Causes and Consequences of Genetic Variability on Sociobiological and Host-Parasite Interactions in Bumblebees

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Frau Anett Huth-Schwarz

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Gutachter /in

1. Prof. Dr. Robin FA Moritz

2. PD Dr. Josef Settele

3. Dr. Juliet Osborne

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Dedicated to

Alexander Schwarz

and my Family

Contents

Chapter 1 - Introduction

Bumblebees are a group of eusocial Hymenoptera which are of great ecological importance due to the pollination services they provide. Besides their role as pollinators in natural ecosystems, bumblebees became more and more important in agricultural ecosystems in recent years where they are increasingly used in green house pollination and bred on an industrial scale (Banda and Paxton 1991; Corbet et al. 1993; Van Doorn and Velthuis 2004). Even though commercially kept bumblebee populations prosper due to this increased usage in agriculture, natural bumblebee populations seem to be affected by the so called "global pollinator decline" (Biesmeijer et al. 2006; Gallai et al. 2009; Potts et al. 2010; Cameron et al. 2011; Ghazoul 2005; Goulson et al. 2010; Williams and Osborn 2009). This is not only a problem for biodiversity conservation and ecosystem functioning but also for the global agricultural sector and food production, as natural bumblebees also provide pollination services in this context. Several factors have been identified to play a key role in worldwide pollinator decline, and increased levels of parasitism are thought to be among the most important drivers (Goulson et al. 2005; Meeus et al. 2011; Potts et al. 2010). Due to their ecological and economic importance bumblebees have gained strong attention by many researchers in the fields of ecology, social insects and genetics in the last decade.

1.1 Classification and distribution

Bumblebees are primitively eusocial Hymenoptera from the family Apidae, tribe Bombini comprising about 250 species and subspecies in 15 suggested subgenera (Williams et al. 2008). They are distributed over five continents and occur in the Western Palaearctic, the Eastern Palaearctic, Oriental, Nearctic and Neotropical region (Williams et al. 2008). Due to their endothermy, bumblebees are primarily distributed in temperate, alpine and arctic zones, as they can regulate their body temperature and thus are active also under harsh climate conditions (Heinrich 1972). Despite of the majority of species living in temperate regions, some species are found in the Tropics of South-East Asia and Central and South America. The colony size of bumblebees is rather small, ranging from about 50 to 2,000 individuals per colony (Sladen 1912; Plath 1934; Michener and LaBerge 1954). Since bumblebees feed on nectar and pollen they are an important group of pollinators in natural as well as agricultural ecosystems (Banda and Paxton 1991, Corbet et al. 1993, Van Doorn and Velthuis 2004).

1.2 Life cycle

The vast majority of bumblebees exhibit an annual life cycle. After emerging from hibernation, the queen seeks to find a suitable site for nest foundation. The first batch of workers is reared by the queen. Brood rearing depends on temperature and food supply, and normally lasts four to five weeks (Alford 1975; Alaux et al. 2004; Cnaani et al. 2002; Duchateau and Velthuis 1988). During pupation of the larvae of the first batch, the queen lays the eggs of the next worker batch. Once the first workers have emerged as adults, the queen stops foraging and stays in the nest. Tasks like foraging and guarding are taken over by some workers while others help the queen to care for the subsequent batches of brood. Over the next weeks the colony increases in size until the production of males and gynes at the end of the season. This moment in colony development has also been termed the "switching point". There are early and late switching colonies and the early switching ones produce mostly males whereas the latter produce mostly gynes (Duchateau and Velthuis 1988; Bourke and Ratnieks 2001).

Some studies indicate that only a few colonies are able to produce reproductives (Cumber 1953; Müller and Schmid-Hempel 1992). It also seems that nest size determines the production of males and gynes. Small colonies often fail to produce any reproductives at all, and only medium- to large-sized colonies produce only males or both males and gynes (Schmid-Hempel 1998). In this last stage of the colony cycle, frequent conflict arises over male production between the queen and the workers. The workers, although unmated, are still able to parthenogenetically produce male offspring. This point in the colony life cycle has been termed the "competition phase" (Duchateau and Velthuis 1988). The young queens leave the nest to forage to increase their body fat. In contrast to these queens, which return regularly to the nest, the males leave the colony once they are sexually mature and do not return to the nest. After mating, the queens hibernate (period of dormancy) to start a new colony in the following year.

1.3 Sex determination

In bumblebees the sex of a given individual is determined by a complementary sex determination system (CSD). If individuals are hemi- or homozygous at the sex-determining locus they develop into males, when they are heterozygous they develop into females. One

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possibility of sex determination in Hymenoptera is the single-locus complementary sex determination (sl-CSD) system (Heimpel and de Boer 2008). If the offspring is diploid but homozygous at the sl-CSD, diploid males develop that are both unviable and sterile or have diploid sperms (Zayed 2009). This genetic load may trigger a "diploid male vortex" leading ultimately to extinction in isolated populations with small sizes or low reproductive rates (Zayed and Packer 2005; Zayed 2009). But to initiate "diploid male vortex" rather specific conditions are required and already extremely low dispersal rates would be sufficient to guarantee the long term survival of a population (Hein et al. 2009). It has been suggested that at least 46 sex determining alleles can be found in a given population of bumblebees (Duchateau et al. 1994). Usually a diploid fertile queen heads a bumblebee colony and produces a) fertilized eggs developing into diploid female offspring, either as virgin queens or as sterile workers and b) unfertilized eggs that develop into haploid male offspring. In natural populations that have a low number of CSD alleles (e.g. due to population fragmentation and reduced genetic diversity) the probability to produce diploid males is increased. It could be repeatedly shown that diploid males do occur in natural bumblebee populations (e.g. Takahashi et al. 2008a; Whidden and Owen 2011).

1.4 Mating frequency and genetic structure of the colony

In social Hymenoptera single mating seems to be the common mating strategy whereas multiple mating appears to be a rare trait and restricted to more derived, highly eusocial Hymenoptera like honeybees, some wasp species, leafcutter ants or army ants (Strassmann 2001). In monogynous social Hymenoptera mating with only one male results in high intracolonial relatedness ($g = 0.75$) (Crozier and Pamilo 1996) which is considered as one of the main factors contributing to the evolution of sociality (Hamilton 1964a, b). Thus, the occurrence of polyandry in social Hymenoptera has gained strong attention from researchers during the last decades (Moritz et al. 1995; Boomsma and Ratnieks 1996; Schmid-Hempel and Schmid-Hempel 2000; Foster and Ratnieks 2001; Payne et al. 2003). Several hypotheses have been put forward to explain the evolution of polyandry as it has not only advantages but also some drawbacks that are excellently reviewed in Strassmann (2001). Possible costs associated with multiple mating are the increased risk of predation, possible injuries by males, additional costs in form of time and energy used for mating flights, reduced female longevity resulting from disease transmission. The advantages of multiple mating in Hymenoptera are

reviewed by Crozier and Fjerdingstad (2001) and could lie e.g. in the compensation of sperm limitation (sperm limitation hypothesis; Cole 1983) or in the increase of the genetic diversity of the colony (herd immunity hypothesis; Schmid-Hempel 1994, genetic polyethism hypothesis; Page et al. 1989). Increased genetic diversity among the offspring could increase pathogen resistance and facilitate task differentiation. However there could be some female choice mechanisms for pre- or post-copulatory discrimination between the males. The advantage could be that the female could choose e.g. the male that has the best genetic match to herself, resulting in a kind of single mating (genetic compatibility hypothesis; Tregenza and Wedell 2000) but requiting post-copulatory discrimination mechanisms have not been proven for social Hymenoptera (Strassmann 2001).

The majority of bumblebee species is monogynous with so far the only exception being *B. atratus*, where the colonies have phases of polygyny (Zucchi 1973) in which the sister queens fight for reproductive dominance (Cameron and Jost 1998; Gonzales et al. 2004). 73 % of the bumblebee species sampled in the wild, which were studied with microsatellites, are singly mated (see Table 1), the remaining 27 % were at least double mated with the siring males contributing unequally to the offspring resulting in an effective mating frequency that is explicitly lower than the observed one (Starr 1984). It seems that monandry is the common mating pattern in bumblebees. The mating frequency of bumblebees seems to depend on various factors (Goulson 2010). Based on the above mentioned theories, it should be in the interest of the queen to mate multiply (herd immunity hypothesis; Schmid-Hempel 1994, sperm limitation hypothesis; Cole 1983) but bumblebee nests last much shorter and are much smaller than e.g. honeybee nests, which questions the need for an increased immunity and for increased sperm storage. The males should try to monopolize a queen to prevent additional matings. Sauter et al. (2001) and Baer et al. (2001) found that male bumblebees in *B. terrestris* apply a so called mating plug, which includes linoleic acid and prevents females from re-mating. Also the mating duration seems to be an important factor besides environmental conditions to facilitate monoandry as the mean mating duration in bumblebees appears to be about 36-44 minutes (Goulson 2010), increasing e.g. the risk of predation. In *B. terrestris* most of this time is used to deposit the mating plug (Duvoisin et al. 1999) which last for three days (Sauter et al. 2001). In the facultative polyandrous bumblebee *B. hypnorum* the mating plug is less effective and just lasts for 6-12 hours (Brown et al. 2002).

Table 1.1. Literature data on the mating frequency and effective mating frequency of bumblebee species studied with molecular tools

Species	Number of fathers	Effective mating frequency (m_e) (Starr 1979)	literature
B. auricomus Robertson	1	1.00	Payne et al. 2003
B. affinis Latreille *S. str.*	1	1.00	Payne et al. 2003
B. fervidus Skorikov	1	1.00	Payne et al. 2003
B. griseocollis Frison	1	1.00	Payne et al. 2003
B. bimaculatus Dalla Torre	1-2	1.00-1.25	Payne et al. 2003
B. impatiens Dalla Torre	1-3	1.00-1.61	Payne et al. 2003
B. mixtus Dalla Torre	4	3.75	Payne et al. 2003
B. ternarius Dalla Torre	2	2.04	Payne et al. 2003
B. vagans Dalla Torre	1	1.00	Payne et al. 2003
B. citrinus Lepeletier	2-3	1.25-3.00	Payne et al. 2003
B. insularis Lepeletier	1	1.00	Payne et al. 2003
B. hypnorum Linnaeus	1-6	1.00-3.13	Paxton et al. 2001
B. hypnorum Linnaeus	1 and more	1.00-1.46	Schmid-Hempel and Schmid-Hempel 2000
B. hypnorum Linnaeus	2-4	1.98-3.46	Estoup et al. 1995,
B. terrestris Linnaeus	1	1.00	Estoup et al. 1995, Schmid-Hempel and Schmid-Hempel 2000
B. lucorum Linnaeus	1	1.00	Estoup et al. 1995, Schmid-Hempel and Schmid-Hempel 2000
B. pratorum Linnaeus	1	1.00	Estoup et al. 1995,
B. lapidaries Linnaeus	1	1.00	Estoup et al. 1995, Schmid-Hempel and Schmid-Hempel 2000
B. sichelii Radoszkowski	1	1.00	Schmid-Hempel and Schmid-Hempel 2000
B. hortorum Linnaeus	1	1.00	Schmid-Hempel and Schmid-Hempel 2000
B. pascuorum Scopoli	1	1.00	Schmid-Hempel and Schmid-Hempel 2000
B. ignites Smith	1	1.00	Takahashi et al. 2008a
B. florilegus Panfilov	1	1.00	Takahashi et al. 2008b
B. deuteronymus Schulz	1	1.00	Takahashi et al. 2010
B. ardens Smith	1	1.00	Kokuvo et al. 2009
B. diversus Smith	1	1.00	Kokuvo et al. 2009
B. honshuensis Tkalcu	1	1.00	Kokuvo et al. 2009
B. wilmattae Cockerell	1-2	1.00-1.70	Huth-Schwarz et al. 2011a

Despite of the majority of bumblebee species which occur in temperate, alpine and arctic zones there are also 14 subgenera which are found in the Tropics of South-East Asia and Central and South America (Williams et al. 2008). The bumblebee species that have established themselves in tropical regions are especially interesting to study as they have to cope with a potentially higher parasite and predator pressure typical for tropical regions - as could be shown for various other taxa (Moller 1998; Nunn and Altizer 2005; Poulin and Rohde 1997; Cumming 2000; Poulin and Morand 2004). Thus tropical bumblebee species might provide a good test system for some of the genetic variability hypotheses for the evolution of multiple mating like the herd immunity hypothesis (Schmid-Hempel 1994).

Information about the mating behaviour of tropical *Bombus* species were only available for *B. atratus* (Garofalo et al. 1986) until recently, where polyandrous mating behaviour was described with up to three matings per queen based on observational data only. However these observations were conducted under highly artificial experimental conditions. Thus, it remains unclear whether multiple mating also occurs under natural conditions, since also behavioural observations in *B. terrestris* proofed less reliable than those based on genetic analyses (Röseler 1973; Estoup et al. 1995; Schmid-Hempel and Schmid-Hempel 2000). The data on the tropical bumblebee *B. willmattae* presented in this thesis is thus the first molecular data set on the mating biology of a tropical bumblebee species.

1.5 Cooperation and conflict

Besides potentially influencing parasite resistance or division of labour the average relatedness among colony members also influences intracolonial conflict and cooperation. A consequence of the haplo-diploid system is that full sisters have an average relatedness of $r = 0.75$ to each other, but to their own female offspring just an average relatedness of 0.5. Based on the high relatedness between supersisters in social haplodiploid species workers should prefer to help their mother to raise more sisters instead of producing their own female offspring (Hamilton 1964a; Hamilton 1972). In the majority of eusocial hymenopteran species the queen is typically the only fertile female that produces both diploid female and haploid male offspring, exhibiting reproductive dominance in the colony. In many species workers are normally sterile and unable to mate but they occasionally activate their ovaries to produce haploid male offspring thus retaining a considerable reproductive capacity (Wilson 1971). Following kin selection theory (Hamilton 1964a, b) it would be best for workers in mongynous and monandrous colonies to produce their own sons ($r = 0.5$) rather than helping to raise their brothers ($r = 0.25$) (Figure 1). Bumblebees are typically monogynous and monandrous (Estoup et al. 1995; Schmid-Hempel and Schmid-Hempel 2000) and a conflict between queen and workers over male production would be facilitated (Hamilton 1972). In evolutionary terms worker reproduction is highly important to ensure gene transfer to the next generation, in case the colony loses the queen and cannot replace the gyne. Worker reproduction can be found in all larger taxa of the eusocial Hymenoptera, in ants, wasps and bees, including bumblebees (Bombini) (Bourke 1988; Hammond and Keller 2004; Ratnieks 1988; Wenseleers and Ratnieks 2006; Williams et al. 2008). In the highly eusocial honeybee (*Apis mellifera*) worker reproduction in queen right colonies is suppressed by the queen,

brood pheromones (Winston and Slessor 1998) and by worker policing. Worker policing is thought to be a consequence of the high queen polyandry which results in low intracolonial relatedness and mutual egg destruction by workers between different half-sibling subfamilies (Ratnieks 1988). Thus, just <1% of the male offspring is worker produced in queen right honeybee colonies (Foster and Ratnieks 2001; Wenseleers et al. 2004).
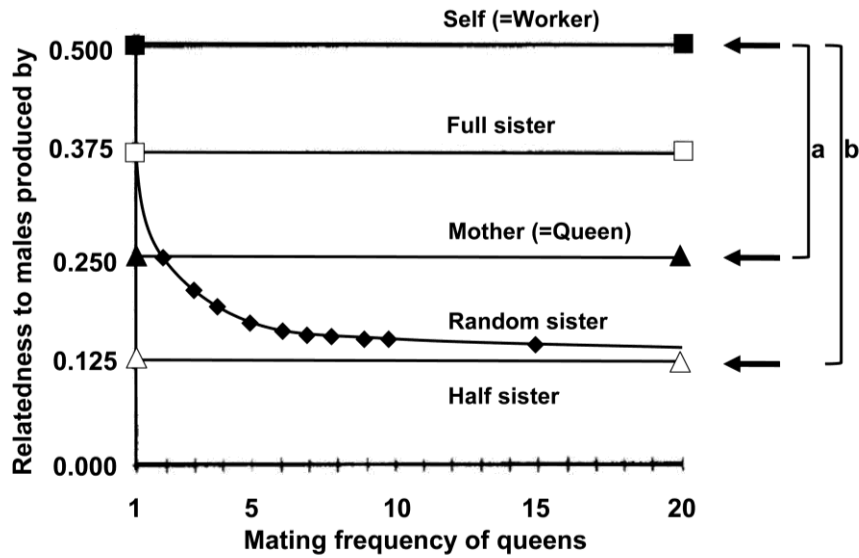

Figure 1. Relatedness between a worker (self) and possible reproductives in haplo-diploid social Hymenoptera (Figure from Ratnieks 1988). (a) Queen-worker conflict over male production is predicted to occur if the worker relatedness (g_{ww}) is above 0.5 and the effective mating frequency (m_e) is below 2. (b) Worker-Worker conflict over male production is predicted to occur if the worker relatedness (g_{ww}) is above 0.5 and the effective mating frequency (m_e) is below 2, although in mongynous and monandrous colonies of social Hymenoptera also other factors than relatedness could cause such a conflict.

In bumblebees, this queen-worker conflict over reproduction has been first described more than 80 years ago (Plath 1923) and most studies so far were performed with *Bombus terrestris* (Alaux et al. 2004; Duchateau and Velthuis 1989; Lopez-Vaamonde et al. 2004; Van Doorn and Heringa 1986). After the switch point in colony life cycle of *B. terrestris* the queen ceases the production of sterile workers and starts producing reproductives. With the onset of male production (competition phase) the queen and workers could exhibit aggressive behaviour towards each other (e.g. destroying egg shells, egg eating, buzzing, attacking, matricide) to maintain or gain reproductive dominance (Van Doorn and Heringa 1986, Van Honk et al. 1981). In spite of this change in worker behaviour, the worker male parentage remains modest with less than 5 % (Alaux et al. 2004, Lopez-Vaamonde et al. 2004) and the queen dominates

male production in *B. terrestris*. In most of the bumblebee species studied so far, the degree of male production by workers in queen-right colonies varied between 5 % and 20 % and reaching a maximum with 50 % (Table 2; Alaux et al. 2004; Lopez-Vaamonde et al. 2004; Takahashi et al. 2008a; Cnaani et al. 2002; Owen and Plowright 1982; Brown et al. 2003; Takahashi et al. 2010).

Table 1.2. Literature data on male production by workers in queen-right colonies of bumblebees studied with molecular tools

species	% males produced by workers	literature
B. impatiens Dalla Torre	9	Cnaani et al. 2002
B. hypnorum Linnaeus	20	Brown et al. 2003
B. terrestris Linnaeus	5	Alaux et al. 2004, Lopez-Vaamonde 2004
B. ignitus Smith	5	Takahashi et al. 2008a
B. deuteronymus Schulz	≤50	Takahashi et al. 2010
B. melanopygus Nylander	19	Owen and Plowright 1982
B. wilmattae Cockerell	85	Huth-Schwarz et al. 2011b

There are two nonexclusive hypotheses on how the queen reaches reproductive dominance. The queen could achieve this via producing a queen pheromone (Duchateau and Velthuis 1988). But queen reproductive dominance could also be controlled just by gains of inclusive fitness by the workers. As long as the queen produces female offspring, workers gain inclusive fitness by helping their mother raising their full sisters. Male production in the early colony cycle would slow colony growth and due to that the production of full sisters (Bourke and Ratnieks 2001). One particular reason for low worker reproduction might be the so called policing behaviour, where workers destroy the eggs laid by other workers, often in association with aggressive behaviour of laying as well as non-laying workers towards each other (Van Doorn and Heringa 1986; Van Honk et al. 1981).

In general, kin selection theory predicts worker policing (Figure 1) to be primarily adaptive in multiple mated species, if intracolonial relatedness is below the critical threshold of r = 0.5 and the effective mating frequency is larger than 2, as already described in *Apis mellifera* (relatedness hypothesis; Starr 1984; Ratnieks 1988). However, worker policing and the absence of worker produced males in general, also occur in single mated species, like in the hornet *Vespa crabro* (Foster et al. 2002) or in the ant *Camponotus floridanus* (Endler et al. 2004) and thus also other factors besides relatedness seem to be of importance (cost hypothesis; Ratnieks 1988). Among these factors are colony size, worker dominance behaviour, colony level costs of worker reproduction and interaction with the queen-worker conflict over the sex ratio leading to a more female biased sex ratio (Wenseleers and Ratnieks 2006). These factors are all in accordance with kin selection theory. A model developed by

Ohtsuki and Tsuji (2009) indicates that worker policing and reproduction depends also on colony growth and development stage. If a colony is in its ergonomic phase (Oster and Wilson 1978) and it is a small colony, then worker reproduction should be self-restraint. Furthermore, also queen policing of worker laid eggs can suppress the reproduction of workers in single mated species with small colony sizes, like it is the case in the paper wasps *Polistes dominulus* (Liebig et al. 2005) and *Polistes chinensis antennalis* (Saigo and Tsuchida 2004) and in the bumblebee *B. terrestris* (Duchateau and Velthuis 1989).

1.6 Bumblebee host genetics and its implication for parasites

Bumblebees as social insects are highly prone to parasites, since living in colonies with many individuals at very close space (Schmid-Hempel 1998) enables fast parasite transmission among the members of a colony (Durrer and Schmid-Hempel 1994; Imhoof and Schmid-Hempel 1999; Lopez-Vaamonde et al. 2004; Sakofski 1990). The defence of social insects against parasites and pathogens proceed on the individual- as well as the group-level. The individual immune defence of social insects is very well studied in the eusocial honeybee. It is based on the innate immune system comprising four immune pathways (Toll, Imd, JNK and JAK/STAT) that show a lack of immune genes compared to *Drosophila melanogaster* and *Anopheles gambiae* (Evans et al. 2006). This lack of immune genes seems to be compensated by group level defence mechanisms described as social immunity (Cremer et al. 2007) like hygienic and social behaviour, social fever and the avoidance of contaminated resources (Fouks and Lattorff 2011).

Bumblebees are parasitized by many species which could be differentiated in seven major parasite groups depending on their population size and generation time (Erler 2012) - among them the intracellular microsporidian parasites *Nosema bombi* (Otti and Schmid-Hempel 2007; Otti and Schmid-Hempel 2008; Rutrecht and Brown 2009) and *Nosema ceranae* (Plischuk et al. 2009; Li et al. 2012) belonging to the group of fungi. Their transmission occur intra- and intercolonial horizontally (Durrer and Schmid-Hempel 1994; Imhoof and Schmid-Hempel 1999; Goodwin et al. 2006; Lopez-Vaamonde et al. 2004; Neumann et al. 2000) and vertically (Betts 1920; Skou et al. 1963; Rutrecht and Brown 2008).

In case of bumblebees and other social insects host genetic variability is, besides the innate immune system and social immunity, one important trait to cope with parasitism and could affect parasitism at various levels of host organismal complexity. This complexity ranges

from the individual over the colony up to population level and several hypotheses have been developed to explain and understand the interaction of host and parasite at each of these levels of organismal complexity. On the individual level, the ploidy of the host seems to influence rates of parasitism. Like in all Hymenoptera the males of bumblebees are haploid and therefore have a reduced allelic variability compared to workers. This so called haploid susceptibility hypothesis (O'Donnell and Beshers 2004) assumes that due to the lack of allelic diversity males are more prone to parasites and pathogens than females. While this hypothesis seems intuitively appealing, the results gained for several bumblebee species and their parasites were diverse. Whereas the prevalence of *N. bombi* in bumblebee species from Europe and North-America sampled from natural populations is higher in males than in females (Gillespie 2010; Shykoff and Schmid-Hempel 1991), Rutrecht and Brown (2009) found no significant differences between the two sexes under laboratory conditions. Also Ruiz-González and Brown (2006) did not find differences between the sexes in an experimental study on *B. terrestris* and its parasite *Crithidia bombi*. The colonies and bees, respectively, used under laboratory conditions were kept under a constant temperature and light regime with ad libitum food supply. In natural population such conditions are never met and variation in food supply is expected to be high, especially for the males which permanently leave the nest after maturing and thus might not be able to compensate food shortage by using nest food resources like workers. Besides the haploid susceptibility hypothesis there are also alternative explanations for sex specific immunity. E.g. Bateman's principle is based on the assumption that males and females have sexually dimorphic life-history strategies (Bateman 1948). The immunocompetence handicap hypothesis (Folstad and Carter 1992) and other related hypotheses (Sheldon and Verhulst 1996) predict that endocrine regulation in adults differs between the sexes resulting in different directions of immunity (reviewed in Rolff 2002). However, it is unclear to what extend these alternative hypotheses are applicable to bumblebees or Hymenoptera in general.

Polyandry by queens of eusocial Hymenoptera directly affects colony level genetic variability (see above). One of the main hypotheses for the evolution of polyandry was developed suggesting that the increased within-colony genetic variability, caused by polyandry, results in an enhanced resistance towards parasites (Schmid-Hempel 1994; Sherman et al. 1988; Keller and Reeve 1994; Mattila and Seeley 2007). On the colony level Baer and Schmid-Hempel (1999) found that high genetic variability significantly decreases the parasite load in *B. terrestris* colonies headed by artificially inseminated queens.

There are two factors that influence parasitism on the population level: host density and genetic variability measured as overall host heterozygosity. It seems that high host genetic variability is not only important at the colony but also at the population level. In declining or isolated bumblebee populations of several species, genetic variability was negatively correlated with parasite prevalence (Cameron et al. 2011; Whitehorn et al. 2011). In social insects the number of colonies (= the number of reproductive queens) rather than the number of sterile workers determines the genetically relevant population size. High host densities are predicted to lead to both high virulence and high transmission rates (Bull, 1994), as suggested for honeybees (Fries and Camazine 2001) as well as bumblebees (Meeus et al. 2011). High pathogen loads have been repeatedly found in commercially bred bumblebee colonies (Colla et al. 2006; Goka et al. 2006).

1.7 Aims of this work

Bumblebees as social insects are an excellent model for studying the impact of genetic variability on various levels of organismal complexity. In this thesis three major topics of bumblebee biology are addressed, ranging from the mating behaviour of queens, the genetic colony structure and queen-worker conflict up to the impact of bumblebee host genetic variability on host-parasite interactions.

These topics are:

How does the mating frequency influence the genetic colony structure in bumblebees?

How does colony genetic variability influence cooperation and conflict in bumblebee societies?

How does host-genetic variability interact with parasite prevalence and degree of parasite infection at various levels of host organismal complexity in bumblebees?

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Chapter 2

Mating frequency and genetic colony structure of the neotropical bumblebee *Bombus wilmattae* (Hymenoptera: Apidae)

Anett Huth-Schwarz[1], Adolfo León[2], Rémy Vandame[3], Robin F. A. Moritz[1], F. Bernhard Kraus[1]

[1]Institut für Biologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 4, 06099 Halle/Saale, Germany
[2]Universidad de Ciencias y Artes de Chiapas, Tuxtla Gutiérrez, Chiapas, Mexico
[3]El Colegio de la Frontera Sur, San Cristóbal de las Casas, Chiapas, Mexico

Abstract

So far, nearly all studies concerning the mating frequency of bumblebees have been conducted with temperate species, showing that single mating seems to be the predominant pattern in bumblebees. Studies involving tropical species, however, are still scarce. Here, we determined the mating frequency of queens of the tropical bumblebee species, *Bombus wilmattae* by using microsatellite genotyping based on a sample of nine colonies from Chiapas, Southern Mexico. A total of 204 workers were genotyped with microsatellite markers to infer the queen genotype and the number of males with which each queen had mated. Two of the nine queens were doubly mated and seven singly mated. In the colonies with the double-mated queens, the distribution of the patrilines was not even, resulting in effective mating frequencies of 1.34 and 1.70, respectively, and an average relatedness of g=0.58±0.06.

Chapter 3

Workers dominate male production in the neotropical bumblebee *Bombus wilmattae* (Hymenoptera: Apidae)

Anett Huth-Schwarz[1,2*], Adolfo León[3], Rémy Vandame[4], Robin FA Moritz[1,5] and
F Bernhard Kraus[1]

Abstract

Background: Cooperation and conflict in social insects are closely linked to the genetic structure of the colony. Kin selection theory predicts conflict over the production of males between the workers and the queen and between the workers themselves, depending on intra-colonial relatedness but also on other factors like colony efficiency, sex ratios, cost of worker reproduction and worker dominance behaviour. In most bumblebee (*Bombus*) species the queen wins this conflict and often dominates male production. However, most studies in bumblebees have been conducted with only a few selected, mostly single mated species from temperate climate regions. Here we study the genetic colony composition of the facultative polyandrous neotropical bumblebee *Bombus wilmattae*, to assess the outcome of the queen-worker conflict over male production and to detect potential worker policing.

Results: A total of 120 males from five colonies were genotyped with up to nine microsatellite markers to infer their parentage. Four of the five colonies were queen right at point of time of male sampling, while one had an uncertain queen status. The workers clearly dominated production of males with an average of 84.9% +/- 14.3% of males being worker sons. In the two doubly mated colonies 62.5% and 96.7% of the male offspring originated from workers and both patrilines participated in male production. Inferring the mother genotypes from the male offspring, between four to eight workers participated in the production of males.

Conclusions: In this study we show that the workers clearly win the queen-worker conflict over male production in *B. wilmattae*, which sets them apart from the temperate bumblebee species studied so far. Workers clearly dominated male production in the singly as well the doubly mated colonies, with up to eight workers producing male offspring in a single colony. Moreover no monopolization of reproduction by single workers occurred.

Chapter 4

Factors influencing *Nosema bombi* infections in natural populations of *Bombus terrestris* (Hymenoptera: Apidae)

Anett Huth-Schwarz [1,2,*], Josef Settele [2], Robin F. A. Moritz [1,3], F. Bernhard Kraus [1]

[1] Institut für Biologie, Martin-Luther-Universität Halle-Wittenberg, Germany
[2] Department of Community Ecology, Helmholtz Centre for Environmental Research (UFZ), Halle, Germany
[3] Department of Zoology and Entomology, University of Pretoria, South Africa

Abstract
Bumblebees are of profound ecological importance because of the pollination services they provide in natural and agricultural ecosystems. Any decline of these pollinators is therefore of great concern for ecosystem functioning. Increased parasite pressures have been discussed as a major factor for the loss of pollinators. One of the main parasites of bumblebees is *Nosema bombi*, an intracellular microsporidian parasite with considerable impact on the vitality of the host. Here we study the effect of host colony density and host genetic variability on *N. bombi* infections in natural populations of the bumblebee *Bombus terrestris*. We sampled males and workers from six *B. terrestris* populations located in an agricultural landscape in Middle Sweden to determine the prevalence and degree of *N. bombi* infections. All individuals were genotyped with five microsatellite markers to infer the colony densities in the sampled populations and the genetic variability of the host population. We confirmed that genetic variability and sex significantly correlate with the degree of infection with *N. bombi*. Males and workers with lower genetic variability had significantly higher infection levels than average. Also colony density had a significant impact on the degree of infection, with high density populations having higher infected individuals.

Highlights

> In this study we examined factors influencing *N. bombi* parasitism in *B. terrestris*. > We determined host genetic variability and colony density in natural populations. > High density and low heterozygosity populations had higher degrees of infection per individual. > Males had both a higher prevalence and a higher spore load than females.

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Chapter 5 – Summary

Bumblebees as social insects are an excellent model for studying the impact of genetic variability on various levels of organismal complexity. First, genetic variability is generally thought to be an important parameter for resistance against parasites and diseases, not only on the individual (O'Donnell and Beshers 2004; Rolff 2002) and population (Cameron et al. 2011; Bull 1994), but also on the colony level (Maynard Smith 1971; Sherman et al. 1988). Second, genetic variability can also influence and determine cooperation or conflict potential among nestmates, which in case of the eusocial Hymenoptera covers both the individual and the colony level (Bourke 1988; Hamilton 1972; Hammond and Keller 2004; Ratnieks 1988; Wenseleers and Ratnieks 2006). The manuscripts united in this PhD thesis investigate three major questions encompassing genetic variability within bumblebees and its causes and consequences at various levels of organismal complexity.

5.1 How does the mating frequency influence the genetic colony structure in bumblebees?

The mating frequency of the queen determines the genetic colony structure in all eusocial Hymenoptera (Crozier and Pamilo 1996; Hamilton 1964; Hamilton 1972). Here we studied the mating frequency and the genetic colony structure of the tropical bumblebee *Bombus wilmattae* which is endemic to the mountain regions of southwest Mexico and Guatemala. In seven out of nine analysed monogynous colonies the queen was single mated while two colonies were headed by double mated queens. The paternities in the two colonies with double mated queens were not evenly distributed, with a significant paternity skew in one of the colonies ($\chi^2 = 4.96$; p = 0.03), resulting in an effective mating frequency of 1.34 for the colony with skewed paternity and 1.70 for the colony without. The average worker relatedness was therefore higher than 0.5 in both colonies (expected from the observed number of matings and evenly distributed paternities), to 0.62 and 0.54, respectively. This was the first study on the mating frequency of a tropical bumblebee species with microsatellite genotyping. The results were highly reliable as the microsatellite markers used were extremely variable, resulting in minute non-detection errors. The sample size of at least 20 workers per colony also guaranteed a robust estimate of the mating frequency and only very rare patrilines might have gone undetected. Thus, even though only 18 % of our analysed colonies had double mated queens, our results show that *B. wilmattae* belongs to the minority of facultative polyandrous bumblebee species. One of the main hypotheses for the evolution

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of polyandry is resistance against parasites (herd immunity hypothesis; Schmid-Hempel 1994; Sherman et al. 1988; Keller and Reeve 1994; Mattila and Seeley 2007), and given the increased parasite load in the tropics (Moller 1998; Nunn and Altizer 2005; Poulin and Rohde 1997; Cumming 2000; Poulin and Morand 2004), species of tropical Hymenoptera might be expected to have a higher probability to evolve polyandry.

However, although *B. wilmattae* is indeed facultative polyandrous, the effective mating frequency over all analysed colonies is barely higher than in colonies with single mated queens. Thus it seems doubtful whether this low degree of polyandry is indeed related to parasite pressure. *B. wilmattae*, being more aggressive than other temperate bumblebee species, may have evolved other strategies than increased genetic variability to cope with potentially higher parasite and predator pressure. One should also keep in mind that multiple mating also bears the risk of predation, possible injuries by males, additional costs in form of time and energy used for mating flights, and reduced female longevity resulting from disease transmission (Strassmann 2001), while single mating reduces such threats. Thus polyandry would have to result in a considerable fitness gain to overcome the costs associated with additional matings. Ultimately this constitutes a trade-off which, depending on the ecological context (climate, predator and parasites pressure) of a given species, could drive evolution towards polyandry or maintain the presumably ancestral state of monandry.

5.2 How does colony genetic variability influence cooperation and conflict in bumblebee societies?

The evolution of conflict and cooperation in insect societies is directly affected by the genetic colony structure. The fundament of kin selection theory is built upon the degree of relatedness amongst the individuals in the colony (Hamilton 1972). Since workers in a bumblebee colony can lay unfertilized eggs and have a higher relatedness to their own sons than to their nephews, conflict is predicted to arise over the production of males between the queen and the workers but also between the workers themselves. Here we investigated the queen-worker conflict in a bumblebee species that is facultative polyandrous, the tropical bumblebee species *B. wilmattae*. In the five studied colonies the workers clearly dominated male production with an average of 84.9 % ± 14.3 % worker produced males. Two of the five colonies were double mated with a worker parentage of 62.5 % and 96.7 %, respectively. Four of the analysed colonies were queen-right at point of sampling and one had an uncertain queen status. Thus matricide as causative for the high male numbers produced by workers could be excluded.

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The colony with the uncertain queen status was a single mated one with a high worker reproduction. As the developmental time of male bumblebees is three to four weeks, it is likely that the male eggs laid by workers were produced in the presence of the queen.

Generally in polyandrous colonies, where the effective mating frequency exceeds the value of 2, and where worker relatedness is below 0.5, worker policing would be expected (Ratnieks 1988), resulting in a high number of queen produced males. In the opposite case (effective mating frequency < 2 and worker relatedness > 0.5) workers should prefer the production of own male offspring resulting in a high number of males produced by workers (Ratnieks 1988) unless the queen suppresses worker reproduction, as found in *B. terrestris* (Van Doorn and Heringa 1986; Van Honk et al. 1981; Alaux et al. 2004; Lopez-Vaamonde et al. 2004). The two *B. wilmattae* colonies with double mated queens showed a medium (60 %) to high (97 %) amount of worker reproduction and no monopolisation of reproduction by single workers. Thus, there was no indication for worker policing, although dominance behaviour by the laying workers could not be excluded as observational records are missing. Furthermore as the effective mating frequency in both colonies was below the critical threshold of 2 and the average worker relatedness > 0.5, worker policing would not be expected to be adaptive. In the single mated colonies the worker reproduction was high, indicating that suppression of worker reproduction by the queen was either absent or ineffective.

Even though the queen-worker conflict is heavily influenced by relatedness (Starr 1984; Ratnieks 1988) also factors like colony growth, developmental stage, worker dominance behaviour, sex ratio and colony level costs of worker reproduction play an important role (Ratnieks 1988; Wenseleers and Ratnieks 2006; Ohtsuki and Tsuji 2009). In the majority of bumblebee species studied so far with molecular tools, the queen gained reproductive dominance over the workers (Alaux et al. 2004; Lopez-Vaamonde 2004, Takahashi et al. 2008; Cnaani et al. 2002; Owen and Plowright 1982; Brown et al. 2003) and thus the tropical bumblebee *B. wilmattae* seems to be an exception with the workers dominating the production of males.

5.3 How does host-genetic variability interact with parasite prevalence and degree of parasite infection at various levels of host organismal complexity?

Genetic variability not only influences conflict and cooperation within colonies of social insects, it is also thought to be an important factor influencing host parasite interactions. Bumblebees as social insects are highly prone to parasites due to their social life style (Schmid-Hempel 1998). Besides social immunity (Cremer et al. 2007) the immune defence

based on host genetic variability (Ebert and Hamilton 1996; Hamilton 1980; Kraus and Page 1998; O'Donnell and Beshers 2004; Rolff 2002; Sherman et al. 1988; Strassmann 2001) seems to be one important trait to cope with parasitism. To study the impact of host-genetic variability on parasite prevalence and degree of parasite infection at various levels of host organismal complexity, *Bombus terrestris* and its intracellular microsporidian parasite *Nosema bombi* were used as study system. *B. terrestris* is an ubiquitous bumblebee species that is distributed over the whole Palaearctic and used for pollination services as it can be easily reared. *B. terrestris* is an excellent model system to study host parasite interactions as it is one of the best studied bumblebee species regarding biology and ecology (Goulson 2010; Schmid-Hempel 1998). *N. bombi* is a worldwide distributed parasite of bumblebees (Otti and Schmid-Hempel 2007; Otti and Schmid-Hempel 2008; Rutrecht and Brown 2009) that can be easily detected via microscopy or with molecular tools (Erler et al. 2011). Here the effect of host genetic variability at the population, colony and individual level of six natural *B. terrestris* populations on the microsporidian parasite *N. bombi* was studied. For the first time it could be shown that in natural *B. terrestris* populations the colony densities were significantly positive correlated with the degree of parasite infection. However, there was no correlation between the prevalence of infection and colony densities. Furthermore the heterozygosity of the inferred queens as a general measure for the heterozygosity of the colonies was significantly negatively correlated with the degree of parasite infection. The degree of parasite infection and prevalence differed between the sexes with males always being higher and more often infected than females. The distribution of the parasite infection was significantly different in females between the populations, which was not the case for the males. It seems not only in rearing facilities with artificially generated high colony densities (Colla et al. 2006; Goka et al. 2006), but also in the wild, that high colony densities facilitate the spread of diseases in bumblebee populations. On the colony level we could confirm the results that were obtained by semi-natural experiments with *B. terrestris* and its parasites (Baer and Schmid-Hempel 1999). Judging from these results it seems that high heterozygosities reduce the rate of parasitism, since the larger high density host populations showed higher degrees of individual host infections than the smaller low density ones. On the individual level the differences in the degree of infection and the prevalence of infection found between the sexes could be either explained by the haploid susceptibility hypothesis, but also by different life strategies of the sexes. In conclusion our results show that genetic variability of the host influences the degree of parasitism at various levels of organismal

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complexity with higher host genetic variability increasing the ability of *B. terrestris* to cope with its parasite *N. bombi*.

In conclusion, the three manuscripts united in this thesis cover several important aspects of bumblebee biology and are the result of three years of field and laboratory work. On the individual and colony level for the first time a tropical bumblebee species was analyzed in detail with molecular ecological tools to determine its mating frequency and the outcome of the queen workers conflict. The third manuscript even included individual, colony and population level aspects of genetic variability and its impact on host parasite interactions. Thus, the studies of this thesis could provide valuable insights to our growing knowledge on this fascinating and important pollinator group.

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Chapter 6 – Zusammenfassung

Hummeln, welche zu den eusozialen Insekten (Hymenoptera) gehören, sind ein exzellentes Modell um den Einfluss der genetischen Variabilität auf verschiedenen organismischen Organisationsebenen zu untersuchen. So wird im Allgemeinen angenommen, dass die genetische Variabilität eine wichtige Rolle bei der Resistenz gegen Parasiten und Krankheiten spielt, und das nicht nur auf der Ebene des Individuums (O'Donnell and Beshers 2004; Rolff 2002) und der Population (Cameron et al. 2011; Bull 1994), sondern auch auf der Ebene der Kolonie (Maynard Smith 1971; Sherman et al. 1988). Desweiteren kann die genetische Variabilität das Kooperations- und Konfliktpotential zwischen den Kolonieangehörigen beeinflussen und determinieren (Bourke 1988; Hamilton 1972; Hammond and Keller 2004; Ratnieks 1988; Wenseleers and Ratnieks 2006). Bei den eusozialen Hymenopteren kann dies sowohl die Individuen- als auch die Kolonieebene betreffen. In den Manuskripten, die in dieser Doktorarbeit zusammengefasst sind, werden drei wesentliche Fragen hinsichtlich der Ursachen und Auswirkungen der genetischen Variabilität am Beispiel der Hummeln auf verschiedenen organismischen Organisationsebenen untersucht.

6.1 Wie beeinflusst die Paarungsfrequenz die genetische Koloniestruktur bei Hummeln?

Bei allen eusozialen Hymenopteren bestimmt die Paarungsfrequenz der Königin die genetische Struktur der Kolonie (Crozier and Pamilo 1996; Hamilton 1964; Hamilton 1972). Sowohl die Paarungsfrequenz als auch die daraus resultierende genetische Struktur der Kolonie wurden im Zuge dieser Arbeit an Hand der Hummelart *Bombus wilmattae* untersucht. *B. wilmattae* gehört zu den tropischen Hummeln und kommt in den montanen Regionen im Südwesten Mexikos und in Guatemala endemisch vor.

In sieben der neun analysierten monogynen *B. wilmattae* Kolonien war die Königin einfach verpaart, während zwei Kolonien eine zweifach verpaarte Königin aufwiesen. In den Kolonien mit den zweifach verpaarten Königinnen waren die Patrilinien innerhalb der Nachkommenschaft nicht gleichmäßig vertreten. Dies resultierte in einer der beiden Kolonien in signifikant verschiedenen Anteilen der Patrilinien innerhalb der Nachkommenschaft ($\chi^2 = 4{,}96$; $p = 0{,}03$) und dementsprechend in einer effektiven Paarungsfrequenz von 1,34. In der Kolonie, in der sich die Anteile der Patrilinien nicht signifikant voneinander unterschieden, entsprach die effektive Paarungsfrequenz 1,70. Die durchschnittliche Verwandtschaft der Arbeiterinnen war dementsprechend in den beiden Kolonien mit mehrfachverpaarten

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Königinnen größer als 0,5 (ausgehend von der beobachteten Anzahl an Paarungen und gleichmäßig verteilten Vaterschaften) und wurde mit 0,62 beziehungsweise 0,54 bestimmt.

In dieser Arbeit wurde zum ersten Mal die Paarungsfrequenz einer tropischen Hummelart mit molekularen Methoden untersucht. Die Ergebnisse können als sehr verlässlich angesehen werden, da sehr variable Mikrosatelliten als molekulare Marker benutzt wurden. Somit ergab sich ein entsprechend kleiner Nichtdedektierungsfehler. Die Probengröße von 20 Arbeiterinnen pro Kolonie garantiert ebenfalls eine robuste Abschätzung der Paarungsfrequenz. Nur sehr seltene Patrilinien könnten unentdeckt geblieben sein. Obwohl nur 18 % der hier analysierten Kolonien doppelt verpaarte Königinnen aufwiesen, zeigen unsere Ergebnisse, dass *B. wilmattae* zu den fakultativ polyandrischen Hummelarten gehört. Polyandrische Hummelarten scheinen aber eher eine Ausnahme innerhalb der Hummeln zu sein, da nur 27 %, der bisher molekular untersuchten Hummelarten, mehrfach verpaarte Königinnen aufweisen.

Eine der Haupthypothesen für die Evolution von Polyandry basiert auf der Annahme der besseren Resistenz gegen Parasiten („herd immunity hypothesis"; Schmid-Hempel 1994; Sherman et al. 1988; Keller and Reeve 1994; Mattila and Seeley 2007). Ausgehend von einer erhöhten Parasitenbelastung in den Tropen (Moller 1998; Nunn and Altizer 2005; Poulin and Rohde 1997; Cumming 2000; Poulin and Morand 2004) könnte sich Polyandry bei eusozialen tropischen Hymenopteren mit einer höheren Wahrscheinlichkeit entwickelt haben. Aber, obwohl *B. wilmattae* in der Tat fakultativ polyandrisch ist, ist die effektive Paarungsfrequenz über alle Kolonien betrachtet kaum höher als in Kolonien mit einfach verpaarten Königinnen. Somit scheint es zweifelhaft, dass der hier gefundene Grad an Polyandry und der sich daraus ergebende Grad an genetischer Variabilität wirklich in Bezug zum Parasitendruck stehen. *B. wilmattae*, die aggressiver als andere temperate Hummelarten ist, könnte daher andere Strategien entwickelt haben um mit einem potentiell erhöhten Parasiten- und Räuberdruck umzugehen. Man sollte sich außerdem vergegenwärtigen, dass Polyandry das Risiko der Prädation, von möglichen Verletzungen durch Männchen, von zusätzlichen Kosten in Form von Zeit und Energie für Paarungsflüge und einer reduzierten Lebensdauer der Weibchen durch die Übertragung von Krankheiten bergen (Strassmann 2001). Monoandry würde solche Risiken reduzieren. Polyandry müßte zu einem solch entscheidenden Fitnessgewinn führen, dass die Kosten, die mit zusätzlichen Paarungen einhergehen, kompensiert werden könnten. Letztendlich stellt dies einen *trade-off* dar, welcher, abhängig vom ökologischen Kontext (Klima, Räuber und Parasitendruck) einer Art, die Evolution von Polyandry begünstigt oder die phylogenetisch ursprüngliche Variante, die Monoandry, beibehält.

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6.2 Wie beeinflusst die genetische Variabilität der Kolonie Kooperationen und Konflikte in der Hummelgesellschaft?

Bei eusozialen Hymenopteren beeinflusst die genetische Struktur der Kolonie, welche auf dem Grad der Verwandtschaft zwischen den Individuen beruht, die Evolution von Kooperationen und Konflikten innerhalb des Sozialgefüges. Der hohe Grad der Verwandtschaft wird als einer der Hauptfaktoren für die Entstehung von Eusozialität innerhalb der Hymenopteren angenommen und bildet die Basis der sogenannten „Kin Selection Theory" (Hamilton 1972). Hummeln gehören ebenfalls zu den eusozialen Hymenopteren. Arbeiterinnen in Hummelkolonien können unbefruchtete Eier legen und haben eine höhere Verwandtschaft zu ihren eigenen Söhnen als zu ihren Neffen. Dies kann einen Konflikt zwischen der Königin und den Arbeiterinnen aber auch zwischen den Arbeiterinnen selbst über die Produktion von Männchen bedingen.

In dieser Arbeit wird der Königin-Arbeiterinnen-Konflikt an Hand der tropischen fakultativ polyandrischen Hummelart *B. wilmattae* untersucht. In den fünf analysierten Kolonien dominierten die Arbeiterinnen klar die Produktion der Männchen mit im Durchscnitt 84,9 % ± 14,3 %. In zwei der fünf Kolonien waren die jeweiligen Königinnen doppelt verpaart. Die Arbeiterinnen dieser polyandrischen Kolonien produzierten 62,5 % beziehungsweise 96,7 % der männlichen Nachkommen. Vier der Kolonien wiesen zum Zeitpunkt der Probennahme eine Königin auf, während eine Kolonie einen unklaren Königinnenstatus hatte. Matrizid kann daher als Ursache für den hohen Männchenanteil, der von Arbeiterinnen produziert wurde, ausgeschlossen werden. Die Kolonie mit dem unklaren Königinnenstatus war monoandrisch und hatte eine hohe Arbeiterinnenreproduktion. Da die Entwicklungsdauer bei Hummelmännchen zwischen drei bis vier Wochen liegt, kann davon ausgegangen werden, dass in dieser Kolonie, die von den Arbeiterinnen produzierten unbefruchteten Eier in Anwesenheit der Königin gelegt wurden. Wie schon erwähnt, basieren Kooperationen und Konflikte im Hymenopterenstaat auf den Verwandtschaftsverhältnissen zwischen den einzelnen Individuen. Ein entsprechender Konflikt kann zwischen den Arbeiterinnen auftreten und sich in Form vom sogenannten „worker policing" äußern.

„Worker policing" würde im Allgemeinen in polyandrischen Kolonien erwartet werden, in denen die effektive Paarungsfrequenz >2 und die Verwandtschaft der Arbeiterinnen <0,5 ist (Ratnieks 1988), was in einer hohen Anzahl Männchen, die von der Königin produziert werden, resultiert. Im umgekehrten Fall (effektive Paarungsfrequenz <2 und Verwandtschaft

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der Arbeiterinnen >0,5) sollten Arbeiterinnen die Produktion ihres eigenen männlichen Nachwuchs bevorzugen, was zu einer hohen Anzahl an Männchen, die von Arbeiterinnen produziert werden, führen würde (Ratnieks 1988). Eine hohe Arbeiterinnenreproduktion würde aber nur möglich sein, solange die Königin die Arbeiterinnenreproduktion nicht unterdrückt, wie es bei *B. terrestris* (Van Doorn and Heringa 1986; Van Honk et al. 1981; Alaux et al. 2004; Lopez-Vaamonde et al. 2004) der Fall ist. Die zwei *B. wilmattae* Kolonien mit den jeweils zweifach verpaarten Königinnen zeigten einen mitteleren (60 %) bis sehr hohen (97 %) Anteil an Arbeiterinnenreproduktion und es wurde keine Monopolisierung der Reproduktion durch einzelne Arbeiterinnen gefunden. Daher gibt es keine Anhaltspunkte für „worker policing", obwohl ein Dominanzverhalten von legenden Arbeiterinnen, aufgrund von fehlenden Beobachtungsdaten, nicht ausgeschlossen werden kann. Desweiteren lag die effektive Paarungsfrequenz in beiden Kolonien unterhalb der kritischen Grenze von 2 und die durchschnittliche Verwandtschaft der Arbeiterinnen über 0,5. Daher würde nicht erwartet werden, dass „worker policing" adaptiv wäre. In den einfach verpaarten Kolonien war die Arbeiterinnenreproduktion ebenfalls hoch, was dafür spricht, dass die Unterdrückung der Arbeiterinnenreproduktion durch die Königin entweder fehlte oder ineffektiv war.

Obwohl der Königin-Arbeiterinnen-Konflikt durch die Verwandtschaftsverhältnisse beeinflusst wird (Starr 1984; Ratnieks 1988), spielen auch Faktoren wie das Wachstum der Kolonie, der Entwicklungsstand der Kolonie, das Dominanzverhalten der Arbeiterinnen, die Geschlechterverhältnisse und die Kosten der Arbeiterinnenreproduktion auf Ebene der Kolonie eine wichtige Rolle (Ratnieks 1988; Wenseleers and Ratnieks 2006; Ohtsuki and Tsuji 2009). Bei den meisten Hummelarten, die bisher mit molekularen Methoden untersucht wurden, hatte die Königin die reproduktive Dominanz inne (Alaux et al. 2004; Lopez-Vaamonde 2004, Takahashi et al. 2008; Cnaani et al. 2002; Owen and Plowright 1982; Brown et al. 2003). Die tropische Hummel *B. wilmattae* scheint somit eine Ausnahme zu sein, da die Arbeiterinnen die Männchenproduktion eindeutig dominieren.

6.3 Wie beeinflusst die genetische Variabilität des Wirtes die Parasitenprävalenz und den Grad der Parasitierung?

Die genetische Variabilität beeinflusst nicht nur Konflikte und Kooperationen innerhalb der Hummelkolonie, sondern auch Wirt-Parasit-Interaktionen. Hummeln sind, wegen ihres eusozialen Lebensstils, stark anfällig für Parasiten (Schmid-Hempel 1998). Scheinbar haben Hummeln, wie auch andere eusoziale Hymenopteren, verschiedene Wege entwickelt mit

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dieser erhöhten Anfälligkeit gegenüber Parasiten zurechtzukommen. Neben der sozialen Immunität scheint die Immunität, die auf der genetischen Variabilität des Wirtes beruht (Ebert and Hamilton 1996; Hamilton 1980; Kraus and Page 1998; O'Donnell and Beshers 2004; Rolff 2002; Sherman et al. 1988; Strassmann 2001), eine wichtige Rolle zu spielen. Um den Einfluss der genetischen Variabilität des Wirtes auf die Parasitenprävalenz und den Grad der Parasitierung auf verschiedenen Ebenen der organismischen Komplexität des Wirtes zu untersuchen, wurde die Hummel *Bombus terrestris* und ihr intrazellulärer, zu den Mikrosporidien gehörender, Parasit *Nosema bombi* als Untersuchungssystem ausgewählt. *B. terrestris* ist eine ubiquitäre über die gesamte Palaearktik verbreitete Hummelart. Da sie einfach zu züchten ist, wird sie für die Bestäubung in Gewächshäusern und im Freiland genutzt. *B. terrestris* stellt ein hervorragendes Modellsystem dar um Wirt-Parasit-Interaktionen zu studieren, da sie bezüglich ihrer Biologie und Ökologie zu den am besten untersuchten Hummelarten gehört (Goulson 2010; Schmid-Hempel 1998). *N. bombi* ist ein weltweit verbreiteter Hummelparasit (Otti and Schmid-Hempel 2007; Otti and Schmid-Hempel 2008; Rutrecht and Brown 2009), der relativ einfach mittels Mikroskopie oder molekularen Methoden erfasst werden kann (Erler et al. 2011).

In dieser Arbeit wurde der Effekt der genetischen Variabilität des Wirtes (*B. terrestris*) auf den Parasiten *N. bombi* untersucht. Dabei wurden drei Organisationsebenen des Wirtes (Population, Kolonie und Individuum) in sechs natürlich vorkommenden Populationen des selbigen betrachtet.

Zum ersten Mal konnte gezeigt werden, dass in natürlich vorkommenden Populationen von *B. terrestris* die Koloniedichte signifikant mit dem Grad der Parasitierung korreliert. Eine Korrelation zwischen der Prävalenz des Parasiten und der Koloniedichte wurde hingegen nicht gefunden.

Desweiteren konnte auf Kolonieebene gezeigt werden, dass die Heterozygotie der hergeleiteten Königinnen (basierend auf den molekularen Daten der Arbeiterinnen) signifikant negativ mit dem Grad der Parasitierung korreliert war. Die Heterozygotie der hergeleiteten Königinnen wurde hierbei als Maß für die Heterozygotie der Kolonien angenommen.

Der Grad der Parasitierung sowie die Prävalenz des Parasiten unterschieden sich, bei Betrachtung der Individuenebene, zwischen den Geschlechtern. Die Männchen waren höher parasitiert als die Weibchen. Die Verteilung der Parasitierung unterschied sich zwischen den Populationen bezogen auf die Weibchen aber nicht bei den Männchen.

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Aus den Ergebnissen kann abgeleitet werden, dass hohe Koloniedichten die Verbreitung von Krankheiten in Hummelpopulationen begünstigen. Wie hier gezeigt werden konnte, scheint dies also nicht nur auf Zuchteinrichtungen mit künstlich generierten hohen Koloniedichten (Colla et al. 2006; Goka et al. 2006), sondern auch auf natürlich vorkommende Populationen zuzutreffen. Auf der Ebene der Kolonie konnten die Ergebnisse, die durch semi-natürliche Experimente mit *B. terrestris* und ihren Parasiten ermittelt wurden (Baer and Schmid-Hempel 1999), bestätigt werden. Auch in natürlichen Hummelpopulationen scheinen hohe Heterozygotien innerhalb der Kolonien den Grad der Parasitierung zu reduzieren. Die Unterschiede, die bezüglich der Prävalenz und dem Grad der Parasitierung auf Individuenebene gefunden worden sind, können zum einen durch die „haploid susceptibility hypothesis", aber auch durch verschiedenartige Lebensstrategien der Geschlechter erklärt werden. Zusammenfassend zeigen unsere Ergebnisse, dass die genetische Variabilität des Wirtes den Grad der Parasitierung auf verschiedenen Ebenen der organismischen Komplexität des Wirtes beeinflusst, wobei eine höhere genetische Wirtsvariabilität die Fähigkeit von *B. terrestris* erhöht mit ihrem Parasiten *N. bombi* zurechtzukommen.

Zusammenfassend kann gesagt werden, dass die drei Manuskripte, die dieser Doktorarbeit zu Grunde liegen, sich mit verschiedenen wichtigen Aspekten der Biologie und Ökologie der Hummeln auseinandersetzen und das Ergebnis von drei Jahren Feld- und Laborarbeit darstellen. Zum ersten Mal wurde eine tropische Hummelart auf der Individuen- und Kolonieebene im Detail mit molekular ökologischen Methoden analysiert um die Paarungsfrequenz der Königin und den Ausgang des Königin-Arbeiterinnen-Konflikts zu untersuchen. Das dritte Manuskript beschäftigt sich mit dem Einfluss der genetischen Variabilität auf Wirt-Parasit-Interaktionen bei Hummeln und betrachtet dabei die Individuen-, Kolonie- und Populationsebene des Wirtes. Somit konnten, basierend auf den Ergebnissen dieser Doktorarbeit, wertvolle Erkenntnisse über die Biologie und Ökologie der Hummeln zu dem wachsenden Wissen über diese wichtige Bestäubergruppe beigetragen werden.

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Appendix

A. Declaration on the authors' contributions

1. Huth-Schwarz A, León A, Vandame R, Moritz RFA, Kraus FB (2011): Mating frequency and genetic colony structure of the neotropical bumblebee Bombus wilmattae (Hymenoptera: Apidae). Apidologie. 42, 519-525. DOI: 10.1007/s13592-011-0038-4

Collecting of samples; molecular analyses, data analyses, statistical analyses, drafting the manuscript

2. Huth-Schwarz A, León A, Vandame R, Moritz RFA, Kraus FB (2011): Workers dominate male production in the neotropical bumblebee *Bombus wilmattae* (Hymenoptera: Apidae). Front Zool 8:13. DOI:10.1186/1742-9994-8-13

Collecting of samples; molecular analyses, data analyses, statistical analyses, drafting the manuscript

3. Huth-Schwarz A, Settele J, Moritz RFA, Kraus FB (2012): Factors influencing Nosema bombi infections in natural populations of Bombus terrestris (Hymenoptera: Apidae). J Invertebr. Pathol. in press.

Project design, collecting of samples; molecular analyses, measurments, data analyses, statistical analyses, drafting the manuscript

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B. Curriculum Vitae

Date of Birth: 10th December 1981

Place of Birth   Halle (Saale)

Marital status: Married

Nationality: german

Higher Education

Since 02/2008   PhD thesis (in progress) in the working group Molecular
                                  Ecology at the MLU Halle-Wittenberg, Germany;
                                  Topic: "The impact of land use on multi-trophic insect systems";
                                  Scholarship from the Helmholtz Interdisciplinary Graduate
                                  School for Environmental Research (HIGRADE)

WS 2006 – SS 2007   Diploma thesis in Nature Conservation (Molecular Ecology);
                                  Topic: "Studies of the ecology and biology of the leafhopper
                                  Zyginidia scutellaris"; (grade:1,4)

WS 2000/2001 – SS 2007   Studies of Biology at the MLU Halle-Wittenberg, Germany
                                  Subjects of study: Molecular Ecology, Behavioural Biology,
                                  Geobotany, Nature Conversation

School Education

1992 – 2000   Grammar school (Latina August Hermann Francke); Abitur
                                  (grade: 1,9)

1988 – 1992   Primary school

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C. <u>Publications</u>

Peer-reviewed Articles

Huth-Schwarz A, Settele J, Moritz RFA, Kraus FB (2012): Factors influencing *Nosema bombi* infections in natural populations of *Bombus terrestris* (Hymenoptera: Apidae). J. Invertebr. Pathol. 2012, doi:10.1016/j.jip.2012.02.003

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Popular Articles

Huth A, Witsack W (2009): Studies on host plant use for feeding and oviposition in *Zyginidia scutellaris* (Herrich-Schäffer, 1838) (Achenorrhyncha, Cicadellidae, Typhlocybinae) (in German). Cicadina 10: 89-100.

D. Oral Presentations

Huth-Schwarz A, Kraus FB, León A, Vandame R, Moritz RFA (2011): Queen worker conflict over male production in the neotropical bumblebee *Bombus wilmattae* (Hymenoptera: Apidae). IUSSI 2011, Papenburg, March 25th – 28th.

Huth-Schwarz A, Kraus FB, León A, Vandame R., Moritz RFA (2010): Genetic colony structure and male production in the neotropical bumblebee *Bombus wilmattae* (Hymenoptera: Apidae). EURBEE 2010, Ankara, September 7th - 9th.

Huth-Schwarz A, Settele J, Moritz RFA (2010): Host-parasite interactions in bumblebees: *Bombus terrestris* and its parasite *Nosema bombi*. 6th DocConference at UFZ 2010, Leipzig, April 26th - 27th.

Huth-Schwarz A, Settele J, Moritz RFA (2009): *Nosema* infection in drones (*Apis mellifera*) of DCAs. 56. Jahrestagung der AG der Inst. Für Bienenforschung e.V. , Schwerin 2009, March 24th - 26th.

E. Poster Presentations

Huth-Schwarz A, Kraus FB, Settele J, Moritz RFA (2010): Factors influencing infection rates of the intracellular parasite *Nosema bombi* in natural populations of the bumblebee *B. terrestris*. IUSSI 2010, Copenhagen, August 9th - 13th.

F. Media Coverage

Carsten Heckmann (2011): Mexikanische Arbeiterhummeln übernehmen königliche Aufgaben. pressemitteilungen.pr.uni-halle.de, 188/2011

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G. Erklärung

Halle (Saale), den 15. März 2012

Hiermit erkläre ich an Eides statt, dass diese Arbeit von mir bisher weder an der Naturwissenschaftlichen Fakultät I - Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion eingereicht wurde.

Ich erkläre weiterhin, 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.

Anett Huth-Schwarz