). The mandibular gland signal of *A. m. capensis* pseudoqueens is not only qualitatively queenlike, but also the produced quantities are similar to that of *A. m. capensis* queens and even higher than those produced by queens of other honey bee subspecies (Hemmling et al. 1979, Crewe and Velthuis 1980, Crewe 1988, Crewe et al. 1990, Wossler 2002). Furthermore, the life expectancy of *A. m. capensis* pseudoqueens is much higher (5 month or longer, Velthuis et al. 1990, Tribe and Allsopp 2001) than that of sterile workers (4-6 weeks, Winston 1987).



Fig. 1: An *A. m. capensis* pseudoqueen eliciting retinue behaviour in the surrounding workers (photo: Stephan Härtel).

4.1 The geographical distribution of A. m. capensis

The Cape honey bee is native to the fynbos region at the Western and Eastern Cape in the South of South Africa (Alpatov 1933, Tribe 1983, Hepburn and Crewe 1991, Hepburn and Radloff 1998). The North of South Africa is part of the endemic region of the adjacent African honey bee, *A. m. scutellata* Lepeletier (Ruttner 1988). The native ranges of *A. m. capensis* and *A. m. scutellata* are separated by an introgression zone, where the two subspecies usually hybridise (Fig. 2; Hepburn and Crewe 1991, Hepburn et al. 1998, Dietemann et al. 2007).

The endemic region of *A. m. capensis* is characterised by suddenly changing (Tribe 1983) and very windy weather, which might cause frequent queen losses, as queens are not able to return to the colony after mating flights. These weather conditions were suggested as cause for the evolution of thelytokous worker reproduction in *A. m. capensis*, which enables workers to requeen a colony from worker laid brood, instead of getting extinct (Moritz 1986). Despite the various fitness benefits and selective advantages resulting from thelytokous worker reproduction, the *A. m. capensis* population remains stable and thelytoky does not spread into other honey bee populations (Crewe and Hepburn 1991, Hepburn et al. 1998), which remained a puzzling issue for evolutionary biologists (Moritz 1986, Greeff 1996).

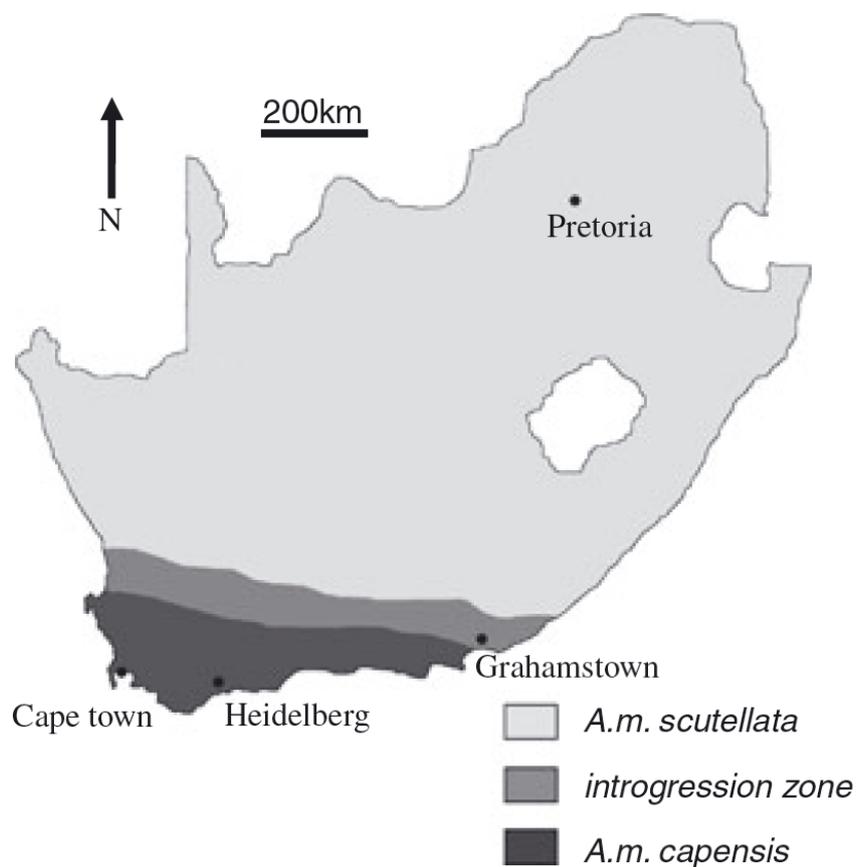


Fig. 2: A map of South Africa with the endemic ranges of *A. m. capensis* and *A. m. scutellata* and the introgression zone, where the two subspecies hybridise. Figure taken from Dietemann et al. (2007).

5 Social parasitism of *A. m. capensis* workers

Evolutionary theory predicts that the thelytoky syndrome, comprising swift ovary activation, queenlike pheromone production and thelytokous worker reproduction (Lattorff and Moritz 2013), in combination with some behavioural traits (Neumann and Hepburn 2002) can facilitate the evolution of selfish behaviour (Hamilton 1964, Greeff 1996). Indeed, *A. m. capensis* workers can explore an additional life history trajectory as social parasites. Cape honey bees are more likely to disperse into foreign colonies than workers of other honey bee subspecies, representing a potential host finding mechanism (Neumann et al. 2001, Neumann and Hepburn 2002, Neumann and Moritz 2002). In the host colonies, they usually stay in areas distant from the queen (Moritz et al. 2002, Neumann and Moritz 2002, Pirk et al. 2002, Neumann et al. 2003b) and develop into laying workers (Moritz et al. 2002, Neumann et al. 2003a). In colonies of their own subspecies, foreign worker laid brood is regularly observed (Härtel et al. 2006b). Especially queenless colonies are susceptible to *A. m. capensis* social parasites (Woyke 1995, Neumann and Hepburn 2002). Indeed, in hopelessly queenless *A. m. capensis* colonies, new queens are more often reared from eggs laid by parasitic workers than from offspring of native workers (Jordan et al. 2008b, Allsopp et al. 2010, Holmes et al. 2010, Moritz et al. 2011). Nevertheless, within the natural *A. m. capensis* population, social parasitism is a rather uncommon phenomenon, which is most likely the result of counterbalancing selection between the individual and the colony level fitness (Härtel et al. 2006b).

More often, inter-subspecies specific social parasitism occurs, as *A. m. capensis* pseudoqueens seem to be resistant to the pheromonal control of queens of other honey bee subspecies (Dietemann et al. 2006, but see Mumoki et al. 2018). In addition, within colonies of other *A. mellifera* subspecies, *A. m. capensis* workers get preferentially fed (Beekman et al. 2000). As a result, Cape honey bee workers can successfully establish themselves as social parasitic pseudoqueens in colonies of *A. m. mellifera*, *A. m. carnica*, *A. m. ligustica*, *A. m. caucasica* and *A. m. scutellata* (Onions 1912, Lundie 1954, Johansmeier 1983, 1992, Velthuis et al. 1990, Koeniger and Wüerkern 1992, Woyke 1995).

5.1 The Capensis Calamity

The most extreme case of social parasitism with strong negative impact was caused by migratory beekeeping in the 1990s, when about 400 *A. m. capensis* colonies were brought into the native region of the adjacent South African honey bee subspecies *A. m. scutellata* (Hepburn and Crewe 1991, Allsopp 1993, Allsopp and Crewe 1993, Neumann and Moritz 2002). A single clonal lineage of *A. m. capensis* workers, descending from a single worker originating from the endemic region of *A. m. capensis* (Kryger 2001, Neumann et al. 2002, Radloff et al. 2002, Baudry et al. 2004, Härtel et al. 2006a, Neumann et al. 2011), spread widely in the area of *A. m. scutellata* (over an area of ~275.000 km², Neumann et al. 2011) and caused substantial colony losses to beekeepers (thousands of colonies per year, Allsopp and Crewe 1993), which is known as ‘Capensis Calamity’ (Allsopp 1992, Allsopp and Crewe 1993, Neumann and Moritz 2002). In the Northern provinces of South Africa, this mass extinction of *A. m. scutellata* colonies still persists today (Pirk et al. 2014), seriously harming commercial beekeepers.

About nine weeks after the initial infestation with *A. m. capensis* social parasites, the host colony dies (Neumann and Hepburn 2002). As soon as several parasitic *A. m. capensis* workers are in the colony, the queen is lost, which might be the result of lethal fights with the *A. m. capensis* pseudoqueen(s) (Moritz et al. 2003). Immediately after, the parasites take over the reproductive monopoly and establish themselves as pseudoqueens in the hive, evading worker policing (Martin et al. 2002a, Neumann et al. 2003a). Almost all individuals of the first parasitic generation have activated ovaries (Härtel et al. 2011). Quickly the number of parasitic *A. m. capensis* workers increases, but the number of *A. m. scutellata* host workers decreases, resulting in an imbalance of the division of labour. Because *A. m. capensis* pseudoqueens do not engage in worker task, but only in reproduction (Hillesheim et al. 1989), the colony more and more lacks regular brood rearing and foraging (Martin et al. 2002b, Neumann and Hepburn 2002). In the end, the colony collapses and ultimately dies, known as ‘dwindling colony syndrome’ (Allsopp 1993, Allsopp and Crewe 1993, Hepburn and Allsopp 1994, Allsopp 1995).

6 Genetic control of thelytoky in *A. m. capensis*

The genetic control of the thelytoky syndrome (Lattorff and Moritz 2013), which constitutes the genetic predisposition of the intraspecific social parasitism of *A. m. capensis*, has been studied for decades. First, based on the results of back-cross experiments, a single recessive locus was suggested to control thelytokous worker reproduction (Ruttner 1988). For these experiments *A. m. capensis* queens were inseminated with a single drone produced by a hybrid queen (*A. m. capensis* x *A. m. carnica*) each, and were kept in mating nuclei. After having successfully produced female offspring, the queens were removed to allow their daughter workers to activate their ovaries and to produce offspring. As the sex ratio (percentage of females to males) of the laying workers' offspring from 110 test colonies followed a bimodal distribution, Ruttner (1988) assumed a single recessive locus control. After genetic tools like microsatellite markers became available, more controlled studies were possible. The first genetic study on the thelytoky control in *A. m. capensis* was done by Lattorff et al. (2005) who performed a double back-cross experiment with a hybrid queen (*A. m. capensis* x *A. m. carnica*) that was inseminated with two different drones (*A. m. capensis* and *A. m. carnica*) to generate laying workers (F2). Using microsatellite genotyping of the F2 laying mother workers and their brood, the mode of parthenogenesis of the laying workers was determined on the individual level. As the segregation of the two modes of parthenogenesis matched a Mendelian inheritance pattern (Fig. 3) and no amphitoky (mixed mode of parthenogenesis) was found, also Lattorff et al. (2005) assumed a single locus control with a recessive thelytoky allele.

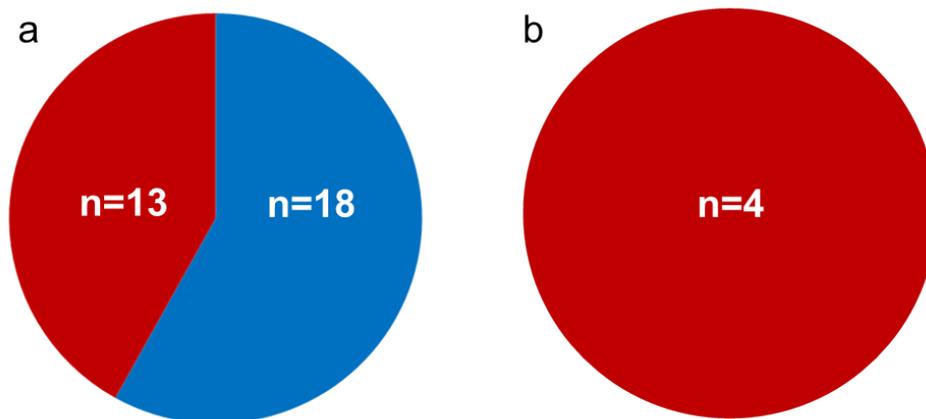


Fig. 3: The results of Lattorff et al. (2005). Given is the number of thelytokous (blue) and arrhenotokous (red) workers among the offspring of the two fathering drones, either (a) *A. m. capensis* or (b) *A. m. carnica*.

Based on the same back-cross mapping population (Lattorff et al. 2005) and a microsatellite QTL study using 546 microsatellite markers, the putative thelytoky locus (*th*) was mapped to chromosome 13 (Lattorff et al. 2007). This locus also seemed to control two additional reproductive traits of *A. m. capensis* workers, the queenlike mandibular gland secretion (amount of 9-ODA) and the early onset of egg-laying (Lattorff et al. 2007). RNAi knockdown experiments revealed that alternative splicing of exons 5 and 7 of the transcription factor *gemini*, a gene located within the target region on chromosome 13, correlated with the degree of ovary activation (Jarosch et al. 2011) and queenlike mandibular gland secretion (Jarosch et al. 2018) in *A. m. carnica* workers. The *thelytoky associated element* (*tae1*), which is a 9 bp deletion flanking the alternatively spliced exon 5 of *gemini* was suggested as the genetic switch controlling the entire process of differential splicing and thus, the mode of parthenogenesis (Jarosch et al. 2011). However, even though, *gemini* appears to be a key regulator of worker reproduction and the establishment of reproductive dominance, it is not controlling worker thelytoky (Chapman et al. 2015, Wallberg et al. 2016).

Moreover, recently, the single locus control of thelytoky was challenged by Chapman et al. (2015) and Wallberg et al. (2016), both claiming a multi-locus control. Chapman et al. (2015) performed various back-crosses using the two South African honey bee subspecies *A. m. capensis* and *A. m. scutellata*. Within these back-cross colonies, the heading queens were removed to allow worker reproduction. Microsatellite genotyping of the collected worker-laid brood was performed to determine the mode of parthenogenesis at the colony-level. As the deduced patterns of thelytoky and arrhenotoky did not segregate in a Mendelian fashion, Chapman et al. (2015) assumed a multi-locus control. The study of Wallberg et al. (2016) used a population-scale genome sequencing approach, comparing *A. m. capensis*, *A. m. scutellata* and *A. m. adansonii* workers. Twelve loci across the whole genome showed high genetic differentiation between *A. m. capensis* and the other two honey bee subspecies. Based on the assumption that all Cape honey bee workers reproduce thelytokously, these multiple loci were suggested to be linked to the thelytoky syndrome and thus, social parasitism in *A. m. capensis* (Wallberg et al. 2016). In summary, the genetic basis of the thelytoky syndrome has been intensely studied, but remains to be controversially discussed and ambiguous.

7 Aims of this thesis

The proximate pheromonal and behavioural mechanisms driving social parasitism in *A. m. capensis* are well understood, but the ultimate causes remain largely elusive. Why does this trait not spread any further? Which is/are the gene/s underpinning the social parasitism of *A. m. capensis*? The thelytoky syndrome provides the predisposition for the social parasitism and understanding the underlying genetic mechanisms is crucial, as it will provide insights into the genetic control of worker reproduction in *A. mellifera* and thus, in the regulation of altruism in eusocial colonies. A new concise mapping population from the Cape of Good Hope Nature Reserve needed to be established to identify the actual thelytoky controlling locus (Chapter I, pp. 15-33). Using microsatellite genotyping and whole genome sequencing in combination with a classical genetic mapping study, I can show whether thelytoky is controlled by a single or multiple loci (Chapter I, pp. 15-33, Chapter II, pp. 34-56). Eventually, also the actual locus controlling the mode of parthenogenesis could be identified (Chapter II, pp. 34-56). Finally, a population genetic model could be inferred, plausibly explaining the stability of the hybrid zone between *A. m. capensis* and *A. m. scutellata* and why thelytoky is not spreading further (Chapter II, pp. 34-56). As it strongly depends on the environment whether *A. m. capensis* workers develop into normal workers or reproductive dominant pseudoqueens, also the gene cascades associated with the transition from a social sterile worker into a reproductive dominant social parasite could be elucidated with my studies (Chapter III, pp. 57-83). Due to its multifaceted basis, this thesis will significantly contribute to the understanding of the genetic mechanisms underlying the social parasitism in *A. m. capensis*.

Thelytoky in Cape honeybees (*Apis mellifera capensis*) is controlled by a single recessive locus

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Abstract

Worker reproduction in *Apis mellifera* typically leads to haploid males produced via arrhenotokous parthenogenesis. An exception are laying workers of the South African Cape honeybee *Apis mellifera capensis*. Due to an abnormal spindle rotation during meiosis *A. m. capensis* workers are able to produce female progeny via thelytokous parthenogenesis. This trait has been suggested to be genetically controlled by a recessive allele at the thelytoky locus (*th*), but this conclusion was recently challenged by Chapman et al. (2015). To clarify the mode of inheritance for thelytokous parthenogenesis in Cape honeybees, we determined the sex of the offspring of 74 *A. m. capensis* workers of a single queen from a colony of the endemic wild population at the Cape of Good Hope Nature Reserve. When we tested individual worker reproduction, parthenogenesis was dimorphic, segregating in a Mendelian fashion supporting the single locus model. We could exclude maternal or paternal effects determining the mode of parthenogenesis. A careful re-analysis of the data of Chapman et al. (2015) also revealed that their data do not contradict the one locus model for the inheritance of thelytoky.

Keywords: thelytoky / worker reproduction / reproductive dominance / asexual reproduction / *gemi*

A single SNP turns a social honey bee (*Apis mellifera*) worker into a selfish parasite

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Abstract

In honey bee colonies, reproduction is monopolised by the queen that pheromonally suppresses ovary activation in her daughter workers. Without such pheromonal control, workers can lay unfertilized eggs that normally develop into males (arrhenotoky). However, some workers of the South African Cape honey bee (*Apis mellifera capensis*) can produce female offspring (thelytoky) and are phenotypically queen-like by possessing a high number of ovarioles, a spermatheca, queen-like pheromones and rapid ovary activation (thelytoky syndrome), allowing them to establish themselves as ‘pseudoqueens’ in queenless colonies and even to parasitically replace queens in foreign colonies. Using a joint mapping and population genomics approach, in combination with a time-course transcript abundance dynamics analysis, we show that a single nucleotide polymorphism (SNP) at the mapped thelytoky locus (*Th*) is associated with the iconic thelytokous phenotype. *Th* forms a linkage group with the gene *ecdysis triggering hormone receptor* (*ethr*) within a non-recombining region under strong selective constraints in the genome. A balanced detrimental allele system plausibly explains why the trait is specific to *A. m. capensis* and cannot easily establish itself into genomes of other honey bee subspecies.

Key words: *A. m. capensis* / thelytoky / worker reproduction / social parasitism / balancing selection

The transcriptomic changes associated with the development of social parasitism in the honeybee *Apis mellifera capensis*

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Abstract

Social insects are characterised by the division of labour. Queens usually dominate reproduction, whereas workers fulfil non-reproductive age-dependent tasks to maintain the colony. Although workers are typically sterile, they can activate their ovaries to produce their own offspring. In the extreme, worker reproduction can turn into social parasitism as in *Apis mellifera capensis*. These intraspecific parasites occupy a host colony, kill the resident queen and take over the reproductive monopoly. Because they exhibit a queenlike behaviour and are also treated like queens by the fellow workers, they are so-called “pseudoqueens”. Here we compare the development of parasitic pseudoqueens and social workers at different time points using fat body transcriptome data. Two complementary analysis methods – a principal component analysis and a time course analysis – led to the identification of a core set of genes involved in the transition from a social worker into a highly fecund parasitic pseudoqueen. Comparing our results on pseudoqueens with gene expression data of honeybee queens revealed many similarities. In addition, there was a set of specific transcriptomic changes in the parasitic pseudoqueens that differed from both, queens and social workers, which may be typical for the development of the social parasitism in *A. m. capensis*.

Keywords: worker reproduction / reproductive dominance / social parasitism / *Apis mellifera capensis* / thelytoky / pseudoqueen

General Discussion

The physiological characteristics of the thelytoky syndrome including swift ovary activation, queenlike pheromone production and thelytokous worker reproduction (Lattorff and Moritz 2013), enable *A. m. capensis* workers to explore the additional life history trajectory as social parasites. The proximate causes underlying the social parasitism in *A. m. capensis* are generally well understood. The ultimate reasons were controversially discussed and remained largely elusive for decades, but were elucidated in the course of this thesis. Based on a concise mapping population, the single locus control of thelytoky could be confirmed and both, the thelytoky controlling locus (*Th*) as well as the exceptional mode of inheritance, were identified. Also the transcriptomic changes associated with the facultative social parasitism, allowing *A. m. capensis* workers to transition from a social sterile worker into a reproductive dominant social parasite, were determined.

In addition, the results of this work provide explanations for two puzzling issues: 1) the presence of worker policing in *A. m. capensis* colonies and 2) the stability of the hybrid zone between *A. m. capensis* and *A. m. scutellata* and why thelytoky is not spreading into other honey bee populations, despite the various fitness benefits and selective advantages resulting from thelytokous worker reproduction.

1 Worker reproduction in *A. m. capensis*

In my studies, workers of a wild endemic *A. m. capensis* colony from the Cape of Good Hope Nature Reserve served as mapping population. For each worker of this mapping population, the mode of parthenogenesis was determined at the individual level. Microsatellite genotyping revealed that they either reproduced thelytokously or arrhenotokously, not significantly deviating from a 1:1 distribution (Chapter I, pp 15-33). In the past, it was assumed that all *A. m. capensis* workers reproduce thelytokously, as it was the only mode of parthenogenesis that was observed in queenless *A. m. capensis* colonies (Verma and Ruttner 1983, Hepburn and Crewe 1991, Hepburn et al. 1998, Neumann et al. 2000). However, these studies were done at the colony and not at the individual level and thus, they might have been biased by the pheromonal competition for the reproductive monopoly among *A. m. capensis* workers in queenless colonies (Moritz et al. 1996, 2000, 2002, 2004, Simon et

al. 2005). A single study reported about very rarely occurring arrhenotokous worker reproduction in queenright *A. m. capensis* colonies (<0.5% of drone brood, Beekman et al. 2009). Based on the predominating thelytokous worker reproduction, evolutionary theory predicted a reduction of worker policing in *A. m. capensis* colonies (Hamilton 1964, 1972, Greeff 1996), as there is no difference in the degree of relatedness between workers and their sisters (offspring of the queen) and their nieces (offspring of their sisters) (both $r=0.25$). Indeed, *A. m. capensis* colonies lacking worker policing have been described in the past (Moritz et al. 1999, Beekman et al. 2002). However, overall worker policing seems to be a common phenomenon in natural *A. m. capensis* colonies (Pirk et al. 2003, Beekman et al. 2002). In contrast to previous assumptions (Hepburn and Crewe 1991, Hepburn et al. 1998, Beekman et al. 2009), this study shows that arrhenotoky might be a common trait among *A. m. capensis* workers, leading to low degrees of intracolony relatedness ($r=0.125$ between workers and the offspring of their sisters) and thus, providing an explanation why worker policing is still present in *A. m. capensis* colonies (Beekman et al. 2002, Pirk et al. 2003), in addition to the theory that it enhances the colony's efficiency (Pirk et al. 2003). The frequent observation of worker produced female offspring (10.5% of all worker brood) in comparison to the rare observation of worker produced male offspring (0.48% of all drone brood) in queenright *A. m. capensis* colonies (Beekman et al. 2009), might indicate selective worker policing of arrhenotokously produced brood only.

2 Genetic mechanisms underpinning thelytokous worker reproduction in *A. m. capensis*

The finding of arrhenotokous worker reproduction in *A. m. capensis* gives rise to a major criticism of the study of Wallberg et al. (2016), which did not determine the mode of parthenogenesis of their experimental *A. m. capensis* workers, but assumed exclusively thelytokous worker reproduction in *A. m. capensis*. Based on the comparison of different honey bee subspecies, they deduced a multi-locus control of the social parasitism in *A. m. capensis* (Wallberg et al. 2016). However, the detected differences between *A. m. capensis* and the two other honey bee subspecies, *A. m. scutellata* and *A. m. adansonii*, are rather related to subspecies specific differences and not the mode of parthenogenesis. Similarly, the re-analysis of the data of Chapman et al. (2015), which was part of this thesis (Chapter I, pp. 15-33), showed that an incorrect data analysis led to the wrong conclusion of a multi-locus

control of thelytoky in *A. m. capensis*. Thus, both studies (Chapman et al. 2015, Wallberg et al. 2016) were lacking crucial experimental setups, which led to wrong assumption and their result of a multi-locus control of thelytoky in *A. m. capensis* can clearly be rejected.

In contrast to all previous studies on the genetic control of the thelytoky syndrome (Ruttner 1988, Lattorff et al. 2005, 2007, Jarosch et al. 2011, Chapman et al. 2015, Wallberg et al. 2016), in which back-cross experiments or comparisons of different honey bee subspecies were performed, in my experiments, a natural *A. m. capensis* colony with thelytokous and arrhenotokous workers served as mapping population. The two modes of parthenogenesis segregated in a Mendelian fashion in both, the whole colony and within each patriline, supporting the single locus control of thelytoky, as suggested by several previous studies (Ruttner 1988, Lattorff et al. 2005, 2007, Jarosch et al. 2011). In addition, any epigenetic mechanisms as well as maternal or paternal factors like imprinting could be excluded (Chapter I, pp. 15-33). As all experimental workers belong to the same subspecies and even to the same colony, the major difference between them is the mode of parthenogenesis. Whole genome sequencing of the laying workers in combination with a classical genetic mapping study, comparing thelytokous and arrhenotokous workers, led to the definite identification of the actual thelytoky controlling locus (*Thelytoky*, *Th*) on chromosome 1. *Th* was inferred to be an uncharacterised gene, which might encode a receptor protein with a signalling domain. A single SNP (single nucleotide polymorphism) within the 3' part of *Th* was identified to be associated with the mode of parthenogenesis, leading to an amino acid substitution from the polar amino acid threonine to the non-polar amino acid isoleucine and thus, substantial structural and likely functional changes of the encoded protein (Chapter II, pp. 34-56). Few similar cases where a single amino acid substitution causes major phenotypic changes are known to date. In maize (*Zea mays*) a single amino acid substitution in a gene that belongs to the SBP-box gene family of transcriptional regulators (*teosinte glume architecture 1*, *tga1*) was identified as origin for the conversion from encased to naked kernels (Wang et al. 2015). In the silk moth *Bombyx mori* L. a single amino acid substitution in a mucin like membrane protein is associated with resistance to the *Bombyx mori* densovirus type 1 (BmDV, Ito et al. 2018), which infects silkworm larvae and destroys their gut tissue (Watanabe et al. 1976). In

comparison to these transitions, the single amino acid substitution associated with worker thelytoky in *A. m. capensis* seems to be particularly exceptional, as it does not only cause a major phenotypic change, but it also opens an additional life history trajectory as social parasite for *A. m. capensis* workers, dramatically enhancing the individual's fitness with far reaching effects. The single amino acid substitution does not only directly affect *A. m. capensis* workers by increasing their fitness, but it also indirectly harms the *A. m. scutellata* host colonies that are destroyed by *A. m. capensis* workers of the parasitic clonal lineage (Kryger 2001, Neumann et al. 2002, Baudry et al. 2004, Neumann et al. 2011). As a result, it does also negatively impact humans who suffer dramatic colony losses (Allsopp and Crewe 1993, Pirk et al. 2014), which might reduce pollination efficacy and crop yield, which in turn might potentially cause loss of profits.

3 Evolutionary perspective on the genetic control of the social parasitism in *A. m. capensis*

3.1 The thelytoky locus under balancing selection

From the evolutionary perspective, this study demonstrated that *Th* lies within a non-recombining region under strong selective constraints in the *A. m. capensis* population, causing a drastically reduced allelic variability with only two alleles present. Following Occam's Razor "*Pluralitas non est ponenda since necessitate*", previous studies assumed a simple mode of inheritance and concluded a recessive thelytoky allele (Ruttner 1988, Lattorff et al. 2005, 2007, Jarosch et al. 2011, Chapter I, pp. 15-33), which was based on the observation of both modes of parthenogenesis among the offspring of crosses between *A. m. capensis* and *A. m. carnica* (Ruttner 1988, Lattorff et al. 2005, Fig. 3). Contradicting these previous assumptions (Ruttner 1988, Lattorff et al. 2005, 2007, Jarosch et al. 2011, Chapter I, pp. 15-33), the results of my classical genetic mapping study (Chapter II, pp. 34-56) revealed that the inheritance pattern of thelytoky in *A. m. capensis* is more complex than previously assumed. It has been shown that the thelytoky allele (Th_{Th}) is dominant and can only function in combination with the specific complementary arrhenotoky allele (Th_{ar}). All thelytokous workers were determined to be heterozygous (Th_{Th}/Th_{ar}) and all arrhenotokous workers to be homozygous (Th_{ar}/Th_{ar}) at *Th* (Fig. 4).

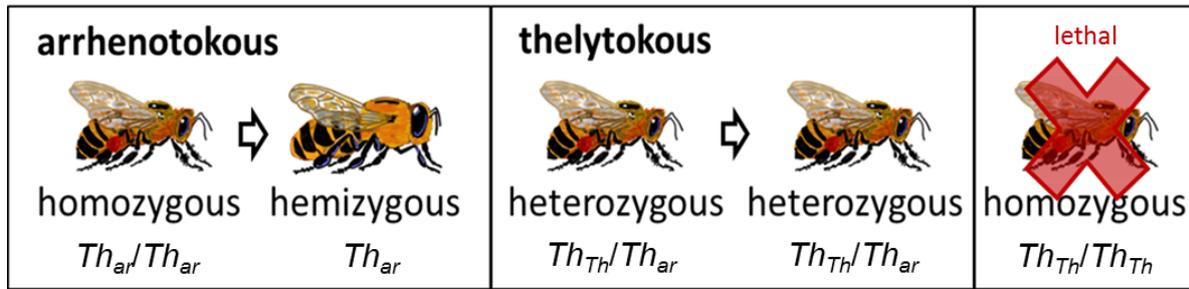


Fig. 4: The biallelic inheritance pattern of *Th* in arrhenotokous and thelytokous *A. m. capensis* workers and the genotypes of their offspring.

Balancing selection was inferred to maintain the equilibrium in the natural *A. m. capensis* population, favouring heterozygous individuals that have maximum fitness (heterozygote advantage/overdominance, Chapter II, pp. 34-56). In general, balancing selection acts on traits controlled by single or tightly linked loci (Charlesworth and Charlesworth 1975) and favours heterozygous individuals, which in turn might facilitate the evolution of dominance (Bourguet 1999). Overall, balancing selection seems to be a frequent evolutionary event as signatures of balancing selection have been described for different loci of many different taxa (Key et al. 2014, Llaurens et al. 2017). In honey bees, the only other known locus that is under balancing selection is the sex determining gene *csd* (*complementary sex determiner*, Hasselmann and Beye 2004), which is complementary determined and characterised by large allelic richness (87 alleles have been identified, Lechner et al. 2014). As in diploid individuals, heterozygosity is essential to result in fertile females, balancing selection favoured this great allelic richness at *csd* to increase the colony's fitness (Lechner et al. 2014).

One of the best studied loci under balancing selection in social insects, is the biallelic odorant binding protein gene *Gp-9* (*General protein-9*, alleles: *Gp-9B* and *Gp-9b*) of the eusocial fire ant *Solenopsis invicta* Buren, which regulates the colony's social organisation (monogyny or polygyny, Keller and Ross 1993, Ross and Keller 1995, 1998, Keller and Ross 1999, Krieger and Ross 2002) and thus, indirectly influences the mode of colony foundation, the colony's reproductive performance and aggression potential (Bourke and Franks 1995, Ross and Keller 1995). Individuals, homozygous for the recessive allele at *Gp-9* (*Gp-9b/Gp-9b*) are not viable (Ross 1997, Wang et al. 2013). Workers

homozygous for the dominant allele ($Gp-9B/Gp-9B$) and workers heterozygous at $Gp-9$ ($Gp-9B/Gp-9b$) differ in their binding properties of the queens' pheromones, enabling them to differentiate between homozygous ($Gp-9B/Gp-9B$) and heterozygous ($Gp-9B/Gp-9b$) queens (Keller and Ross 1998, Krieger and Ross 2002). Colonies consisting of workers that are all homozygous for the dominant allele ($Gp-9B/Gp-9B$) only tolerate a single queen that is also homozygous for the dominant allele ($Gp-9B/Gp-9B$). Colonies comprising both, workers that are heterozygous ($Gp-9B/Gp-9b$) and workers that are homozygous for the dominant allele ($Gp-9B/Gp-9B$) accept several queens as long as all of them are heterozygous at $Gp-9$ ($Gp-9B/Gp-9b$) (Fig. 5, Keller and Ross 1998, Ross and Keller 1998, Krieger and Ross 2002).

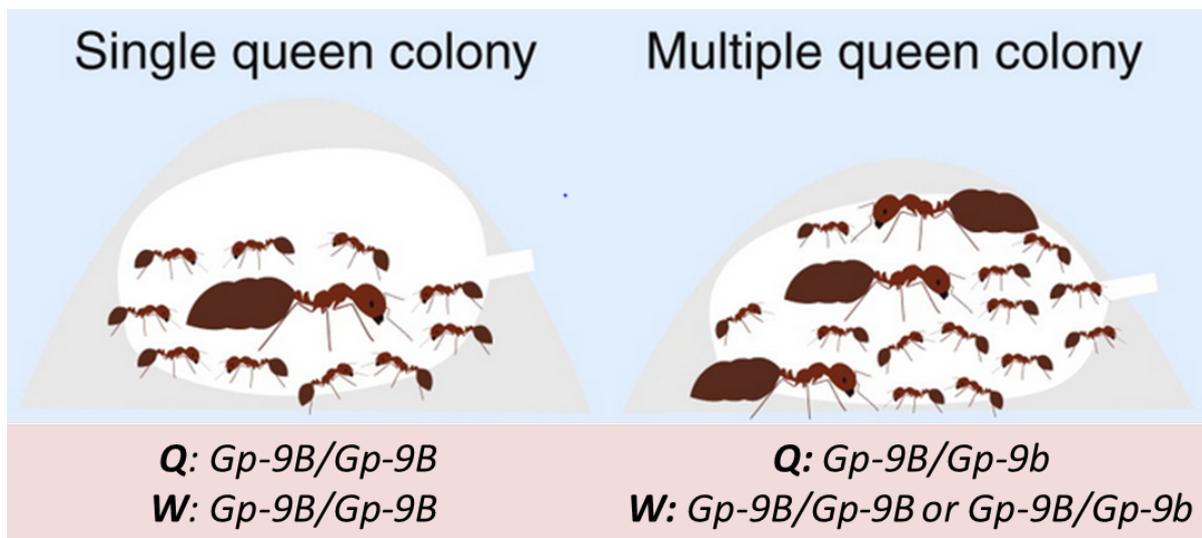


Fig. 5: The genotypes of queens (Q) and workers (W) in single and multiple queen colonies of the fire ant *S. invicta*. Figure modified from Libbrecht and Kronauer (2014).

3.2 The balanced detrimental allele system at *Th*

Like the gene $Gp-9$ in *S. invicta*, Th in *A. m. capensis* is characterised by a biallelic inheritance pattern. The single arrhenotoky allele (Th_{ar}) found in *A. m. capensis* appears to serve as ‘rescue allele’ for the thelytoky allele (Th_{Th}), which seems to be highly detrimental, if not even functionally sterile or lethal, in homozygous individuals (Th_{Th}/Th_{Th}) (Fig. 4). Any other combination was determined to result in non-functional (Th_{Th}/Th_{+}) or arrhenotokous phenotypes (Th_{ar}/Th_{+}), which is not just the result of this study but also in full agreement with all published previous crossing experiments of *A. m. capensis* with other honey bee subspecies (de Villiers 1883, Lattorff et al. 2005, Jordan et al.

2008a, Oldroyd et al. 2014, Chapman et al. 2015). Also the colonies 9 and 20 of the study of Chapman et al (2015) that were inconsistent with a single recessive thelytoky locus (as discussed in Chapter I, pp. 15-33) are compatible with a dominant thelytoky allele and the inferred balanced detrimental allele system.

The single arrhenotoky allele (Th_{ar}) found in *A. m. capensis* was only present at low frequencies in the adjacent *A. m. scutellata* population, as these two subspecies usually hybridize in the introgression zone (Hepburn and Crewe 1991, Hepburn et al. 1998, Dietemann et al. 2007). The absence of this allele in more distant *A. m. scutellata* populations and other honey bee subspecies, explains the unsuccessful attempts of breeding between *A. m. capensis* and other honey bee subspecies like *A. m. carnica* (de Villiers 1883, Lattorff et al. 2005) (Chapter II, pp. 34-56). For instance, in the experiment of Lattorff et al. (2005), among the offspring of the *A. m. capensis* drone 31 laying workers (13 arrhenotokous, 18 thelytokous) were found. But among the offspring of the *A. m. carnica* drone (both drones crossed with hybrid queen *A. m. capensis* x *A. m. carnica*) only four arrhenotokous workers were detected (Fig. 3). The absence of any thelytokous workers among the offspring of the *A. m. carnica* drone might be likely the result of an incompatibility of the thelytoky (Th_{Th}) and the arrhenotoky allele (Th_+) of the *A. m. carnica* drone.

The inferred balanced detrimental allele system in combination with the limited distribution of the thelytoky allele complementing arrhenotoky allele (Th_{ar}), plausibly explains the stability of the hybrid zone between *A. m. capensis* and *A. m. scutellata* and why thelytoky cannot easily establish itself into the genomes of other honey bee populations, which have been determined to be characterised by great allelic richness (various Th_+ alleles) at Th (Chapter II, pp. 34-56).

3.3 The thelytoky allele as a selfish element

Selfish genes or alleles cause beneficial phenotypes and thus, increase their probability to be transmitted to the next generation (Dawkins 1976). Given this definition, also the thelytoky allele (Th_{Th}) can be regarded as a selfish genetic element, as it controls worker thelytoky and thus, asexual reproduction which results in genetically identical offspring (Onions 1912, Moritz and Haberl 1994, Baudry et al. 2004), all carrying the thelytoky allele (Th_{Th}). Following Hamilton's inclusive fitness

theory (1964), the arrhenotoky allele (Th_{ar}) might act as altruistic antagonist to the thelytoky allele (Th_{Th}) and thus, its spread is also determined by its antagonistic function to the selfish genetic element. As in *A. m. capensis*, the colony's productivity is negatively correlated with the number of dominant bees (Hillesheim et al. 1989), even under queenright conditions (Korst and Velthuis 1982), colony level selection might contribute to the detected high frequency of Th_{ar} in the *A. m. capensis* population (Chapter II, pp. 34-56).

3.4 The transcriptomic changes associated with the social parasitism in *A. m. capensis*

Favoured by extreme selection, the social parasitic clonal lineage of *A. m. capensis* could evolve in the North of South Africa, seriously harming the endemic *A. m. scutellata* population (Neumann and Moritz 2002, Allsopp 1992, Pirk et al. 2014). Even though the *A. m. capensis* social parasite and the *A. m. scutellata* host belong to the same species and can potentially hybridise (Hepburn and Crewe 1991, Hepburn and Radloff 1998, Hepburn et al. 1998, Dietemann et al. 2007), sympatric reproductive barriers completely isolate them from one another. As the parasitic clonal lineage of *A. m. capensis* exclusively reproduces thelytokously (Baudry et al. 2004, Neumann et al. 2011), parasitic workers are not mated and male offspring is lacking. The parasite's female offspring can potentially develop into queens. However, gene flow and hybridisations rarely occur (Neumann et al. 2011). Thus, reproductive isolation in combination with extreme selection favoured the evolution of the highly virulent clonal lineage of *A. m. capensis*.

In general, social parasitism evolved many times in social insects of the order Hymenoptera (Schmid-Hempel 1998, Hölldobler and Wilson 1990). Usually, the social parasite and its host are closely related taxa (Emery's rule, Emery 1909) and most social parasites engage in reproduction (Wilson 1971). Thus, comparisons between social parasites and female hosts will gain insights into the evolution of social parasitism, for which two theoretical models have been suggested in the past (De Visser and Krug 2014, Cini et al. 2015, Smith et al. 2015). According to the "Phenotype Deletion Model", social parasitism evolved by suppressing the worker phenotype and only fostering the queen phenotype. The "Phenotype Shift Model" assumes that social parasites express new or modified phenotypes, different to both queens and workers (Cini et al. 2015). As the social parasitism of *A. m.*

capensis is facultative and strongly depends on the environment, it provides a model system in which individuals from the same taxa and from the same caste with the same physiological and genetic conditions can be compared. In my thesis, I took advantage of this beneficial system and used it to identify the transcriptomic changes associated with the transition from a sterile social worker into a reproductive dominant social parasite and compared them to both, queens and social workers. In the host colonies, *A. m. capensis* pseudoqueens resemble real queens and as expected, it has been shown that *A. m. capensis* pseudoqueens and queens are characterised by many similar gene expression patterns, such as for genes related to ovary activation, immune response and the antioxidative stress response. However, also parasite specific transcriptomic changes that might be associated with the developmental trajectories of social parasites, enabling them to outcompete the host queen and to gain the reproductive monopoly, have been determined. These results support a combination of the two theoretical models for the evolution of social parasitism, the “Phenotype Deletion Model” and “Phenotype Shift Model” (Cini et al. 2015), in *A. m. capensis* (Chapter III, pp. 57-83).

Similar to *A. m. capensis*, many other social parasitic ant, wasp and bee species are known that take over the reproductive monopoly in the host colony and pheromonally control the host workers to the benefit of their own offspring (Wilson 1971, Buschinger 1986, 2009, Carpenter and Perera 2006, Cervo 2006, Brandt et al. 2007, Brasero et al. 2018, Hines and Cameron 2010, Lhomme and Hines 2018). However, only a few comprehensive studies, addressing the genetic control of social parasitism, exist (Cini et al. 2015, Smith et al. 2015, Alleman et al. 2018). In social parasites of the ant genera *Pogonomyrmex* and *Vollenhovia*, full genome sequence analyses revealed no evidence for the “Phenotype Deletion Model” (Smith et al. 2015). However, within closely related species, phenotypic differences are more likely controlled by differential gene regulation than sequence changes or deletions of genes (Britten and Davidson 1969, King and Wilson 1975). Thus, studies about gene regulatory mechanisms (transcriptomic, proteomic, epigenetic studies or studies about regulatory elements like microRNAs) are required (Cini et al. 2015). In the well studied host-parasite system of the paper wasps *Polistes sulcifer* Zimmerman (obligate social parasite) and *P. dominula* Christ (host) (Choudhary et al. 1994), gene expression analyses revealed profound differences between the two

species, supporting the “Phenotype Shift Model” (Cini et al. 2015). However, the authors of this study acknowledge that their result is just preliminary and that full transcriptome analyses are required to unambiguously evaluate the evolutionary model of the social parasitism in this host-parasite-system (Cini et al. 2015). The present predominating lack of comprehensive studies about the genetic control of social parasitism additionally emphasizes the importance of my transcriptome studies, as they provide first insights into the evolution of social parasitism in social insects.

In conclusion, in the course of my thesis, I took advantage of state of the art next generation sequencing (NGS) techniques in combination with full genome and transcriptome analyses to unravel the ultimate causes underpinning the social parasitism of *A. m. capensis*, expanding the overall understanding of the evolution of social parasitism as well as of the evolution of sociality in general. In the studied system, a single SNP seemed to be sufficient to break down the evolutionary successful concept of sociality, impressively demonstrating that peculiar conditions, which break the random segregation of genes, can easily destroy even most robust evolutionary stable strategies.

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Appendix D – Curriculum Vitae

Personal information

Name: Denise Aumer
Date of Birth: 05/07/1987
Place of Birth: Pegnitz, Germany
Nationality: German

Education

- 2013-present** **PhD candidate**, Martin-Luther-Universität Halle-Wittenberg, Germany
Advisor: Prof Dr Dr h. c. Robin F. A. Moritz
- 2012** **MSc Biology**, Universität Leipzig, Germany
Focus: Evolution of Animals, Genetics
Master thesis: “Investigations on the horizontal transmission of *Wolbachia* in bees”
Reviewers: Prof Dr Martin Schlegel, Prof Dr Christoph Bleidorn
- 2010** **Bsc Biology**, Universität Bayreuth, Germany
Focus: Ecological and Organismic Biology
Bachelor thesis: “Effects of region, plant diversity and crops on the reproductive success of the Red Maso bee *Osmia bicornis*”
Reviewers: Prof Dr Ingolf Steffan-dewenter, Prof Dr Konrad Dettner
- 2006** **A-Level (Abitur)**, Markgräfin-Wilhelmine-Gymnasium, Bayreuth, Germany

Research visits

- 02/16-05/16** **University of Pretoria**, Pretoria, South Africa (lab of Prof Dr Christian W. W. Pirk)
- 10/15-12/15** **University of Pretoria**, Pretoria, South Africa (lab of Prof Dr Christian W. W. Pirk)

08/13-12/13 **ARC Plant Protection Research Institute**, Stellenbosch, South Africa (lab of Dr Mike H. Allsopp)

Internships and Courses

2017 **Orthology Workshop**, Westfälische Wilhelms-Universität Münster, Germany

2016 **Transcriptome analyses Workshop**, Universität Freiburg, Germany
Introduction to Bioinformatics, Westfälische Wilhelms-Universität Münster, Germany

2015 **Life History Modelling Workshop**, University of Groningen, Netherlands

Conference presentations

2017 **XVI. ESEB** (European Society for Evolutionary Biology), Groningen, Netherlands

2016 **6th European Meeting of the IUSSI** (International Union for the Study of Social Insects), Helsinki, Finland
7th EurBee (European Association for Bee Research) Congress, Cluj Napoca, Romania

2015 **4th Central European Meeting of the IUSSI** (International Union for the Study of Social Insects), Lichtenfels, Germany

2014 **6th EurBee** (European Association for Bee Research) Congress, Murcia, Spain
61. Jahrestagung der Arbeitsgemeinschaft der Institute für Bienenforschung, Marburg, Germany

2013 **60. Jahrestagung** der Arbeitsgemeinschaft der Institute für Bienenforschung, Würzburg, Germany
Erster Afrikatag, Halle/Saale, Germany

Appendix E – Publication List

Peer reviewed publications:

Hertel J, Bartschat S, Wintsche A, Otto C, **the students of the bioinformatics computer lab**, Stadler PF (2012): Evolution of the let-7 micro-RNA family. *RNA Biology* **9**: 1-11

Rueppell O, **Aumer D**, Moritz RFA (2016): Ties between ageing plasticity and reproductive physiology in honey bees (*Apis mellifera*) reveal a positive relation between fecundity and longevity as consequence of advanced social evolution. *Curr Opin Insect Sci* **16**: 64-68

Aumer D, Allsopp MH, Lattorff HMG, Moritz RFA, Jarosch-Perlow A (2017) Thelytoky in Cape honeybees (*Apis mellifera capensis*) controlled by a single recessive locus. *Apidology* **48**: 401-410

Aumer D, Mumoki FN, Pirk CWW, Moritz RFA (2018): The transcriptomic changes associated with the development of social parasitism in the honeybee *Apis mellifera capensis*. *Naturwissenschaften* **105**: 22

Submitted publications:

Aumer D, Stolle E, Allsopp M, Mumoki FN, Pirk CWW, Moritz RFA (*submitted*): A single SNP turns a social honeybee (*Apis mellifera*) worker into a selfish parasite. *PNAS*

Appendix F – Eidesstattliche Erklärung

Halle, den 31.07.2018

Hiermit erkläre ich an Eides statt, dass diese Arbeit von mir bisher weder bei der Naturwissenschaftlichen Fakultät I – Biowissenschaften – 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 selbstständig und nur unter Zuhilfenahme der angegebenen Quellen und Hilfsmittel angefertigt habe. Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen sind als solche kenntlich gemacht worden.

Denise Aumer