Molecular Analysis of Host-Parasite Interaction in the Bumblebee *Bombus terrestris* (Linnaeus, 1758)

D i s s e r t a t i o n

zur Erlangung des akademischen Grades

doctor rerum naturalium (Dr. rer. nat.)

vorgelegt der

Naturwissenschaftlichen Fakultät I -
Biowissenschaften

der Martin-Luther-Universität Halle-Wittenberg

von

Herrn Silvio Erler

geb. am 08.09.1983 in Karl-Marx-Stadt

Gutachter / in:

1. Prof. Dr. Ingemar Fries

2. Prof. Dr. Robert J. Paxton

3. Prof. Dr. Paul Schmid-Hempel

Promotionsgesuch eingereicht am: 18.10.2011

Tag der öffentlichen Verteidigung: 03.02.2012

‘‘Many kinds of small animals, chiefly insects, are to be found in humble-bees' nests. Some of these are chance visitors, with no particular business there, but others are dependent in some way upon the humble-bees, and several belonging to this class are very injurious to them, devouring the larvae and pupae.’’

F.W.L. Sladen, 1912

(The humble-bee – its life-history and how to domesticate it.)

Contents

Chapter 1 - General Introduction

1.1 The bumblebee *Bombus terrestris*

Bumblebees, especially the buff-tailed bumblebee *Bombus terrestris*, play a central role in
1) pollination of wild flowers, crops, fruits and vegetables, and
2) represent an established model organism to study the evolution of sociality, parasitism and host-parasite interaction in social insects.

The bumblebee *B. terrestris* produces annual colonies, headed by a single queen which is singly or rarely multiple-mated (Payne et al., 2003; Schmid-Hempel and Schmid-Hempel, 2000). These colonies are funded in early spring by a hibernated queen and first brood care is done exclusively by the queen. Later in the season, workers contribute to brood care and maintain the colony. At the end of the season (late summer) only sexuals are produced and, after mating, queens hibernate until the next season (Sladen, 1912). During the colony development all different castes and stages of bumblebees are prone to different types of parasite infections. The restricted size of a single colony, 50 - 600 individual bees, and high density of hosts will also increase the accessibility for parasites and pathogens. Such density-dependent parasite pressure is not only the problem for large scale breeding of bumblebee colonies commercially (Velthuis and van Doorn, 2006), but also under laboratory conditions.

The bumblebee as host organism can easily be characterized by their population dynamics and population structure. They are haplo-diploid organisms, with females (queens and workers) being diploid (2n) and males (drones) being haploid (n) (Crozier, 1975; Trivers and Hare, 1976). The simple kinship system between colony members can be used to estimate colony affiliation, by including population genetic tools, e.g. highly polymorphic microsatellite markers (Estoup et al., 1995, 1996; Reber-Funk et at., 2006; Stolle et al., 2009). Sibship reconstruction is further enhanced, because *B. terrestris* queens are exclusively singly mated. Therefore, *B. terrestris* provides an excellent model organism to study host-parasite interactions; compared to ant, wasp, or honeybee species which sometimes show high levels of multiple mating (Strassmann, 2001). Studying host-parasite interactions at the colony level is greatly facilitated because genetically similar individuals determine colony performance, and colonies can be split to compare different treatments.

Recent efforts of the Bombus Annotation Group will drive forward the annotation of the *B. terrestris* genome; see NCBI: Bter_1.0 gene prediction sets (Refseq, RNAseq data, AUGUSTUS and GeneID sets). The nearly complete gene set, together with the known EST-libraries (Sadd et al., 2010) and the genetic map of *B. terrestris* (Stolle et al., 2011; Wilfert et al., 2006), provide several opportunities to study this host organism more intensively by using molecular tools.

The simple life-cycle, the possibility to maintain experimentally treated colonies in the lab, the availability of a huge amount of genetic identical individuals, the haplo-diploid sex determination system, and the nearly complete covered genome makes the bumblebee *B. terrestris* an ideal model organism to study host-parasite interactions.

1.2 Parasites, bumblebees and social parasitism

Social insects, in general, are prime targets for parasites due to their abundance, family structure, and persistent colonies (Schmid-Hempel, 1998). Eggs, larva, pupa, worker, drones, and queens represent stages which might be infected by viruses, bacteria, fungi, protozoa, nematodes, parasitoids, mites or other colony specific parasites (Schmid-Hempel, 1995).

Parasite transmissions between colony members allow a fast parasite spread, in correlation with fast performing colony growth during the short season of host availability. Parasite transmission (horizontal and / or vertical) controls the intensity of infection and parasite effects on the host population (Ebert and Herre, 1996). Parasite infestation, growth, transmission and development depend mostly on host colony size / population. Increasing numbers of colony members lead to increased parasite spread and *vice versa*. Generation time and body size mainly control the population size, so increasing body size of the parasite (e.g., from viruses to insects) induces substantial reduction in effective population size (Lynch, 2007). Density dependent host-parasite development, as it might occur in bumblebees, is controlled by virulence of the parasite and defence of the host (Anderson and May, 1981).

In general, small parasites were detected with higher densities in the host due to smaller generation time compared to parasites with complex life cycles. Grouping the different bee parasite species in seven major parasite groups (viruses, bacteria, fungi, protozoa, nematodes, insect parasitoids, and ectoparasites) and analysing census population size (N_c) of the parasite per bee (normalised for parasites per bee, by using an average bumble bee colony size of 300 - 600 bees, if the number of parasites per bee was not available) and

generation time of the respective parasite group, showed a significant correlation for all the seven groups (N = 7, r = 0.894, p = 0.007, Figure 1).

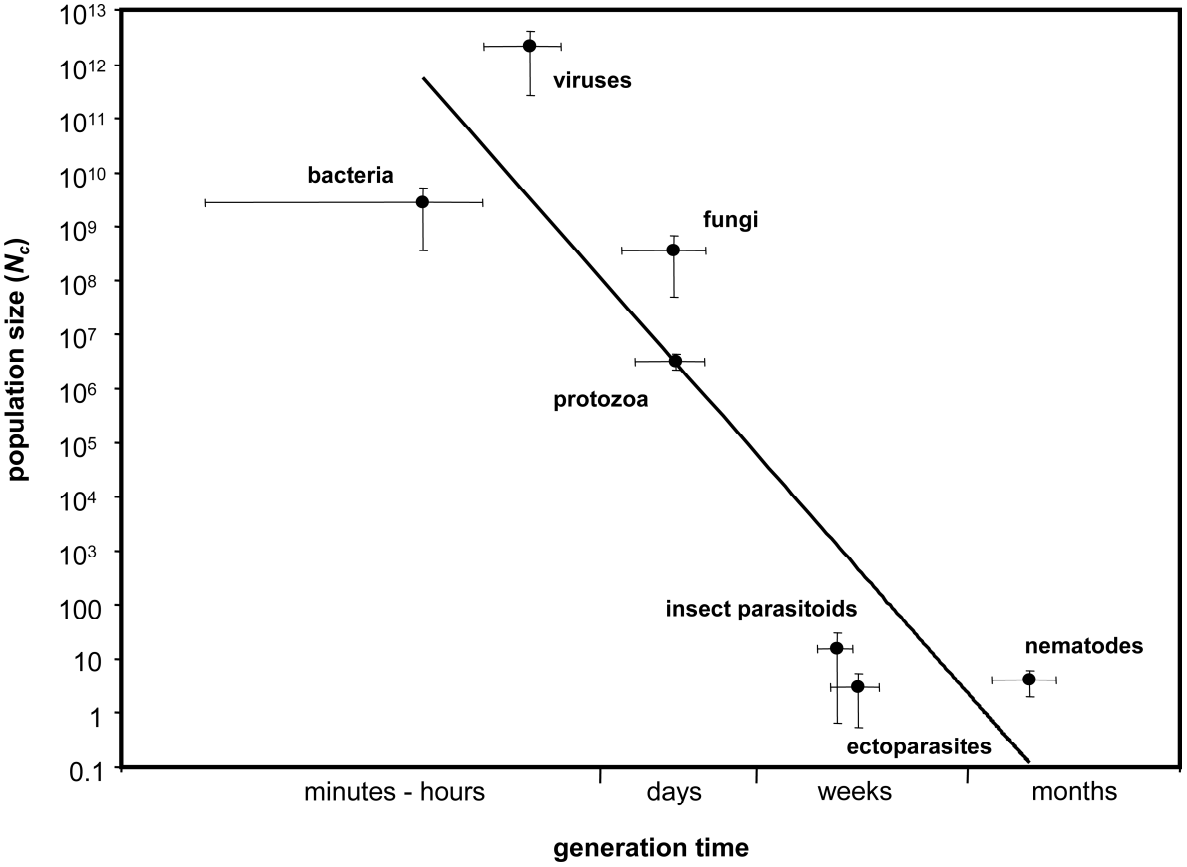


Figure 1: Comparative analysis of generation time and census population size (N_c) over all groups of bumblebee parasites. Census population size of the individual parasites were used according to published data and calculated as population size per bee, independent of localization of the parasite. In order to have a wide range of data, some groups also include non-bumblebee or non-insect data (ectoparasites, bacteria and viruses). For each group (black dots) the average values are plotted on logarithmic scales. Additionally, standard errors for generation time and population size were calculated. Generation time was plotted in minutes and modified to improve visualization. Power regression analysis showed a significant correlation between both factors (N = 7, r = 0.894, p = 0.007). (* marked references in the reference list were used to construct Figure 1)

As mentioned before, bumblebee parasites belong to all known groups of parasites from intracellular viruses to macro-parasites like mites and hymenopteran parasitoids (Table 1, see page 7) (Schmid-Hempel, 1998, 2001). Several types of parasites are already used to study these interactions, e.g., the gut parasite *Crithidia bombi*, the Microsporidium *Nosema bombi* and typical representatives of fungi, gram-positive and gram-negative bacteria (Imhoof and Schmid-Hempel, 1998; McIvor and Malone, 1995; Lipa and Triggiani, 1988; Sadd and Schmid-Hempel, 2006, 2007).

However, bumblebees are not only vulnerable to infections with the listed parasites and pathogens (see Table 1, page 7), they can also be parasitised by brood parasites (social parasitic cuckoo bumblebees) (Fisher, 1988; van Honk et al., 1981). Host and parasite share the same but time shifted annual life cycle and environmental conditions. In late spring (April-July) the hibernated cuckoo bumblebee queen invades the host colony. In a few cases, the host queen might be tolerated, but mostly the host queen is killed by the cuckoo bumblebee queen. After the takeover of the host colony, the cuckoo queen starts laying her own eggs and the workers of the host take care on the brood of the parasite (Alford, 1975; Sladen, 1912). As the cuckoo bumblebee parasitise only a subset of all available colonies, their population size might be lower than the size of their hosts. This might have strong negative effects on the genetic diversity of the cuckoo bumblebees and might increase genetic drift.

Bombus terrestris is exclusively parasitised by *Bombus* (*Psithyrus*) *vestalis*. Location and excavation of nests of such a population is nearly impossible, as most bumblebee species have cryptic underground nests in abandoned mammal nests (Goulson, 2003; Sladen, 1912).

1.3 Parasites and pollinator decline

Parasite infections have recently been claimed to be one of the most important factors for worldwide decline of pollinators (honey bees and bumblebees) (Bromenshenk et al., 2010; Cameron et al., 2011; Goulson et al., 2008; Meeus et al., 2011; Potts et al., 2010; vanEngelsdorp et al., 2009).

Microsporidian pathogens like *Nosema apis*, *N. bombi* and *N. ceranae* might play the key role for global honey and bumblebee decline and the so called CCD (‘Colony Collapse Disorder’) (Bromenshenk et al., 2010; Cameron et al., 2011). Short generation times and huge population sizes will increase the effectiveness of infections with *Nosema* spp. This host-parasite system demonstrates that host populations might be regulated by the effect of the parasite. Density-dependent (N_c) parasite effects can cause dramatic decline or extinction of the host population (Martin-Hernández et al., 2007; Paxton et al., 2007).

The generalist parasites, *Nosema* spp., are able to infect different species of honey and bumblebees. Parasite transmission might occur horizontally (via infected flowers or beekeeping equipment) or vertical (within the host colony). It also depends on host availability. Estimating the infection status of a colony assumes sensitive and specific methods to measure spore titre and to determine the correct *Nosema* species, as co-infections

are quite common (Chen et al., 2009; Paxton et al., 2007). Different molecular and non-molecular methods have been described to detect and characterise the different *Nosema* species more or less specific and sensitive (de Graaf et al., 1994; Klee et al., 2006, 2007; Larsson, 2005; Martin-Hernández et al., 2007; Webster et al., 2004). However, little is known about the limits of detection. The evaluation of detection limits might influence the outcome of molecular diagnostics and bee health monitoring.

1.4 Host-parasite interactions

Host-parasite interactions are shaped by a wide range of biological, ecological, and environmental factors. Variations in parasite load and virulence, as well as variations in host susceptibility and immune responses control host-parasite dynamics under natural conditions (Anderson and May, 1982; Gandon et al., 2001).

Host finding, infection, establishment, growth, reproduction and transmission are the major steps of the parasites life cycle. Parasites might be transmitted directly, via vectors, horizontally or vertically, depending on the life cycle and the host species (Schmid-Hempel, 2011). To perform a complete life cycle, parasites developed different strategies: (1) harm the host (proteases, toxins, virulence factors or damage host tissue) and / or (2) evasion of the host immune system (recognition and killing) to survive host defence (Schmid-Hempel, 2005, 2009). Both strategies help the parasite to survive in / on the host and allow the host to react on parasite infections and develop new defence mechanisms. Therefore, the hosts developed a huge arsenal of weapons to fight parasite attack. These weapons are based on behavioural, chemical, immune system mediated and other molecular defence mechanisms. Using molecular methods provide unique tools to measure host-parasite interaction on different levels. Host defence against parasites allows using a wide rage of parameters on the part of the host: parasite clearance, immunocompetence of the host, susceptibility, recognition, recovery, resistance, and robustness of the host (Schmid-Hempel, 2011).

Insect hosts and all other invertebrates attack parasites by means of their innate immune system (Hultmark, 1993; Siva-Jothy et al., 2005). Surface molecules of the parasite (e.g., antigenic peptides, carbohydrates and lipids) switch on the innate immune system by up- or down-regulation of immune related pathways (Toll, Imd, JNK and JAK/STAT). So the immune system activation results in an anti-parasite response via melanization, proteasome-dependent degradation, apoptosis, expression of antimicrobial peptides (AMPs) and cytotoxic enzymes (Evans et al., 2006). Different components of the hosts' immune response might act

as short-term clearance or as long-lasting protection. Humoral and cellular compounds cooperate and interact, mostly temporal staggered, to kill the invading parasite. Maternal or paternal immune priming might increase the success of fighting against a known parasite (Roth et al., 2009; Sadd and Schmid-Hempel, 2007; Zanchi et al., 2011).

Comparing the number of genes related to the innate immune system of completely sequenced social (71-97, honey bee and ants (Bonasio et al., 2010; Honeybee Genome Sequencing Consortium, 2006; Smith et al., 2011a, b; Suen et al., 2011)) vs. non-social insects (203-417, beetle, fly, lepidopterans, mosquitoes (Adams et al., 2000; Arensburger et al., 2010; Holt et al., 2002; International Silkworm Genome Consortium, 2008; Nene et al., 2007; Tribolium Genome Sequencing Consortium, 2008)) revealed a strong reduction in immunity components on site of the social insects.

This effect might be compensated by individual and / or group-level traits which can be found in social insect colonies, e.g., 'social immunity', hygienic behaviour, social behaviour (brood care, grooming), 'social fever', foraging antibiotic compounds like propolis or avoiding contaminated food resources (Cremer et al., 2007; Fouks and Lattorff, 2011; Schmid-Hempel, 2011; Simone et al., 2009). The innate immune system of the bumblebee *Bombus terrestris* is partly characterised by sequencing of EST-libraries (Sadd et al., 2010).

Study questions

The knowledge from *B. terrestris* population genetics, genomics – including the innate immune system, different ways of parasite transmission and interaction with social parasitic cuckoo bumblebees represent a useful system to answer the following major questions, which are addressed in the next three chapters:

1. How strong is the impact of parasitism for social parasites and how diverse are host and parasite species?

2. What is the limit of parasite detection and how specific are such detection systems?

3. Is the host innate immune system influenced by parasite dynamics and does the host defence system show temporal patterns?

Table 1: Parasites described for bumblebees. The different species were grouped according to species, genera or organism group and, additionally to location in the host and / or place of reproduction. For some groups (e.g. fungi) only few examples are given, which were included in Figure 1; for the remaining species rare data were available on population size and generation time.

Colony	**Single Bumblebee**		
	Ectoparasite	**Endoparasite**	
		Extracellular	**Intracellular**
Lepidoptera	**Sacrophagidae (Diptera)**	**Conopidae (Diptera)**	**Virus**
Ephestia kuehniella	*Boettcharia litorosa*	*Conops flavipes*	Acute bee paralysis virus
Vitula edmandsae	*Brachicoma* spp.	*Physocephala* spp.	Black queen cell virus
	Helicobia morionella	*Sicus ferrugineus*	Deformed wing virus
			Entomopox virus
	Acari	**Braconidae (Hymenoptera)**	Israeli acute paralysis virus
	Locustacarus buchneri	*Syntretus splendidus*	Kashmir bee virus
		Eulophidae (Hymenoptera)	Sacbrood virus
		Melittobia acasta	
		Torymidae (Hymneoptera)	
		Monodontomerus montivagus	
		Protozoa	**Protozoa**
		Crithidia bombi	*Apicystis bombi*
		Crithidia expoeki	*Nosema bombi*
		Nematoda	
		Sphaerularia bombi	
		Fungi	
		Beauveria bassiana	
		Metarhizium anisopliae	
		Bacteria	
		Aerobacter cloaca	
		Spiroplasma spp.	

References (* marked references were used to construct Figure 1)

Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD et al. (2000) The genome sequence of *Drosophila melanogaster*. Science, 287: 2185-2195.

Alford DV (1975) Bumblebees. Davis-Poynter, London, UK.

Al-Mazraawi MS (2007) Impact of the entomopathogenic fungus *Beauveria bassiana* on the honey bees, *Apis mellifera* (Hymenoptera: Apidae). World Journal of Agricultural Sciences, 3: 07-11.*

Anaderson DL and Trueman JWH (2000) *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Experimental and Applied Acarology, 24: 165-189.*

Anderson RM and May RM (1981) The population dynamics of microparasites and their invertebrate hosts. Philosophical Transactions of the Royal Society of London. Series B, 291: 451-524.

Anderson RM and May RM (1982) Coevolution of hosts and parasites. Parasitology, 85: 411-426.

Arensburger P, Megy K, Waterhouse RM, Abrudan J, Amedeo P et al. (2010) Sequencing of *Culex quinquefasciatus* establishes a platform for mosquito comparative genomics. Science, 330: 86-88.

Aronstein KA and Murray KD (2010) Chalkbrood disease in honey bees. Journal of Invertebrate Pathology, 103: S20-S29.*

Ayvaz A and Karabörklü S (2008) Effect of cold storage and different diets on *Ephestia kuehniella* Zeller (Lep:Pyralidae). Journal of Pesticide Science, 81: 57-62.*

Bonasio R, Zhang G, Ye C, Mutti NS, Fang X et al. (2010) Genomic comparison of the ants *Camponotus floridanus* and *Harpegnathos saltator*. Science, 329: 1068-1071.

Boncristiani HF, Di Prisco G, Pettis JS, Hamilton M and Chen YP (2009) Molecular approaches to the analysis of deformed wing virus replication and pathogenesis in the honey bee, *Apis mellifera*. Virology Journal, 6: 221.*

Bromenshenk JJ, Henderson CB, Wick CH, Stanford MF, Zulich AW et al. (2010) Iridovirus and Microsporidian Linked to Honey Bee Colony Decline. PloS ONE, 5(10): e13181.

Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N et al. (2011) Patterns of widespread decline in North American bumble bees. Proceedings of the National Academy of Sciences USA, 108: 662-667.

Chen Y, Evans JD, Zhou L, Boncristiani H, Kimura K et al. (2009) Asymmetrical coexistence of *Nosema ceranae* and *Nosema apis* in honey bees. Journal of Invertebrate Pathology, 101: 204-209.

Clark TB (1977) *Spiroplasma* sp., a new pathogen in honey bees. Journal of Invertebrate Pathology, 29: 112-113.*

Clark TB, Whitcomb RF, Tully JG, Mouches C, Saillard C et al. (1985) *Spiroplasma melliferum*, a new species from the honeybee (*Apis mellifera*). International Journal of Systematic Bacteriology, 35: 296-308.*

Cremer S, Armitage SAO and Schmid-Hempel P (2007) Social Immunity. Current Biology, 17: R693-R702.

Crozier RH (1975) Animal Cytogenetics, 3 Insecta (7) Hymenoptera. Gebruder Borntraeger, Berlin and Stuttgart, 95p.

de Carvalho CJB and de Mello-Patiu CA (2008) Key to the adults of the most common forensic species of Diptera in South America. Revista Brasileira de Entomologia, 52: 390-406.*

de Graaf DC, Raes H, Sabbe G, de Rycke PH and Jacobs FJ (1994) Early Development of *Nosema apis* (Microspora: Nosematidae) in the Midgut Epithelium of the Honeybee (*Apis mellifera*). Journal of Invertebrate Pathology, 63: 74-81.

de Miranda JR, Cordoni G and Budge G (2010) The Acute bee paralysis virus-Kashmir bee virus-Israeli acute paralysis virus complex. Journal of Invertebrate Pathology, 103: S30-S47.*

Duguid JP and Wilkinson JF (1953) The influence of cultural conditions on polysaccharide production by *Aerobacter aerogenes*. Journal of General Microbiology, 9: 174-189.*

Ebert D and Herre EA (1996) The evolution of parasitic diseases. Parasitology Today, 12: 96-101.

Estoup A, Scholl A, Pouvreau A and Solignac M (1995) Monoandry and polyandry in bumble bees (Hymenoptera; Bombinae) as evidenced by highly variable microsatellites. Molecular Ecology, 4: 89-93.

Estoup A, Solignac M, Cornuet JM, Goudet J and Scholl A (1996) Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. Molecular Ecology, 5: 19-31.

Evans JD, Aronstein K, Chen YP, Hetru C, Imler J-L et al. (2006) Immune pathways and defence mechanisms in honey bees *Apis mellifera*. Insect Molecular Biology 15: 645-656.

Fisher RM (1988) Observations on the behaviours of three European cuckoo bumble bee species (*Psithyrus*). Insectes Sociaux, 35: 341-354.

Fouks B and Lattorff HMG (2011) Recognition and avoidance of contaminated flowers by foraging bumblebees (*Bombus terrestris*). PLoS ONE (*in press*), DOI: 10.1371/journal.pone.0026328.

Gandon S, Jansen VA and van Baalen M (2001) Host life history and the evolution of parasite virulence. Evolution, 55: 1056-1062.

Genersch E and Aubert M (2010) Emerging and re-emerging viruses of the honey bee (*Apis mellifera* L.). Veterinary Research, 41: 54.*

González JM, Terán JB and Matthews RW (2004) Review of the biology of *Melittobia acasta* (Walker) (Hymenoptera: Eulophidae) and additions on development and sex ratio of the species. Caribbean Journal of Science, 40: 52-61.*

Goulson D (2003) Bumblebees - Their Behaviour and Ecology. Oxford University Press, Oxford, UK.*

Goulson D, Lye GC and Darvill B (2008) Decline and conservation of bumble bees. Annual Review of Entomology, 53: 191-208.

Grissel EE (2007) Torymidae (Hymenoptera: Chalcidoidea) associated with bees (Apoidea), with a list of chalcidoid bee parasitoids. Journal of Hymenoptera Research, 16: 258-259.*

Holt RA, Subramanian GM, Halpern A, Sutton GG, Charlab R et al. (2002) The genome sequence of the malaria mosquito *Anopheles gambiae*. Science, 298: 129-149.

Honeybee Genome Sequencing Consortium (2006) Insights into social insects from the genome of the honeybee *Apis mellifera*. Nature, 443: 931-949.

Hultmark D (1993) Immune reactions in *Drosophila* and other insects: a model for innate immunity. Trends in Genetics, 9: 178-183.

Imhoof B and Schmid-Hempel P (1998) Single colon and mixed clone infections versus host environment in *Crithidia bombi* infecting bumblebees. Parasitology, 117: 131-136.

International Silkworm Genome Consortium (2008) The genome of a lepidopteran model insect, the silkworm *Bombyx mori*. Insect Biochemistry and Molecular Biology, 38: 1036-1045.

Khelifa M, Massé D, Blanc S and Drucker M (2010) Evaluation of the minimal replication time of *Cauliflower mosaic virus* in different hosts. Virology, 396: 238-245.*

Klee J, Tay WT and Paxton RJ (2006) Specific and sensitive detection of *Nosema bombi* (Microsporidia: Nosematidae) in bumble bees (*Bombus* spp.; Hymenoptera: Apidae) by PCR of partial rRNA gene sequences. Journal of Invertebrate Pathology, 91: 98-104.

Klee J, Besana AM, Genersch E, Gisder S, Nanetti A et al. (2007) Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. Journal of Invertebrate Pathology, 96: 01-10.

Larsson JIR (2005) Fixation of microsporidian spores for electron microscopy. Journal of Invertebrate Pathology, 90: 47-50.

Lanzi G, de Miranda JR, Boniotti MB, Cameron CE, Lavazza A et al. (2006) Molecular and biological characterization of deformed wing virus of honeybees (*Apis mellifera* L.). Journal of Virology, 80: 4998-5009.*

Lipa JJ and Triggiani O (1988) *Crithidia bombi* sp. n. a flagellated parasite of a bumble-bee *Bombus terrestris* L. (*Hymenoptera, Apidae*). Acta Protozoologica, 27: 287-290.*

Lipa JJ and Triggiani O (1996) *Apicystis* gen nov and *Apicystis bombi* (Liu, Macfarlane & Pengelly) comb nov (Protozoa:Neogregarinida), a cosmopolitan parasite of *Bombus* and *Apis* (Hymenoptera: Apidae). Apidologie, 27: 29-34.*

Locatelli DP and Limonta L (1998) Development of *Ephestia kuehniella* (Zeller), *Plodia interpunctella* (Hübner) and *Corcyra cephalonica* (Stainton) (Lepidoptera:Pyralidae) on kernels and wholemeal flours of *Fagopyrum esculentum* (Moench) and *Triticum aestivum* L. Journal of Stored Products Research, 34: 269-276.*

Lynch M (2007) The origins of genome architecture. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.

Madel G. (1966) Beiträge zur Biologie von *Sphaerularia bombi* LEON DUFOUR 1837. Zeitschrift für Parasitenkunde, 28: 99-107.*

Manning JE and Wolstenholme DR (1976) Replication of kinetoplast DNA of *Crithidia acanthocephali*. The Journal of Cell Biology, 70: 406-418.*

Martin-Hernández R, Meana A, Prieto L, Salvador AM, Garrido-Bailón E et al. (2007) Outcome of Colonization of *Apis mellifera* by *Nosema ceranae*. Applied and Environmental Microbiology, 73: 6331-6338.

McCoy RE, Davis MJ and Dowell RV (1981) In vivo cultivation of Spiroplasmas in larvae of the greater wax moth. Phytopathology, 71: 408-411.*

McIvor CA and Malone LA (1995) *Nosema bombi*, a microsporidian pathogen of the bumble bee *Bombus terrestris* (L.). New Zealand Journal of Zoology, 22: 25-31.*

Meeus I, Brown MJF, de Graaf DC and Smagghe G (2011) Effects of invasive parasites on bumble bee declines. Conservation Biology, 25: 662-671.

Moino A, Alves SB, Lopes RB, Neves PMOJ, Pereira RM et al. (2002) External development of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in the subterranean termite *Heterotermes tenuis*. Scientia Agricola, 59: 267-273.*

Nene V, Wortman JR, Lawson D, Haas B, Kodira C et al. (2007) Genome sequence of *Aedes aegypti*, a major arbovirus vector. Science, 316: 1718-1723.

Oldroyed BP (1999) Coevolution while you wait: *Varroa jacobsoni*, a new parasite of western honeybees. Trends in Ecology & Evolution, 14: 312-315.*

Palacios G, Hui J, Quan PL, Kalkstein A, Honkavuori KS et al. (2008) Genetic Analysis of Israel Acute Paralysis Virus: Distinct Clusters Are Circulating in the United States. Journal of Virology, 82: 6209-6217.*

Paxton RJ, Klee J, Korpela S and Fries I (2007) *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. Apidologie, 38: 558-565.

Payne CM, Laverty TM and Lachance MA (2003) The frequency of multiple paternity in bumble bee (*Bombus*) colonies based on microsatellite DNA at the B10 locus. Insectes Sociaux, 50: 375-378.

Poinar GO and van der Laan PA (1972) Morphology and life history of *Sphaerularia bombi*. Nematologica, 18: 239-252.*

Popp M and Lattorff HMG (2011) A quantitative in vitro cultivation technique to determine cell number and growth rates in strains of *Crithidia bombi* (Trypanosomatidae), a parasite of bumblebees. Journal of Eukaryotic Microbiology, 58: 07-10.*

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O et al. (2010) Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution. 25: 345-353.

Reber-Funk C, Schmid-Hempel R and Schmid-Hempel P (2006) Microsatellite loci for *Bombus* spp. Molecular Ecology Notes, 6: 83-86.

Ribière M, Olivier V and Blanchard P (2010) Chronic bee paralysis: A disease and a virus like no other? Journal of Invertebrate Pathology, 103: S120-S131.*

Robledo-Monterrubio M, Alatorre-Rosas R, Viniegra-González G and Loera O (2009) Selection of improved *Beauveria bassiana* (Bals.) Vuill. strains based on 2-deoxy-D-glucose resistance and physiological analysis. Journal of Invertebrate Pathology, 101: 222-227.*

Rosenkranz P, Aumeier P and Ziegelmann B (2010) Biology and control of *Varroa destructor*. Journal of Invertebrate Pathology, 103: S96-S119.*

Roth O, Sadd BM, Schmid-Hempel P and Kurtz J (2009) Strain-specific priming of resistance in the red flour beetle, *Tribolium castaneum*. Proceedings of the Royal Society B 276: 145-151.

Sadd BM and Schmid-Hempel P (2006) Insect immunity shows specificity in protection upon secondary pathogen exposure. Current Biology. 16: 1206-10.

Sadd BM and Schmid-Hempel P (2007) Facultative but persistent transgenerational immunity via the mother's eggs in bumblebees. Current Biology, 17: R1046-R1047.

Sadd BM, Kube M, Klages S, Reinhardt R and Schmid-Hempel P (2010) Analysis of a normalised expressed sequence tag (EST) library from a key pollinator, the bumblebee *Bombus terrestris*. BMC Genomics, 11: 110.

Schmid-Hempel P (1995) Parasites and social insects. Apidologie, 26: 255-271.

Schmid-Hempel P (1998) Parasites in social insects. Princeton University Press, Princeton, New Jersey, USA.*

Schmid-Hempel P (2001) On the evolutionary ecology of host–parasite interactions: addressing the question with regard to bumblebees and their parasites. Naturwissenschaften, 88: 147-158.

Schmid-Hempel P (2005) Natural insect host–parasite systems show immune priming and specificity: puzzles to be solved. BioEssays, 27: 1026-1034.

Schmid-Hempel P (2009) Immune defence, parasite evasion strategies and their relevance for 'macroscopic phenomena' such as virulence. Philosophical Transactions of the Royal Society B, 364: 85-98.

Schmid-Hempel P (2011) Evolutionary Parasitology - The integrated study of infections, immunology, ecology and genetics. Oxford University Press Inc., New York, USA.

Schmid-Hempel R and Schmid-Hempel P (2000) Female mating frequencies in *Bombus* spp. from Central Europe. Insectes Sociaux, 47: 36-41.

Simpson L, Simpson AM and Wesley RD (1974) Replication of the kinetoplast DNA of *Leishamnia tarentolae* and *Crithidia fasciculate*. Biochimica et Biophysica Acta, 349: 161-172.*

Simone M, Evans JD and Spivak M (2009) Resin collection and social immunity in honey bees. Evolution, 63: 3016-3022.

Siva-Jothy MT, Moret Y and Rolff J (2005) Insect immunity: An evolutionary ecology perspective. Advances in Insect Physiology, 32: 01-48.

Sladen FWL (1912) The humble-bee - Its life-history and how to domesticate it. London, Macmillan and co., limited, St.Martin´s street, London, UK.

Smith CD, Zimin A, Holt C, Abouheif E, Benton R et al. (2011a) Draft genome of the globally widespread and invasive Argentine ant (*Linepithema humile*). Proceedings of the National Academy of Sciences USA, 108: 5673-5678.

Smith CR, Smith CD, Robertson HM, Helmkampf M and Zimin A (2011b) Draft genome of the red harvester ant *Pogonomyrmex barbatus*. Proceedings of the National Academy of Sciences USA, 108: 5667-5672.

Strassmann J (2001) The rarity of multiple mating by females in the social Hymenoptera. Insectes Sociaux, 48: 01-13.

Stolle E, Rohde M, Vautrin D, Solignac M, Schmid-Hempel P et al. (2009) Novel microsatellite DNA loci for *Bombus terrestris* (Linnaeus, 1758). Molecular Ecology Resources, 9: 1345-1352.

Stolle E, Wilfert L, Schmid-Hempel R, Schmid-Hempel P, Kube M et al. (2011) A second generation genetic map of the bumblebee *Bombus terrestris* (Linnaeus, 1758) reveals slow genome and chromosome evolution in the Apidae. BMC Genomics, 12: 48.

Suen G, Teiling C, Li L, Holt C, Abouheif E et al. (2011) The genome sequence of the leaf-cutter ant *Atta cephalotes* reveals insights into its obligate symbiotic lifestyle. PLoS Genetics, 7(2):e1002007.

Traver BE and Fell RD (2011) Prevalence and infection intensity of *Nosema* in honey bee (*Apis mellifera* L.) colonies in Virginia. Journal of Invertebrate Pathology, 107: 43-49.*

Tribolium Genome Sequencing Consortium (2008) The genome of the model beetle and pest *Tribolium castaneum*. Nature, 452: 949-955.

Trivers RL and Hare H (1976) Haplodiploidy and the evolution of the social insects. Science, 191: 249-263.

vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E et al. (2009) Colony Collapse Disorder: A descriptive study. PLoS ONE, 4(8): e6481.

van Honk C, Röseler PF, Velthuis HHW and Malotaux M (1981) The conquest of a *Bombus terrestris* colony by a *Psithyrus vestalis* female. Apidologie, 12: 57-67.

Velthuis HHW and van Doorn A (2006) A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. Apidologie, 37: 421-451.

Webster TC, Pomper KW, Hunt G, Thacker EM and Jones SC (2004) *Nosema apis* infection in worker and queen *Apis mellifera*. Apidologie 5: 49-54.

Wilfert L, Gadau J and Schmid-Hempel P (2006) A core linkage map of the bumblebee *Bombus terrestris*. Genome, 49: 1215-1226.

Yarwood CE (1956) Generation Time and the Biological Nature of Viruses. The American Naturalist, 90: 97-102.*

Yoneda M, Furuta H, Kanbe Y, Tsuchida K, Okabe K et al. (2008) Reproduction and transmission within a colony of bumblebee tracheal mite *Locustacarus buchneri* (Acari: Podapolipidae) in *Bombus terrestris* (Hymenoptera: Apidae). Applied Entomology and Zoology, 43: 391–395.*

Yourth CP and Schmid-Hempel P (2006) Serial passage of the parasite *Crithidia bombi* within a colony of its host, *Bombus terrestris*, reduces success in unrelated hosts. Proceedings of the Royal Society B, 273: 655-659.*

Youssef NN and McManus WR (1991) In vitro culture of *Ascosphaera aggregata* (Ascosphaeraceae), a pathogen of the alfalfa leafcutting bee *Megachile rotundata* (Apidae). Journal of Invertebrate Pathology, 58: 335-347.*

Zanchi C, Troussard J-P, Martinaud G, Moreau J and Moret Y (2011) Differential expression and costs between maternally and paternally derived immune priming for offspring in an insect. Journal of Animal Ecology, DOI: 10.1111/j.1365-2656.2011.01872.x.

Online reference

http://www.textbookofbacteriology.net/growth.html*

Chapter 2

The degree of parasitism of the bumblebee (*Bombus terrestris*) by cuckoo bumblebees (*Bombus* (*Psithyrus*) *vestalis*)

Silvio Erler* and H. Michael G. Lattorff

Institut für Biologie, Molekulare Ökologie, Martin-Luther-Universität Halle-Wittenberg,

Hoher Weg 4, 06099 Halle (Saale), Germany

* e-mail: silvio.erler@zoologie.uni-halle.de

Abstract

Host-parasite systems are characterised by coevolutionary arms races between host and parasite. Parasites are often the driving force, as they replicate much faster than their hosts and have shorter generation times and larger population sizes, resulting in higher mutation rates per time interval. This scenario does not fit all host–parasite systems. Socially parasitic cuckoo bumblebees (*Bombus* (*Psithyrus*) *vestalis*) parasitise colonies of *Bombus terrestris* share most life history characteristics with their hosts. As they parasitise only a subset of all available colonies, their population size should be lower than that of their hosts. This might have strong negative effects on the genetic diversity of *B. vestalis* and their adaptability. Here, we study for the first time the population structure of a *Bombus / Bombus* (*Psithyrus*) system. Highly polymorphic DNA markers were used to reconstruct sibships from individuals collected in the wild. The analysis of the host and parasite populations revealed a rate of parasitism of about 42% (range 33-50%). The population size of *B. vestalis* was lower compared to their hosts, which was also reflected in low within-group genetic distance. An analysis of the reconstructed queen genotypes revealed more supersisters amongst the *B. vestalis* queens when compared to the *B. terrestris* host. The data suggest that *B. vestalis* females and males do not disperse over long distances. This shows a potential for local adaptation to their hosts.

Keywords: Social parasite, *Psithyrus*, Bumblebees, Sibship reconstruction

Received: 26 May 2009 / Revised: 1 March 2010 / Accepted: 25 March 2010 / Published online: 22 April 2010

Insectes Sociaux (2010) 57: 371-377

Supplementary Material:

http://www.springerlink.com/content/j0813787818g3406/MediaObjects/40_2010_93_MOESM1_ESM.pdf

DOI: 10.1007/s00040-010-0093-2

Chapter 3

Comparative analysis of detection limits and specificity of molecular diagnostic markers for three pathogens (Microsporidia, *Nosema* spp.) in the key pollinators *Apis mellifera* and *Bombus terrestris*

Silvio Erler*, Stefanie Lommatzsch and H. Michael G. Lattorff

Institut für Biologie, Molekulare Ökologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 4, 06099 Halle (Saale), Germany

* e-mail: silvio.erler@zoologie.uni-halle.de

Abstract

Global pollinator decline has recently been discussed in the context of honey and bumble bees infections from various pathogens including viruses, bacteria, microsporidia and mites. The microsporidian pathogens *Nosema apis*, *N. ceranae* and *N. bombi* may in fact be major candidates contributing to this decline. Different molecular and non-molecular detection methods have been developed, however, a comparison, especially of the highly sensitive PCR based methods, is currently lacking. Here we present the first comparative quantitative real-time PCR study of 9 *Nosema* spp. primers within the framework of primer specificity and sensitivity. With the help of dilution series of defined numbers of spores we reveal six primer pairs amplifying *N. apis*, six for *N. bombi* and four for *N. ceranae*. All appropriate primer pairs detected an amount of at least 10^4 spores, the majority of which were even as sensitive to detect such low amounts as 10^3 to 10 spores. Species specificity of primers was observed for *N. apis* and *N. bombi*, but not for *N. ceranae*. Additionally, we did not find any significant correlation for the amplified fragments with PCR efficiency or the limit of detection. We discuss our findings on the background of false positive and negative results using quantitative real-time PCR. On the basis of these results future research might be based on appropriate primer selection depending on the experimental needs. Primers may be selected on the basis of specificity or sensitivity. Pathogen species and load may be determined with higher precision enhancing all kinds of diagnostic studies.

Received: 14 July 2011 / Accepted: 2 September 2011 / Published online: 17 September 2011

Parasitology Research (2011) Online First

Online Resources:

http://www.springerlink.com/content/8h52j61740956w67/MediaObjects/436_2011_2640_MOESM1_ESM.pdf

DOI: 10.1007/s00436-011-2640-9

Chapter 4

Dynamics of Immune System Gene Expression upon Bacterial Challenge and Wounding in a Social Insect (*Bombus terrestris*)

Silvio Erler*, Mario Popp, H. Michael G. Lattorff

Institut für Biologie, Molekulare Ökologie, Martin-Luther-Universität Halle-Wittenberg,

Hoher Weg 4, 06099 Halle (Saale), Germany

* e-mail: silvio.erler@zoologie.uni-halle.de

Abstract

The innate immune system which helps individuals to combat pathogens comprises a set of genes representing four immune system pathways (Toll, Imd, JNK and JAK/STAT). There is a lack of immune genes in social insects (e.g. honeybees) when compared to Diptera. Potentially, this might be compensated by an advanced system of social immunity (synergistic action of several individuals). The bumble bee, *Bombus terrestris*, is a primitively eusocial species with an annual life cycle and colonies headed by a single queen. We used this key pollinator to study the temporal dynamics of immune system gene expression in response to wounding and bacterial challenge. Antimicrobial peptides (AMP) (abaecin, defensin 1, hymenoptaecin) were strongly up-regulated by wounding and bacterial challenge, the latter showing a higher impact on the gene expression level. Sterile wounding down-regulated TEP A, an effector gene of the JAK/STAT pathway, and bacterial infection influenced genes of the Imd (relish) and JNK pathway (basket). Relish was up-regulated within the first hour after bacterial challenge, but decreased strongly afterwards. AMP expression following wounding and bacterial challenge correlates with the expression pattern of relish whereas correlated expression with dorsal was absent. Although expression of AMPs was high, continuous bacterial growth was observed throughout the experiment. Here we demonstrate for the first time the temporal dynamics of immune system gene expression in a social insect. Wounding and bacterial challenge affected the innate immune system significantly. Induction of AMP expression due to wounding might comprise a pre-adaptation to accompanying bacterial infections. Compared with solitary species this social insect exhibits reduced immune system efficiency, as bacterial growth could not be inhibited. A negative feedback loop regulating the Imd-pathway is suggested. AMPs, the end product of the Imd-pathway, inhibited the up-regulation of the transcription factor relish, which is necessary for effector gene expression.

Received: 15 October 2010 / Accepted: 24 February 2011 / Published: 29 March 2011

PLoS ONE (2011) 6(3): e18126

Supporting Information: http://www.plosone.org/article/info:doi/10.1371/journal.pone.0018126

DOI: 10.1371/journal.pone.0018126

Chapter 5 – Synopsis

Insects, especially social insects, belong to the best studied groups with respect to life-history evolution, behavioural ecology, sexual selection and host-pathogen interactions (Hölldobler and Wilson, 1990; Moritz and Southwick, 1992; Schmid-Hempel, 1998). The reciprocal control of life histories is the central aspect in host-parasite interactions (Anderson and May, 1979a, b, 1981). Parasite fitness is completely dependent on its success in infecting and reproducing in a host; and interactions with the host may determine the size of parasite populations.

Social insects and their parasites provide a huge variety of research topics related to the social structure and close relatedness of colony members and its influence on parasite transmission (Schmid-Hempel, 1955, 1998).

The topics covered in this thesis are the development and application of new molecular tools, which were used to improve our understanding of host-parasite interactions:

1. Estimation of host and parasite population structure and size by means of highly polymorphic microsatellites and sibship reconstruction (*B. terrestris* and the cuckoo bumblebee *B. vestalis*, Chapter 2).
2. Determination of parasite loads per host individual with species specific and highly sensitive detection methods (*Apis mellifera*, *B. terrestris* and the microsporidians *Nosema apis*, *bombi* and *ceranae*, Chapter 3).
3. Quantification of host immune response and *in vivo* measurement of parasite development (*B. terrestris* and the gram-negative bacteria *E. coli*, Chapter 4).

5.1 How strong is the impact of parasitism for social parasites and how diverse are host and parasite species?

Host-parasite interactions and their evolutionary consequences are usually studied in hosts and their microparasites. However, less attention has been paid to ‘equally sized’ systems with respect to their generation times and population sizes. This can be studied excellently using parasitic cuckoo bumblebees which take over colonies of *B. terrestris* (Kilner and Langmore, 2011; van Honk et al., 1981). Estimating the prevalence of cuckoo bumblebees within host populations requires the excavation of all host colonies, a task that is almost impossible due to the hidden location of colonies (Carvell et al., 2008; Müller and Schmid-

Hempel, 1992; Sladen, 1912). For socially parasitic cuckoo bumblebees, the prevalence has been estimated as 30 to 100% using artificially placed host colonies. Unfortunately, these values might be an overestimate due to enhanced host finding (Carvell et al., 2008; Müller and Schmid-Hempel, 1992). Here, we demonstrate that the combination of population genetic tools and appropriate statistical methods allow for an estimation of the number of infected host colonies under natural conditions for the host bumblebee *B. terrestris* and the obligate specialist brood parasite *B. vestalis* (see Chapter 2). Sampling free flying drones of the parasite species and foraging workers and / or drones of the host species allow for precise sibship assignment using their genotypes derived from highly polymorphic markers. The estimated prevalence was 33-50% indicating a much smaller population size for *B. vestalis*. Lower genetic distance between parasite queens compared to host queens supported by the discovery of more super-sisters for the parasite might be a sign for local adaptation due to the absence of long distance dispersal of the queens. Recently, our expected host-parasite adaptation for the *Bombus / Bombus* (*Psithyrus*) system was confirmed for six couples of bumblebees and cuckoo bumblebees (Antonovics and Edwards, 2011). This study also found that parasite population sizes lay still under the population sizes of the host, which enforces the concept that parasitic cuckoo bumblebees are specialists and might be exposed more strongly to effects of genetic drift. However, despite the smaller population size no signs for inbreeding were detected in the parasite population (see Chapter 2).

5.2 What is the limit of parasite detection and how specific are such detection systems?

For the system *Bombus / Bombus* (*Psithyrus*) the host and parasite share the same life-cycle (generation time) but differ in population size. Hence, most of the common parasites show shorter generation times due to less complex life-cycles and might have a higher impact on host colony decline. Recent studies highlighted *Nosema* spp. as a major cause of pollinator decline or ‘Colony Collapse Disorder’ (CCD) - the sudden loss of all individuals from hives, in honeybees (Bromenshenk et al., 2010; Cameron et al., 2011). Estimates for the prevalence should be based on huge sample sizes in order to avoid false negatives. On the other hand, differentiation between distinct *Nosema* species using microscopical techniques is time consuming and not always reliable. PCR-based methods allow testing huge amounts of samples and offers the possibility of differentiating between species and, probably also, between genotypes within species (Chaimanee et al., 2011; Klee et al. 2007; Martin-Hernández et al., 2007; Medici et al., 2011; Sagastume et al., 2011). Unfortunately, all known

methods were established with different aims and thus largely differ with respect to the methodology used for DNA extraction and further downstream applications, which might results in false positive or negative results.

Therefore, we compared nine different *Nosema* spp. primer pairs under defined standard conditions (see Chapter 3); using the same method for spore and DNA extraction for three *Nosema* species. Moreover, a dilution series was used to quantify the sensitivity of the molecular markers. Nearly all primer sets showed high sensitivity and, for each parasite species, highly specific primer sets were found. Using this standard assay simplifies *Nosema*-infection experiments. Defined numbers of *Nosema* spores are needed to infect 50% of healthy honey bees (ID 50). This number differs between *N. apis* (~390 spores) and *N. ceranae* (~85 spores) (Forsgren and Fries, 2010). At least one of the nine tested primer sets detects such a low amount of spores for both parasite species. Standardized DNA extraction and subsequent analysis using either species specific or highly sensitive primer sets will enhance future research on the impact of *Nosema* on 'CCD' in honeybees or bumblebee decline (Cameron et al., 2011; Goulson et al., 2008; Meeus et al., 2011; Potts et al., 2010).

5.3 Is the host innate immune system influenced by parasite dynamics and does the host defence system show temporal patterns?

Another challenge is the determination of parasite performance and the temporal process during infections. In combination with an analysis of the hosts' response towards the parasite, it will enhance the understanding of the molecular processes involved in resistance or susceptibility of hosts as well as the virulence of parasites.

We were able to quantify pathogen dynamics in a social insect during the course of infection with the gram-negative bacteria *E. coli* (see Chapter 4). The bumblebee immune system was not able to reduce the bacterial growth completely during 24 hours post-infection, as previous studies described (Haine et al., 2008). This might be due to a lack of immune genes in social insects compared to solitary insects (Evans et al, 2006). We focussed our study on the so far unknown innate immune system of bumblebees. The *B. terrestris* innate immune system effector gene expression (antimicrobial peptides-AMPs) showed significant up-regulation after non-septic wounding and bacterial infection. Additionally, other effector and regulatory genes (e.g., TEP A, basket) were also influenced by either injection or bacterial infection. Recently, similar temporal dynamics of gene expression (signalling, enzymatic processes and respiration) were shown for controlled infections of *B. terrestris* with the gut parasite

Crithidia bombi. As expected from our study, long-lasting protection was shown for only a few genes (Riddell et al., 2011). Expression of AMPs in response to bacterial and trypanosome infection belongs to the late group (> 12h) and hence might also be important for immune priming.

Large population sizes of the parasite are not necessary to activate host immune response, because only small amount of surface related molecules (e.g. carbohydrates, lipids and peptides) are needed for activation (Beckage, 2008). Parasite recognition did not correlate with the amount of parasites. Yet, the amount of effector gene expression might directly correlate with the amount of parasites, as shown for *B. terrestris* and *E. coli* (see Chapter 4). Therefore, harming the host and consequently decreasing host fitness depends directly on parasites generation time and resulting population size. The combination of finding significant differences in immune gene expression due to wounding and micro-parasite infection enforces the theory that the host might modulate its immune system by ascertainment of a combination of signals from pathogens and damaged tissue (Lazzaro and Rolff, 2011).

Not to be neglected in this context is the major fact that social insects showed less innate immune system genes than non-social insects. Only 17-48% of the immune genes known from other, non-social insects are found in the sequenced genomes of bees and ants (see introduction for references). Innate immunity at the individual level and non-immunological defence mechanisms acting at the group level coexist and together represent the heritable entire immune competence of invertebrate hosts (Parker et al., 2011). Consequently, the correlation between parasite growth and host defence in the bumblebee *B. terrestris* has to be analysed in the light of a reduced innate immune gene repertoire and the group level defences summarized as ‘social immunity’ (Cremer et al., 2007).

Finally, we developed and applied successfully molecular tools to increase the knowledge of interaction between social insects, as host organism, and their parasites. Sufficient highly polymorphic microsatellites can be used to characterise easily the cuckoo bumblebee / *Bombus* system and to study bumblebee decline in general. Estimated detection limits for *Nosema* spp. and therewith species differentiation will help to find the ‘CCD’ triggering mechanisms in honey bees and the mechanisms behind the global bumblebee decline. The new knowledge about the *B. terrestris* innate immune system gene expression, temporal dynamics of bacterial infections and effects of non-septic wounding will expand the field of determining host immune responses towards parasite attacks.

Chapter 6 – Zusammenfassung

Insekten, insbesondere soziale Insekten, gehören zur Gruppe der am intensivsten studierten Organismen, vor allem im Kontext der Evolution der Individualentwicklung, der Verhaltensökologie, der sexuellen Selektion und der Wirt-Parasit-Interaktionen (Hölldobler und Wilson, 1990; Moritz und Southwick, 1992; Schmid-Hempel, 1998). Die gegenseitige Kontrolle der Individualentwicklung spielt bei Wirt-Parasit Interaktionen eine zentrale Rolle (Anderson und May, 1979a, b, 1981). Die Fitness des Parasiten ist zum einem davon abhängig, ob der Wirt erfolgreich infiziert werden kann, und zum anderen von einer erfolgreichen Reproduktion, in oder auf dem Wirt. Diese und andere Interaktionen mit dem Wirt bestimmen möglicher Weise die Populationsgröße des Parasiten. Wegen ihrer sozialen Strukturen und der sehr engen Verwandtschaftsverhältnisse der einzelnen Mitglieder einer Kolonie, welche insbesondere die Übertragung von Parasiten beeinflussen (Schmid-Hempel, 1995, 1998), bieten soziale Insekten und ihre Parasiten eine große Auswahl an Forschungsfeldern.

Die verschiedenen Kapitel dieser Arbeit beschäftigen sich mit der (Weiter-) Entwicklung und Anwendung neuer molekularer Methoden, die unser Verständnis von Wirt-Parasit-Interaktionen erweitern sollen:

1. Bestimmung von Wirt- und Parasit-Populationsstrukturen, und deren Größe, unter Zuhilfenahme hoch polymorpher Mikrosatelliten, sowie Rekonstruktion verwandtschaftlicher Verhältnisse (*B. terrestris* und die Kuckuckshummel *B. vestalis*, Kapitel 2)
2. Ermittlung der Parasitenanzahl pro Wirtstier mittels artspezifischer und hoch sensitiver Detektionsmethoden (*Apis mellifera*, *B. terrestris* und die Mikrosporidien *Nosema apis*, *bombi* und *ceranae*, Kapitel 3)
3. Quantifizierung der Wirtsimmunantwort und *in vivo* Messungen zur Parasiten-entwicklung (*B. terrestris* und das gram-negative Bakterium *E.coli*, Kapitel 4)

6.1 Wie stark beeinfussen Sozialparasiten Wirt-Parasit-Interaktionen und wie stark unterscheiden sich Wirte und Parasiten?

Wirt-Parasit-Interaktionen und deren evolutive Bedeutung werden meist an Wirten und Mikroparasiten studiert. Wenig Aufmerksamkeit wird jedoch dabei auf „gleich große“ Systeme, bezüglich Generationszeiten und Populationsgrößen, gelegt. Diese Systeme können allerdings anhand von parasitischen Kuckuckshummeln mit dem Wirt *B. terrestris* leicht untersucht werden (Kilner und Langmore, 2011; van Honk et al., 1981). Derzeit erfordern Untersuchungen zum Vorkommen solch eines Parasiten innerhalb einer Wirtspopulation das Ausgraben aller Wirtskolonien. Dies ist jedoch nahezu unmöglich, da die Kolonien sehr versteckt liegen (Carvell et al., 2008; Müller und Schmid-Hempel, 1992; Sladen, 1912). Für künstlich aufgestellte Wirtskolonien wurde, innerhalb einer Wirtspopulation, ein Parasiten-vorkommen von 30 bis 100% ermittelt. Diese Werte könnten allerdings, verglichen mit der natürlichen Situation, zu hoch liegen, da die Wirtsfindung für den Parasiten in den aufgestellten Kolonien stark erleichtert wird (Carvell et al., 2008; Müller und Schmid-Hempel, 1992).

Diese Arbeit zeigt, dass durch die Kombination von populationsgenetischen Werkzeugen und passenden statistischen Methoden, die Anzahl infizierter Wirtskolonien bestimmt werden kann. Dies erfolgte für die Wirtshummel *B. terrestris* und ihren obligaten, spezialisierten Brutparasiten *B. vestalis* unter natürlichen Bedingungen (siehe Kapitel 2). Das Sammeln frei fliegender Drohnen der Parasiten bzw. der Arbeiterinnen und / oder der Dronen des Wirts, ermöglicht nach Genotypisierung mittels hoch polymorpher Marker exakte Verwandtschaftsanalysen.

Die in unserer Studie bestimmte Häufigkeit des Auftretens der parasitischen Hummel *B. vestalis* liegt zwischen 33-50%, was für eine sehr viel kleinere Populationsgröße des Parasiten im Vergleich zum Wirt spricht. Die geringere genetische Distanz zwischen den parasitischen Königinnen, verglichen mit den Wirtsköniginnen, unterstützt die Vermutung, dass bei den parasitischen Hummeln lokale Adaption vorliegt. Diese Vermutung wird weiterhin durch die Tatsache belegt, dass mehr ‚*super-sisters*’ gefunden wurden. Lokale Adaption parasitischer Königinnen ist charakterisiert durch das Fehlen von Ausbreitung über lange Distanzen hinweg. Vor Kurzem wurde unsere vermutete Wirt-Parasiten-Adaption für das *Bombus / Bombus* (*Psithyrus*) System für sechs weitere Hummel-Kuckuckshummelpaare bestätigt (Antonovics und Edwards, 2011). Des Weiteren zeigte die Studie von Antonovics und Edwards (2011), dass die Populationsgröße des Parasiten immer unter der

Populationsgröße des Wirtes lag. Dies verstärkt die Theorie, dass parasitische Kuckuckshummeln spezialisiert sind und das sie Effekten durch genetischen Drift sehr viel stärker ausgesetzt sind. Unabhängig von der kleineren Populationsgröße, haben wir keine Anzeichen für Inzucht in den parasitischen Populationen finden können (siehe Kapitel 2).

6.2 Wo liegen die Grenzen für Parasitendetektion und wie spezifisch sind diese Systeme?

Innerhalb des *Bombus / Bombus* (*Psithyrus*) Systems teilen sich Wirt und Parasit den gleichen Lebenszyklus (Generationszeit), haben aber unterschiedliche Populationsgrößen. Die meisten der bekannten Parasiten haben jedoch durch weniger komplexe Lebenszyklen kürzere Generationszeiten als der Wirt und somit möglicherweise einen größeren Einfluss auf das Aussterben der Wirtskolonie. Derzeitige Arbeiten favorisieren *Nosema* spp. als Hauptursache für das Sterben von Bestäubern oder den so genannten ‚*Colony Collapse Disorder*' (*CCD*) bei Honigbienen (Bromenshenk et al., 2010; Cameron et al., 2011). Dies bezeichnet das komplette Sterben aller Individuen einer Kolonie. Bestimmungen des Befallsgrades des Wirts sollten auf einer großen Probenanzahl beruhen, um falsch negative Ergebnisse zu vermeiden. Allerdings ist es ziemlich zeitaufwendig und nicht immer sehr zuverlässig *Nosema* Arten mit mikroskopischen Methoden zu unterscheiden. Eine bessere Möglichkeit verschiedene Arten zu identifizieren, stellen PCR-basierte Methoden dar. Diese erlauben u.a. die Untersuchung sehr großer Probenmengen und bieten die Möglichkeit verschiedene Genotypen innerhalb einer Art zu ermitteln (Chaimanee et al., 2011; Klee et al. 2007; Martin-Hernández et al., 2007; Medici et al., 2011; Sagastume et al., 2011). Leider wurden alle bisher bekannten PCR-basierten Methoden für verschiedene Fragestellungen entwickelt und unterscheiden sich daher sehr stark, besonders im Methodischen, wie z.B. der DNA-Extraktion und weiterer nachfolgender Anwendungen. Diese Unterschiede könnten zu falsch positiven oder falsch negativen Ergebnissen führen.

Um diese Problematik zu umgehen, haben wir neun verschiedene *Nosema* spp. Primerpaare unter standardisierten Bedingungen miteinander verglichen (siehe Kapitel 3). So wurde z.B. für die drei verschiedenen *Nosema* Arten dieselbe Extraktionsmethode für Sporen-DNA angewandt. Des Weiteren wurde eine Verdünnungsreihe genutzt, um die Sensitivität der genutzten Marker zu quantifizieren. Fast alle untersuchten Primerpaare zeigten eine hohe Sensitivität und für jede Parasitenart wurde zusätzlich auch ein hoch artspezifisches Primerpaar ermittelt. Dieser dargestellte Standardtest soll auf *Nosema*-Infektionen basierende Experimente vereinfachen und erleichtern. Um 50% (ID 50) einer gesunden

Honigbienengruppe mit *Nosema* spp. zu infizieren, ist eine bestimmte Sporenanzahl notwendig. Diese Anzahl variiert zwischen *N. apis* (~390 Sporen) und *ceranae* (~85 Sporen) (Forsgren und Fries, 2010). Zumindest eines der neun getesteten Primerpaare konnte solch geringe Sporenmengen für beide Parasitenarten detektieren. Standardisierte DNA-Extraktionen und nachfolgende Analysen mittels artspezifischer und hoch sensitiver Primerpaare werden weitere Forschungen auf diesem Gebiet ermöglichen, und weiterhelfen herauszufinden welchen Einfluss *Nosema* spp. auf ‚*CCD*' bei Honigbienen und das Aussterben verschiedener Hummelarten hat (Cameron et al., 2011; Goulson et al., 2008; Meeus et al., 2011; Potts et al., 2010).

6.3 Beeinflussen Parasitendynamiken das Wirtsimmunsystem und zeigen Abwehrmechanismen der Wirte zeitliche Anpassungen?

Eine weitere Herausforderung stellt die Erforschung der Parasitenentwicklung und des zeitlichen Prozesses während der Infektion dar. Neue Erkenntnisse auf diesem Gebiet, kombiniert mit der Analyse der Wirtsantwort auf parasitische Infektionen, werden die molekularen Prozesse, welche in Wirtsresistenz, Wirtsanfälligkeit oder Virulenz des Parasiten involviert sind, weiter aufklären.

Wir konnten die Pathogendynamik in einem sozialen Insekt während der Infektion mit dem gram-negativen Bakterium *E. coli* quantifizieren (siehe Kapitel 4). Im Gegensatz zu anderen Studien (Haine et al., 2008), war das Immunsystem der Hummel nicht in der Lage, das bakterielle Wachstum innerhalb von 24 Stunden nach der Infektion komplett zu reduzieren. Dies könnte auf die geringe Anzahl von Immungenen in sozialen Insekten, verglichen mit nicht-sozialen Insekten, zurückzuführen sein (Evans et al, 2006). Deshalb konzentrierten wir uns innerhalb unserer Studie auf die bis dahin noch unbekannten Gene des angeborenen Immunsystems der Hummel. Die Genexpression von Effektorgenen des angeborenen Immunsystems von *B. terrestris* (antimikrobielle Peptide (AMPs)) zeigte eine signifikante Hochregulierung nach nicht septischer Verwundung und bakterieller Infektion. Zusätzlich analysierte Effektor- und regulatorische Gene (z.B. TEP A, basket) wurden ebenfalls durch Injektion und bakterielle Infektion beeinflusst. Eine kürzlich veröffentlichte Studie zeigte einen ähnlichen zeitlichen Verlauf der Genexpression (z.B. Signalgene, Gene für enzymatische Prozesse und der Atmung) nach kontrollierter Infektion von *B. terrestris* mit dem Darmparasiten *Crithidia bombi*. Wie bereits aus unserer Studie erwartet, wurde der langanhaltende Schutz nur für einige wenige Gene gezeigt (Riddell et al., 2011). AMPs als

Immunantwort auf bakterielle und Trypanosomeninfektionen gehören zur Gruppe der später agierenden Gene (> 12 Stunden) und könnten außerdem für das so genannte ‚*immune priming*' wichtig sein, dem vererbbaren Immunstatus der Eltern.
Um die Wirtsimmunantwort auszulösen, werden nur geringe Mengen an Oberflächenmolekülen (z.B. Kohlenhydrate, Fette, Eiweiße) benötigt (Beckage, 2008). Die Erkennung von Parasiten korreliert demnach nicht mit der Anzahl an Parasiten; aber die Menge exprimierter Effektorgene korreliert möglicherweise direkt mit der Anzahl an Parasiten, wie für *B. terrestris* und *E. coli* gezeigt (siehe Kapitel 4).
Somit basiert die Schädigung des Wirtes und die folgliche Abschwächung der Wirtsfitness direkt auf der Generationszeit des Parasiten und der daraus folgenden Populationsgröße. Die signifikanten Unterschiede in der Immungenexpression nach Verletzung und Mikroparasiteninfektion verstärken die Annahme, dass Wirte ihr Immunsystem durch die Ermittlung von Pathogensignalen und verletztem Gewebe anpassen können (Lazzaro and Rolff, 2011).
In diesem Zusammenhang darf jedoch nicht vergessen werden, dass soziale Insekten weniger Gene des angeborenen Immunsystems aufweisen als nicht-soziale Insekten. Lediglich 17-48% der bekannten Immungene von nicht-sozialen Insekten wurden bisher in sequenzierten Genomen von Bienen und Ameisen gefunden (Referenzen siehe Einleitung). Angeborene Immunität auf Individuenebene koexistiert mit nicht immunologischen Abwehrmechanismen auf Gruppenebene. Dies repräsentiert zusammen das komplette vererbbare immunologische Potenzial von wirbellosen Wirten (Parker et al., 2011). Somit muss der Zusammenhang zwischen Parasitenwachstum und Wirtsabwehr in der Hummel *B. terrestris* in Anbetracht eines reduzierten Immungenrepertoirs und des Faktors „Gruppe" weiter analysiert werden. Abwehrmechanismen, die durch das Zusammenleben in Gruppen begründet sind, werden als '*social immunity*' zusammengefasst (Cremer et al., 2007).

Im Rahmen dieser Arbeit ist es uns gelungen neue molekulare Methoden zu etablieren und bereits bekannte Methoden auf weitere Fragestellungen anzuwenden. Dies bietet nun die Möglichkeit, Wirt-Parasit-Interaktionen für soziale Insekten und ihre Parasiten noch weiter zu ergründen.
Hoch polymorphe Mikrosatelliten erwiesen sich als sehr hilfreich, Kuckuckshummeln und ihre Wirte auf Populationsebene zu charakterisieren. Diese Methode kann auch genutzt werden, um den weltweiten Rückgang diverser Hummelarten zu messen. Des Weiteren konnten Detektionsgrenzen für die molekulare Analyse von *Nosema* spp. Infektionen bestimmt werden. Einige der genutzten Marker konnten auch zur Artunterscheidung

verwendet werden. Mechanismen, die der Auslöser des weltweit bekannten ‚*CCD*'-Phänomens bei Honigbienen sind sowie der drastische Rückgang verschiedener Hummelarten können mit den gezeigten Methoden weiter erforscht werden. Um die Genexpression des angeborenen Immunsystems der dunklen Erdhummel *B. terrestris* besser zu verstehen, erwiesen sich Injektions- und Infektionsstudien als nützlich. Auch jene zeitlichen Prozesse, die nach bakterieller Infektion oder Verletzung induziert werden, geben Hinweise darauf, wie Wirte auf einen Parasitenangriff reagieren können.

References (Chapter 5 and 6)

Anderson RM and May RM (1979a) Population biology of infectious diseases: Part I. Nature, 280: 361-367.

Anderson RM and May RM (1979b) Population biology of infectious diseases: Part II. Nature, 280: 455-461.

Anderson RM and May RM (1981) The population dynamics of microparasites and their invertebrate hosts. Philosophical Transactions of the Royal Society of London. Series B, 291: 451-524.

Antonovics J and Edwards M (2011) Spatio-temporal dynamics of bumblebee nest parasites (*Bombus* subgenus *Psythirus* ssp.) and their hosts (*Bombus* spp.). Journal of Animal Ecology, 80: 999-1011.

Beckage NE (2008) Insect Immunology. Academic Press, Elsevier, San Diego, California, USA.

Bromenshenk JJ, Henderson CB, Wick CH, Stanford MF, Zulich AW et al. (2010) Iridovirus and Microsporidian Linked to Honey Bee Colony Decline. PloS ONE, 5(10): e13181.

Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N et al. (2011) Patterns of widespread decline in North American bumble bees. Proceedings of the National Academy of Sciences, 108: 662-667.

Carvell C, Rothery P, Pywell RF and Heard MS (2008) Effects of resource availability and social parasite invasion on field colonies of *Bombus terrestris*. Ecological Entomology, 33: 321-327.

Chaimanee V, Chen Y, Pettis JS, Scott Cornman R and Chantawannakul P (2011) Phylogenetic analysis of *Nosema ceranae* isolated from European and Asian honeybees in Northern Thailand. Journal of Invertebrate Pathology, 107: 229-233.

Cremer S, Armitage SAO and Schmid-Hempel P (2007) Social Immunity. Current Biology, 17: R693-R702.

Evans JD, Aronstein K, Chen YP, Hetru C, Imler J-L et al. (2006) Immune pathways and defence mechanisms in honey bees *Apis mellifera*. Insect Molecular Biology 15: 645-656.

Forsgren E, Fries I (2010) Comparative virulence of *Nosema ceranae* and *Nosema apis* in individual European honey bees. Veterinary Parasitology, 170: 212-217.

Goulson D, Lye GC and Darvill B (2008) Decline and conservation of bumble bees. Annual Review of Entomology, 53: 191-208.

Haine ER, Moret Y, Siva-Jothy MT and Rolff J (2008) Antimicrobial Defense and Persistent Infection in Insects. Science, 322: 1257-1259.

Hölldobler B and Wilson EO (1990) The ants. Harvard Univ. Press, Cambridge, Massachusetts, USA.

Kilner RM and Langmore NE (2011) Cuckoos *versus* hosts in insects and birds: adaptations, counter-adaptations and outcomes. Biological Reviews, DOI: 10.1111/j.1469-185X.2010.00173.x.

Klee J, Besana AM, Genersch E, Gisder S, Nanetti A et al. (2007) Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. Journal of Invertebrate Pathology, 96: 1-10.

Lazzaro BP and Rolff J (2011) Immunology. Danger, microbes, and homeostasis. Science, 332: 43-44.

Martin-Hernández R, Meana A, Prieto L, Salvador AM, Garrido-Bailón E et al. (2007) Outcome of colonization of *Apis mellifera* by *Nosema ceranae*. Applied and Environmental Microbiology, 73: 6331-6338.

Medici SK, Sarlo EG, Porrini MP, Braunstein M and Eguaras MJ (2011) Genetic variation and widespread dispersal of *Nosema ceranae* in *Apis mellifera* apiaries from Argentina. Parasitology Research, DOI: 10.1007/s00436-011-2566-2.

Meeus I, Brown MJF, de Graaf DC and Smagghe G (2011) Effects of invasive parasites on bumble bee declines. Conservation Biology, 25: 662-671.

Moritz RFA and Southwick EE (1992) Bees as superorganisms: an evolutionary reality. Springer-Verlag, Berlin, Germany.

Müller CB and Schmid-Hempel P (1992) Correlates of reproductive success among field colonies of *Bombus lucorum*: the importance of growth and parasites. Ecological Entomology, 17: 343-353.

Parker BJ, Barribeau SM, Laughton AM, de Roode JC and Gerardo1 NM (2011) Non-immunological defense in an evolutionary framework. Trends in Ecology & Evolution, 26: 242-248.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O et al. (2010) Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution. 25: 345-53.

Riddell CE, Sumner S, Adams S and Mallon EB (2011) Pathways to immunity: temporal dynamics of the bumblebee (*Bombus terrestris*) immune response against a trypanosomal gut parasite. Insect Molecular Biology, 20: 529-540.

Sagastume S, del Aguila C, Martín-Hernández R, Higes M and Henriques-Gil N (2011) Polymorphism and recombination for rDNA in the putatively asexual microsporidian *Nosema ceranae*, a pathogen of honeybees. Environmental Microbiology, 13: 84-95.

Schmid-Hempel P (1995) Parasites and social insects. Apidologie, 26: 255-271.

Schmid-Hempel P (1998) Parasites in social insects. Princeton University Press, Princeton, New Jersey, USA.

Sladen FWL (1912) The humble-bee - Its life-history and how to domesticate it. London, Macmillan and co., limited, St.Martin´s street, London, UK.

van Honk C, Röseler PF, Velthuis HHW and Malotaux M (1981) The conquest of a *Bombus terrestris* colony by a *Psithyrus vestalis* female. Apidologie, 12: 57-67.

Danksagung

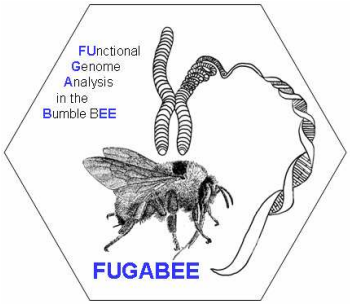
Zu Beginn möchte ich mich bei allen bedanken, die mir während der Zeit der Doktorarbeit immer mit Rat und Tat zur Verfügung standen.

Herrn Prof. Dr. Robin F.A. Moritz und Herrn Dr. H. Michael G. Lattorff danke ich für die Möglichkeit, die Doktorarbeit in dieser Arbeitsgruppe zu diesem faszinierenden Thema durchführen zu können. Außerdem bedanke ich mich bei ihnen für die stets bereitwillige Betreuung und insbesondere für die kreativen und hilfreichen Diskussionen während der Analyse von Daten, des Schreibens von Publikationen, des Zusammenschreibens dieser Arbeit und der Vorbereitung von Konferenzbeiträgen.
Ein großer Dank geht auch an meine Co-Autoren, alle Doktoranden im Projekt FUGABEE, die Studenten der diversen Praktika inner- und außerhalb des Labors, sowie die Bachelor- und Diplomstudenten; insbesondere Sophie Helbing, Stefanie Lommatzsch und Jeanny Richter.
Der Arbeitsgruppe Molekulare Ökologie danke ich für das tolle Arbeitsklima und die ständige Gesprächsbereitschaft aller, besonders bei Problemen im Labor – also „Danke“ Antje, Denise, Dieter, Eckart und Petra.

Nun möchte ich mich noch bei all meinen lieben Freunden, insbesondere bei Claudi und Kati, bedanken für alle Ablenkungen durch Unternehmungen und Reisen bzw. für die immer verlässlichen Abendgestaltungen. Man konnte sich immer, in guten und schlechten Zeiten, auf euch verlassen. Schön, dass es euch gibt, wir werden sehen, was uns die Zukunft bringt.

Zum Schluss geht der Dank an meine ganze Familie und meine Freundin Anja, die immer hinter mir standen und wissen wollten, was es denn Neues aus der „Hummelforschung“ gibt.

Die Arbeit wurde finanziert durch das BMBF-Projekt: „Functional analysis of disease resistance genes in bumble bees (Bombus terrestris)” (Koordinator: Dr. H. Michael G. Lattorff, FKZ: 0315126).



Appendix

A. Declaration on the Author Contributions

Erler S. and Lattorff H.M.G. (2010): The degree of parasitism of the bumblebee (*Bombus terrestris*) by cuckoo bumblebees (*Bombus (Psithyrus) vestalis*). *Insectes Sociaux* 57 (4): 371-377. DOI: 10.1007/s00040-010-0093-2.

I participated in the design of the project, collected and genotyped the samples, performed the analyses and wrote the paper. H. M. G. Lattorff participated in the design of the project, the analyses, writing of the paper, supervised the work and provided helpful discussions.

Erler S., Popp M. and Lattorff H.M.G. (2011): Dynamics of Immune System Gene Expression upon Bacterial Challenge and Wounding in a Social Insect (*Bombus terrestris*). *PLoS ONE* 6(3): e18126. DOI: 10.1371/journal.pone.0018126.

I participated in the design of the project, performed the infections and qPCR, performed the analyses and wrote the paper. M. Popp participated in the infection experiments and writing of the paper. H.M.G. Lattorff participated in the design of the project, the analyses, writing of the paper, supervised the work and provided helpful discussions.

Erler S., Lommatzsch S. and Lattorff H.M.G. (2011): Comparative analysis of detection limits and specificity of molecular diagnostic markers for three pathogens (Microsporidia, *Nosema* sp.) in the key pollinators *Apis mellifera* and *Bombus terrestris*. *Parasitology Research*. DOI: 10.1007/s00436-011-2640-9.

I participated in the design of the project, extracted the samples, performed the analyses and wrote the paper. S. Lommatzsch participated in the qPCR experiments and writing of the paper. H.M.G. Lattorff participated in the design of the project, the analyses, writing of the paper, supervised the work and provided helpful discussions.

B. Curriculum Vitae

Date of birth	08.09.1983
Place of birth	Karl-Marx-Stadt (now Chemnitz), Saxony, Germany
Marital status	single
Nationality	german
School Education	
1990 - 1994	Elementary school - Dr.-Salvador-Allende, Chemnitz
1994 - 2002	Secondary school - Gottfried-Leibniz, Chemnitz Degree: Abitur (Grade: 2.5, 'good')
Civilian service	10/2002 – 05/2003, Botanical garden Chemnitz (as substitute for military service)
Higher Education	
10/2003 - 03/2008	**Studies of Biology,** Martin-Luther-University Halle-Wittenberg Major: zoology, Minors: genetics, immunology, cell-biochemistry **Diploma thesis**: Institute for Biology-Zoology, Molecular Ecology, Advisor: Prof. Dr. H.-H. Kaatz Title: Analysis of the mitochondrial genome of *Schistocerca gregaria gregaria* (Forskåhl, 1775)' Degree: Diploma (Grade: 1.2, 'very good')
Since 04/2008	**Ph. D. student,** Martin-Luther-University Halle-W. Institute for Biology-Zoology, Molecular Ecology Advisor: Prof. Dr. Robin F.A. Moritz Funded by the BMBF-Project: 'Functional analysis of disease resistance genes in bumble bees (*Bombus terrestris*)' (Coordinator: Dr. H. Michael G. Lattorff, FKZ: 0315126 to HMGL) Title: Molecular Analysis of Host-Parasite Interaction in the Bumblebee *Bombus terrestris* (Linnaeus, 1758)
Since 09/2011	**Research assistant**, Martin-Luther-University Halle-W., Institute for Biology-Zoology, Molecular Ecology Funded by the BMELV-Project: 'FIT BEE'

C. Publication List

Peer-reviewed Articles

Erler S., Ferenz H.-J., Moritz R.F.A. and Kaatz H.-H. (2010): Analysis of the mitochondrial genome of *Schistocerca gregaria gregaria* (Orthoptera: Acrididae). *Biological Journal of the Linnean Society* 99 (2): 296-305. DOI: 10.1111/j.1095-8312.2009.01365.x.

Erler S. and Lattorff H.M.G. (2010): The degree of parasitism of the bumblebee (*Bombus terrestris*) by cuckoo bumblebees (*Bombus (Psithyrus) vestalis*). *Insectes Sociaux* 57 (4): 371-377. DOI: 10.1007/s00040-010-0093-2.

Erler S., Popp M. and Lattorff H.M.G. (2011): Dynamics of Immune System Gene Expression upon Bacterial Challenge and Wounding in a Social Insect (*Bombus terrestris*). *PLoS ONE* 6(3): e18126. DOI: 10.1371/journal.pone.0018126.

von Einem S., **Erler S.**, Bigl K., Frerich B. and Schwarz E. (2011): The pro-form of BMP-2 exhibits a delayed and reduced activity when compared to mature BMP-2. *Growth Factors* 29 (2-3): 63-71. DOI: 10.3109/08977194.2011.561798.

Erler S., Lommatzsch S. and Lattorff H.M.G. (2011): Comparative analysis of detection limits and specificity of molecular diagnostic markers for three pathogens (Microsporidia, *Nosema* sp.) in the key pollinators *Apis mellifera* and *Bombus terrestris*. *Parasitology Research*. DOI: 10.1007/s00436-011-2640-9.

Richter J., Helbing S., **Erler S.** and Lattorff H.M.G. (2011): Social context dependent immune gene expression in bumblebees (*Bombus terrestris*). *Behavioral Ecology and Sociobiology* (submitted).

Popp M., **Erler S.** and Lattorff H.M.G. (2011) Seasonal variability of prevalence and occurrence of multiple infections shapes the population structure of *Crithidia bombi*, an intestinal parasite of bumblebees (*Bombus* spp.). *Journal of Evolutionary Biology* (submitted).

Erler S., Popp M., Wolf S., Moritz R.F.A. and Lattorff H.M.G. (2011) Sex, horizontal transmission and multiple hosts prevent local adaptation of *Crithidia bombi*, a parasite of bumblebees (*Bombus* sp.). *Journal of Animal Ecology* (*in preparation*).

Popular Articles

Popp M., **Erler S.**, Lattorff H.M.G. (2009): FUGABEE: Funktionelle Analyse von Krankheitsresistenzgenen bei der Erdhummel (*Bombus terrestris*). *Genomxpress* 4.09: 7-10

D. Oral Presentations

Erler S., Popp M., Lattorff H.M.G.: Immune challenge in the bumble bee *Bombus terrestris*, 102. Annual Meeting of Deutsche Zoologische Gesellschaft - DZG (Regensburg, Germany - September 2009)

Erler S., Lattorff H.M.G.: The degree of parasitism of the host-bumble bee *B. terrestris* by *Psithyrus vestalis*, 1st Central European Meeting of the International Union for the Study of Social Insects - IUSSI (Fraueninsel in Lake Chiemsee, Germany - October 2009)

Erler S., Lattorff H.M.G.: Molecular diagnosis of *Nosema* – what's the limit of detection?, COLOSS Workshop: NOSEMA DISEASE: LACK OF KNOWLEDGE AND WORK STANDARDIZATION (Guadalajara, Spain - October 2009)

Erler S., Popp M., Lattorff H.M.G.: Immune gene expression analysis in the bumble bee *Bombus terrestris*, 57. Jahrestagung der Arbeitsgemeinschaft der Institute für Bienenforschung e.V. (Herne, Germany - March 2010)

Erler S., Popp M., Lattorff H.M.G.: Gene expression analysis after septic and non-septic injury in the bumble bee *Bombus terrestris*, 15th PhD Meeting of Evolutionary Biology of the DZG (Freiburg i.B., Germany - April 2010)

Erler S., Lattorff H.M.G.: Evolution of antimicrobial peptides in bumble bees, 4th European Conference of Apidology - EurBee (Ankara, Turkey - September 2010)

Erler S., Popp M., Lattorff H.M.G.: Immune response in a social insect (*Bombus terrestris*) in response to bacterial challenge and wounding, 2nd Central European Meeting of the International Union for the Study of Social Insects - IUSSI (Papenburg, Germany - March 2011)

Erler S., Lattorff H.M.G.: Quantitative PCR - Detection limits for pollinator pathogens *Nosema sp.*, 58. Jahrestagung der Arbeitsgemeinschaft der Institute für Bienenforschung e.V. (Berlin, Germany - March 2011)

E. Poster Presentations

Erler S., Popp M., Lattorff H.M.G.: Frequent host switching of *Crithidia bombi*, a parasite of bumblebees (*Bombus* sp.), on a microgeographical scale, ESF-FWF Conference: The Impact of the Environment on Innate Immunity: The Threat of Diseases (Obergurgl, Austria - May 2009)

Erler S., Popp M., Lattorff H.M.G.: Comparitive population genetics between bumblebees and their trypanosomatid parasites *Crithidia* reveals frequent horizontal transmission, 12th Congress of the European Society for Evolutionary Biology - ESEB (Turin, Italy - August 2009)

Erler S., Popp M., Lattorff H.M.G.: Parasite resistance in the bumble bee *Bombus terrestris* at the molecular level, XVI International Congress of the International Union for the Study of Social Insects - IUSSI (Copenhagen, Denmark - August 2010)

Erler S., Popp M., Lattorff H.M.G.: Gene expression and conservation of bumble bee antimicrobial peptides, 3. FUGATO-Statusseminar (Kassel, Germany - February 2011)

Erler S., Lattorff H.M.G.: Shared environment in a host-parasite relationship leads to adaptive evolution of immune system genes, 13th Congress of the European Society for Evolutionary Biology - ESEB (Tübingen, Germany - August 2011)

Erklärung

Halle (Saale), den 18. Oktober 2011

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.

Silvio Erler