Reciprocal adaptations between the microsporidian gut pathogen *Nosema ceranae* and its honeybee host *Apis mellifera*

Dissertation

zur Erlangung des
Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

der

Naturwissenschaftlichen Fakultät I – Biowissenschaften –

der Martin-Luther-Universität
Halle-Wittenberg,

vorgelegt

von Herrn Christoph Kurze

geb. am 14.05.1986 in Greifswald

Gutachter/in:

1. Prof. Dr. Dr. h.c. Robin F.A. Moritz (Martin-Luther-Universität Halle-Wittenberg, Germany)
2. Prof. Dr. Peter Neumann (Universität Bern, Switzerland)
3. Prof. Dr. Dirk C. de Graaf (Ghent University, Belgium)

Promotionsverfahren eröffnet am 1.04.2016

Tag der öffentlichen Verteidigung: 13.07.2016

CONTENTS

Chapter I – General introduction

Review: Parasite resistance and tolerance in honeybees at the individual and social level

Authors

Christoph Kurze[a,*], Jarkko Routtu[a], Robin F.A. Moritz[a, b, c]

Affiliations

[a] Institute for Biology, Martin Luther University Halle-Wittenberg, Hoher Weg 4, 06099 Halle (Saale), Germany

[b] German Institute for Integrative Biodiversity Research (iDiv), Deutscher Platz 5e, 04103 Leipzig, Germany

[c] Department of Zoology and Entomology, University of Pretoria, Roper St., 0002 Pretoria, South Africa

*Corresponding author

E-Mail address: christoph.kurze@zoologie.uni-halle.de

Abstract

Organisms living in large groups, such as social insects, are particularly vulnerable to parasite transmission. However, they have evolved diverse defence mechanisms which are not only restricted to the individual's immune response, but also include social defences. Here, we review cases of adaptations at the individual and social level in the honeybee *Apis mellifera* against the ectoparasitic mite *Varroa destructor* and the endoparasitic microsporidians *Nosema ceranae* and *Nosema apis*. They are considered important threats to honeybee health worldwide. We highlight how individual resistance may result in tolerance at the colony level and vice versa.

Keywords: Innate immunity; Social immunity; Honeybee parasites; *Varroa*; *Nosema*

Zoology (2016); accepted 23rd of March 2016

DOI: 10.1016/j.zool.2016.03.007

Chapter II

Nosema spp. infections cause no energetic stress in tolerant honeybees

Authors: Christoph Kurze[1*], Christopher Mayack[1], Frank Hirche[2], Gabriele I. Stangl[2], Yves Le Conte[3], Per Kryger[4], and Robin F.A. Moritz[1,6,7]

Affiliations

[1]Institute for Biology, Martin-Luther-Universität Halle-Wittenberg, 06099 Halle (Saale), Germany

[2]Institute of Agricultural and Nutritional Sciences, Martin-Luther-Universität Halle-Wittenberg, 06120 Halle (Saale), Germany

[4]INRA, UR 406 Abeilles et Environnement, 84914 Avignon Cedex 9, France

[5]Department of Agroecology Flakkebjerg, Aarhus University, 4200 Slagelse, Denmark

[6]German Institute for Integrative Biodiversity Research (iDiv), 04103 Leipzig, Germany

[7]Department of Zoology and Entomology, University of Pretoria, 0002 Pretoria, South Africa

*Correspondence to: christoph.kurze@zoologie.uni-halle.de

Abstract

Host-pathogen coevolution leads to reciprocal adaptations, allowing pathogens to increase host exploitation or hosts to minimise costs of infection. As pathogen resistance is often associated with considerable costs, tolerance may be an evolutionary alternative. Here, we examined the effect of two closely related and highly host dependent intracellular gut pathogens, *Nosema apis* and *Nosema ceranae*, on the energetic state in *Nosema* tolerant and sensitive honeybees facing the infection. We quantified the three major haemolymph carbohydrates fructose, glucose, and trehalose using high-performance liquid chromatography (HPLC) as a measure for host energetic state. Trehalose levels in the haemolymph were negatively associated with *N. apis* infection intensity and with *N. ceranae* infection regardless of the infection intensity in sensitive honeybees. Nevertheless, there was no such association in *Nosema* spp.

infected tolerant honeybees. These findings suggest that energy availability in tolerant honeybees was not compromised by the infection. This result obtained at the individual level may also have implications at the colony level where workers in spite of a *Nosema* infection can still perform as well as healthy bees, maintaining colony efficiency and productivity.

Keywords
host-parasite interaction, immune response, energetic stress, adaptation, fitness cost

Parasitology Research (2016); accepted 4th of March 2016
DOI 10.1007/s00436-016-4988-3

Chapter III

Nosema tolerant honeybees (*Apis mellifera*) escape parasitic manipulation of apoptosis

Authors

Christoph Kurze[1*], Yves Le Conte[2], Claudia Dussaubat[2], Silvio Erler[1], Per Kryger[3], Oleg Lewkowski[1], Thomas Müller[4], Miriam Widder[4], and Robin F.A. Moritz[1,5,6]

Affiliations

[1]Institute for Biology, Martin -Luther-Universität Halle-Wittenberg, Halle (Saale) Germany

[2]UR 406 Abeilles et Environnement, INRA, Avignon, France

[3]Department of Agroecology, Aarhus University, Flakkebjerg, Denmark

[4]Department of Internal Medicine IV, Martin-Luther-Universität Halle-Wittenberg, Halle (Saale), Germany

[5]German Institute for Integrative Biodiversity Research (iDiv), Leipzig, Germany

[6]University of Pretoria, Department of Zoology and Entomology, Pretoria, South Africa

*Corresponding author E-mail: christoph.kurze@zoologie.uni-halle.de

Abstract

Apoptosis is not only pivotal for development, but also for pathogen defence in multicellular organisms. Although numerous intracellular pathogens are known to interfere with the host's apoptotic machinery to overcome this defence, its importance for host-parasite coevolution has been neglected. We conducted three inoculation experiments to investigate in the apoptotic respond during infection with the intracellular gut pathogen *Nosema ceranae*, which is considered as potential global threat to the honeybee (*Apis mellifera*) and other bee pollinators, in sensitive and tolerant honeybees. To explore apoptotic processes in the gut epithelium, we visualised apoptotic cells using TUNEL assays and measured the relative expression levels of subset of candidate genes involved in the apoptotic machinery using qPCR. Our results suggest that *N. ceranae* reduces apoptosis in sensitive honeybees by enhancing *inhibitor*

of apoptosis protein-(*iap*)-*2* gene transcription. Interestingly, this seems not be the case in *Nosema* tolerant honeybees. We propose that these tolerant honeybees are able to escape the manipulation of apoptosis by *N. ceranae*, which may have evolved a mechanism to regulate an anti-apoptotic gene as key adaptation for improved host invasion.

Keywords: programmed cell death, host-parasite interaction, selection, susceptibility, immune defence

PLoS ONE (2015); accepted 21st of September 2015

DOI 10.1371/journal.pone.0140174

Chapter IV

Mechanisms of parasitic manipulation and how to escape them: differential proteomics of *Nosema*–honeybee interactions

Christoph Kurze[*1,2]†, Ryan Dosselli[2], Julia Grassl[2], Yves Le Conte[3], Per Kryger[4], Boris Baer[2] and Robin F.A. Moritz[1,5,6]†

Affiliations

[1]Martin–Luther–Universität Halle–Wittenberg, Institute for Biology/Molecular Ecology, Hoher Weg 4, 06120 Halle (Saale) Germany

[2]Centre for Integrative Bee Research (CIBER) and ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia, Bayliss Building (M316), Crawley, Western Australia 6009, Australia

[3]INRA, UR 406 Abeilles et Environnement, Site Agroparc, 84914 Avignon Cedex 9, France

[4]Aarhus University, Department of Agroecology/Section of Entomology and Plantpathology, Flakkebjerg, 4200, Slagelse, Denmark

[5]German Institute for Integrative Biodiversity Research (iDiv), Bio City, 04103 Leipzig, Germany

[6]University of Pretoria, Department of Zoology and Entomology, Pretoria, 0002, South Africa

*Correspondence to: christoph.kurze@zoologie.uni-halle.de

†Current address: Martin–Luther–Universität Halle–Wittenberg, Institute for Biology/Molecular Ecology, Hoher Weg 4, 06120 Halle (Saale) Germany

Summary

Host manipulation is a common strategy by parasites to reduce host defence responses and enhance development, host exploitation, reproduction and ultimately transmission success. As these parasitic modifications reduce host fitness, natural selection is predicted to result in counter adaptions of the host, which may eventually lead to Red Queen dynamic. Comparing two lineages of the honeybee *Apis mellifera*, one tolerant and the other sensitive towards the microsporidian gut parasite *Nosema ceranae*; we compared the underlying host-parasite interactions on the proteome level. We found that *Nosema* infections affected the abundance of 10 out of 661 protein spots studied, which were more abundant in sensitive compared to tolerant honeybees. Infections of *Nosema* resulted in an up-regulation of the honeybee's energy metabolism. There was an increased abundance of proteins with immune defence functions in infected tolerant honeybee compared to sensitive ones. We also detected three *Nosema* proteins (*N. ceranae* HSP 70 and two uncharacterised proteins), which provide key candidate genes involved during host invasion.

Key index words: host-parasite interaction; *Apis mellifera*; *Nosema ceranae*; tolerance; proteome; coevolution

Scientific Reports; submitted 23rd of March 2016

Chapter V

Synthesis

Due to long co-existence and continuous antagonistic interactions between parasites and their hosts, natural selection has shaped a variety of very complex and intimate relationships (Schmid-Hempel, 2011). Parasites have independently evolved multiple strategies to invade and exploit a broad spectrum of host taxa, which can consequently severely reduce the fitness of their hosts (Moore, 2002; Schmid-Hempel, 2011). Host manipulation is a strategy often employed by parasites to increase their replication/reproduction and transmission success (e.g. Bruchhaus et al., 2007; Moore, 2002; Thomas et al., 2010). Thus, it is not surprising that the selection pressure imposed by a parasite may result in reciprocal adaptations of the host, eventually leading to either avoidance, resistance (i.e. reducing the infection) or tolerance of an infection (i.e. limiting the harm by the parasite for a given infection intensity) (Råberg et al., 2009). Although resistance is the best-known adaptive host response, this strategy does not necessarily increase host fitness as immune responses can also impose high costs for the host (Lochmiller and Deerenberg, 2000; Schmid-Hempel, 2005). In the last decade, tolerance has been recongnized by evolutionary ecologists as an important alternative strategy in animals (Råberg et al., 2007).

In social insects host defence mechanisms are typically more complex than in solitary individuals as they are not only limited to the individual, but also achieve defences against parasites at the social level (known as ‘social immunity’, Cremer et al., 2007). Especially, honeybee health and associated parasites have been studied intensively and present an important model system for host-parasite interactions (Ball and Bailey, 1991; Evans and Schwarz, 2011; Fries, 2010; Rosenkranz et al., 2010). This is primarily because the important role bees play in crop pollination and ecosystem functioning (Klein et al., 2007; Potts et al., 2010). Thus, I reviewed our current knowledge of resistance and tolerance mechanisms at the individual and the colony level in the honeybee *Apis mellifera* in chapter I (general introduction). In this chapter, I primarily focused on two globally crucial infectious agents, the ectoparasitic mite *Varroa destructor* and the intracellular gut pathogen *Nosema ceranae*, which have clearly predominated scientific discussion in the context of honeybee colony declines over the past decade. Notably, individual resistance against *Varroa* (i.e. mite's delayed egg-laying) may result in tolerance at the colony level, if for example only a fraction of the colony are resistant (Le Conte et al., 2007; Locke and Fries, 2011; Locke et al., 2012). On the other hand, individual tolerance against *N. ceranae* (i.e. they develop high infection intensities, but energy metabolism (chapter II) and survival (Huang et al., 2012) was not impaired) may appear resistant at the colony level (Hatjina et al., 2014) in case parasite transmission would be limited or even prevented (chapter I).

Since empirical data describing tolerance mechanisms in animals are scares (Råberg, 2014), I compared bees from a *Nosema* tolerant linage with sensitive bees in my dissertation (chapters II, III and IV). The *Nosema* tolerant honeybees were the result of an intensive breeding programme conducted by beekeepers in Denmark over two decades (Hatjina et al., 2014). Interestingly, although *Nosema* prevalence decreased by more than 50 % in those colonies (Hatjina et al., 2014), *Nosema ceranae* still developed similarly high infection intensities in individuals of the tolerant linage compared to honeybees of the sensitive linage in laboratory controlled inoculation experiments (Huang et al., 2012; chapter II, III and IV). Nevertheless, survival experiments clearly showed that *Nosema* tolerant honeybees had a significantly higher survival than sensitive honeybees (Huang et al., 2012).

The work presented in this thesis provides novel insights into the reciprocal adaptations between the microsporidian gut parasite *N. ceranae* and its honeybee host. In particular, I have been focusing on the molecular interactions between this intracellular fungal pathogen and its honeybee host (chapters III and IV). I conducted a series of inoculation experiments to take snapshots of the intimate interplay between *N. ceranae* and the honeybee in the relatively early phase of an established infection. International collaboration with several colleagues allowed me to integrate research covering energetics, quantitative genetics, immunohistochemistry and proteomics to gain a more holistic understanding of:

1. What effects *N. apis* and *N. ceranae* infections have on the available energy budget in the *Nosema* sensitive and tolerant honeybees? (chapter II)
2. How does *N. ceranae* manipulate the sensitive honeybee to its own advantage? (chapter III and IV)

3. What is the adaptive mechanism in the *Nosema* tolerant honeybee linage? (chapter III and IV)

My data on the energetic consequences of *Nosema* infections clearly showed that infections with both *N. apis* and *N. ceranae* significantly reduced the availability of trehalose in the haemolymph in sensitive honeybees (chapter II). Trehalose is the major energy store in honeybees and other insects and can be used to measure for the host energetic state (Blatt and Roces, 2001; Thompson, 2003). Hence, these results supports the notion that *Nosema*-infected honeybees experience nutritional and energetic stress (Mayack and Naug, 2009, 2010; Moffett and Lawson, 1975), which may ultimately lead to decreased survival (Dussaubat et al., 2012; Higes et al., 2007). My study further extends their results as I included honeybees of the tolerant lineage (chapter II), in which the survival was not negatively affected by *N. ceranae* infections (Huang et al., 2012). Interestingly, I did not detect any significant effect of neither *N. apis* nor *N. ceranae* infection on trehalose levels in honeybee of the tolerant lineage. This may indicate an adaptive mechanism that maintains the energy availability in the haemolymph in spite of an infection (chapter II). Possibly, this might be also crucial for the maintenance of the host immune response and may help explaining why bees from the tolerant linage better withstood Nosemosis than *Nosema* sensitive honeybees (Huang et al., 2012).

Also my proteomic analyses using 2D-DIGE (2-Dimensional Differential In-Gel Electrophoresis) followed by subsequent mass spectrometry for protein identification (chapter IV) showed that the host energy metabolism is affected by *N. ceranae* infections. I detected an increase of abundance for three central proteins of the energy metabolism (cytochrome C oxidase subunit 6A1, alpha-glucosidase precursor and ATP synthase subunit beta) in the infected honeybees, which were more pronounced in individuals of the sensitive than of the tolerant linage. As the enhanced abundance of these proteins may lead to increase ATP production in the host, this would be clearly beneficial for *N. ceranae*, which are highly dependent on the ATP supply from their host (Williams, 2009). Thus, my results support the notion that *N. ceranae* manipulates the host's energy metabolism to increase its own fitness (Vidau et al., 2014). Maintaining energy homeostasis in tolerant honeybees might not only improve the health at the individual level, but may also increase the general performance and consequently the overall fitness of the whole colony (chapters II).

To identify and develop an understanding of the underpinning mechanisms for *Nosema* tolerance, I studied the host-parasite interactions in the midgut of both sensitive and tolerant honeybees (chapter III and IV). As apoptosis (most common form of programmed cell death) plays a crucial role in the host immune response, it is not surprising that numerous intracellular pathogens, including microsporidia, commonly manipulate apoptosis (Bruchhaus et al., 2007; Faherty and Maurelli, 2008; Mocarski et al., 2012). Data documenting how a host may escape this manipulation is largely unexplored. Hence, I first prepared longitudinal histological sections of the midgut epithelium and visualized apoptotic cells using TUNEL assays (labelling of signal- and double-strand DNA nicks). This allowed me to quantify any alterations in apoptosis associated with *N. ceranae* infections and whether this might differ between host lineages (chapter III). I found a significantly decreased proportion of apoptotic cells in *N. ceranae*-infected sensitive honeybees after 6 days post infection (dpi), which confirmed results by Higes *et al.* (2013) after 10 dpi. Nevertheless, there was no significant difference between *Nosema*-infected tolerant honeybees compared to the controls. This may suggests that the host's apoptotic machinery plays a central role in the host pathogenesis of *Nosema* infections.

Extending this data even further, I measured the relative gene expressions of nine candidate genes from the apoptotic cascade, which were predicted from the fruit fly *Drosophila melanogaster* (Hay et al., 2004). The most striking result, however, was a ten-fold increased expression of the *inhibitor of apoptosis protein 2* gene (*iap-2*) in the *Nosema*-infected sensitive honeybees compared to all other treatment groups. This observation is in agreement with previous in vitro studies, where protozoan and bacterial infections were also associated with enhanced *iap* gene expression and resulted in the inhibition of apoptosis in host cells *in vitro* (Binnicker et al., 2003; Molestina et al., 2003; Pedron et al., 2003). Although I found no clear signal for the inhibition of the apoptosis using quantitative proteomics (chapter IV), the detection of *Nosema* HSP70 in sensitive honeybees might indicate a mechanisms by which *N. ceranae* manipulates its host cell (chapter IV; Vidau et al., 2014). HSP70 may trigger the activation of the key transcription factor NF-κB in the host cell (Joly et al., 2010), which may increases IAP abundance, which will then potentially binds to host cell caspases, inhibiting their activity, and ultimately leads to an inhibition of apoptosis (Binnicker et al., 2003; Molestina et al., 2003). Furthermore, the proteome data revealed also significantly higher abundance of an uncharacterized *N. ceranae* protein in the sensitive honeybees, which would be an interesting candidate for future research. Studying the actual functions of those *Nosema* proteins might be very helpful in developing a better understanding of the molecular cross-talk between Nosema and the honeybee. In the end it might even unveil the fundamental mechanism for host invasion in microsporidia.

Overall my dissertation describes how an intracellular pathogen affects its host and how a tolerant host is able to “live with its enemy” using the *Nosema*-honeybee system. Despite the never ending desire for higher resolution of the molecular host-pathogen interactions major pathways of host tolerance could be identified. Future research should also focus on the role of microbiota in shaping a metaorganism, i.e. microbial community associated with the host. Understanding how the microbiome may positively affect honeybees and their associated pathogen may shed new light on bee health (Katsnelson, 2015). For example, Forsgren *et al.* (2010) discovered a new lactic acid bacteria (LAB) in the honey stomach, which beneficial properties against the bacterium *Paenibacillus larvae* in vitro and in American foulbrood infected honeybee larvae in vivo. Another study showed that the core of the gut microbiota is crucial for the protection against virulent trypanosome *Critidia bombi* in bumblebees (Koch and Schmid-Hempel, 2011). These studies highlight that host health is not only limited to the host’s immune system, but pathogen defence is extended by the host microbiota. Thus, studying host-microbiota interactions may also unravel novel insights into the evolutionary ecology of host-parasite (co)evolution.

References

Ball, B., Bailey, L., 1991. Viruses of honey bees, in: Amdams, J.R., Bonami, J.R. (Eds.), Atlas of Invertebrate Viruses. CRC Press, Boca Raton, pp. 525-551.

Binnicker, M.J., Williams, R.D., Apicella, M.A., 2003. Infection of human urethral epithelium with *Neisseria gonorrhoeae* elicits an upregulation of host anti-apoptotic factors and protects cells from staurosporine-induced apoptosis. Cell. Microbiol. 5, 549-560. doi:10.1046/j.1462-5822.2003.00300.x

Blatt, J., Roces, F., 2001. Haemolymph sugar levels in foraging honeybees (*Apis mellifera carnica*): dependence on metabolic rate and in vivo measurement of maximal rates of trehalose synthesis. J. Exp. Biol. 204, 2709-2716.

Bruchhaus, I., Roeder, T., Rennenberg, A., Heussler, V.T., 2007. Protozoan parasites: programmed cell death as a mechanism of parasitism. Trends Parasitol. 23, 376-383. doi:10.1016/j.pt.2007.06.004

Cremer, S., Armitage, S.A., Schmid-Hempel, P., 2007. Social immunity. Curr. Biol. 17, R693-R702.

Dussaubat, C., Brunet, J.-L., Higes, M., Colbourne, J.K., Lopez, J., Choi, J.-H., Martín-Hernández, R., Botias, C., Cousin, M., McDonnell, C., Bonnet, M., Belzunces, L.P., Moritz, R.F.A., Le Conte, Y., Alaux, C., 2012. Gut pathology and responses to the microsporidium *Nosema ceranae* in the honey bee *Apis mellifera*. Plos One 7, e37017. doi:10.1371/journal.pone.0037017

Evans, J.D., Schwarz, R.S., 2011. Bees brought to their knees: microbes affecting honey bee health. Trends Microbiol. 19, 614-620. doi:10.1016/j.tim.2011.09.003

Faherty, C.S., Maurelli, A.T., 2008. Staying alive: bacterial inhibition of apoptosis during infection. Trends Microbiol. 16, 173-180. doi:10.1016/j.tim.2008.02.001

Forsgren, E., Olofsson, T.C., Vasquez, A., Fries, I., 2010. Novel lactic acid bacteria inhibiting *Paenibacillus larvae* in honey bee larvae. Apidologie 41, 99-108. doi:10.1051/apido/2009065

Fries, I., 2010. *Nosema ceranae* in European honey bees (*Apis mellifera*). J. Invertebr. Pathol. 103, S73-S79. doi:10.1016/j.jip.2009.06.017

Hatjina, F., Bienkowska, M., Charistos, L., Chlebo, R., Costa, C., Dražić, M.M., Filipi, J., Gregorc, A., Ivanova, E.N., Kezić, N., Kopernicky, J., Kryger, P., Lodesani, M., Lokar, V., Mladenovic, M., Panasiuk, B., Petrov, P., Rai, S., Smodis Skerl, M.I., Vejsns, F., Wilde, J., 2014. A review of methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies. J. Apicult. Res. 53, 337-363. doi:10.3896/IBRA.1.53.3.02

Hay, B.A., Huh, J.R., Guo, M., 2004. The genetics of cell death: Approaches, insights and opportunities in *Drosophila*. Nat. Rev. Genet. 5, 911-922. doi:10.1038/nrg1491

Higes, M., García-Palencia, P., Martín-Hernández, R., Meana, A., 2007. Experimental infection of *Apis mellifera* honeybees with *Nosema ceranae* (Microsporidia). J. Invertebr. Pathol. 94, 211-217. doi:10.1016/j.jip.2006.11.001

Higes, M., Juarranz, A., Dias-Almeida, J., Lucena, S., Botías, C., Meana, A., García-Palencia, P., Martín-Hernández, R., 2013. Apoptosis in the pathogenesis of *Nosema ceranae* (Microsporidia: Nosematidae) in honey bees (*Apis mellifera*). Environ. Microbiol. Rep. 5, 530-536. doi:10.1111/1758-2229.12059

Huang, Q., Kryger, P., Le Conte, Y., Moritz, R.F.A., 2012. Survival and immune response of drones of a Nosemosis tolerant honey bee strain towards *N. ceranae* infections. J. Invertebr. Pathol. 109, 297-302. doi:10.1016/j.jip.2012.01.004

Joly, A.-L., Wettstein, G., Mignot, G., Ghiringhelli, F., Garrido, C., 2010. Dual Role of Heat Shock Proteins as Regulators of Apoptosis and Innate Immunity. J. Innat. Immun. 2, 238-247. doi:10.1159/000296508

Katsnelson, A., 2015. Microbiome: The puzzle in a bee's gut. Nature 521, S56-S56. doi:10.1038/521S56a

Klein, A.-M., Vaissiere, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. P. Roy. Soc. B. 274, 303-313. doi:10.1098/rspb.2006.3721

Koch, H., Schmid-Hempel, P., 2011. Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. P. Natl. Acad. Sci. USA 108, 19288-19292. doi:10.1073/pnas.1110474108

Le Conte, Y., De Vaublanc, G., Crauser, D., Jeanne, F., Rousselle, J.-C., Becard, J.-M., 2007. Honey bee colonies that have survived *Varroa destructor*. Apidologie 38, 566-572. doi:10.1051/apido:2007040

Lochmiller, R.L., Deerenberg, C., 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88, 87-98. doi:10.1034/j.1600-0706.2000.880110.x

Locke, B., Fries, I., 2011. Characteristics of honey bee colonies (*Apis mellifera*) in Sweden surviving *Varroa destructor* infestation. Apidologie 42, 533-542. doi:10.1007/s13592-011-0029-5

Locke, B., Le Conte, Y., Crauser, D., Fries, I., 2012. Host adaptations reduce the reproductive success of *Varroa destructor* in two distinct European honey bee populations. Ecol. Evol. 2, 1144-1150. doi:10.1002/ece3.248

Mayack, C., Naug, D., 2009. Energetic stress in the honeybee *Apis mellifera* from *Nosema ceranae* infection. J. Invertebr. Pathol. 100, 185-188. doi:10.1016/j.jip.2008.12.001

Mayack, C., Naug, D., 2010. Parasitic infection leads to decline in hemolymph sugar levels in honeybee foragers. J. Insect. Physiol. 56, 1572-1575. doi:10.1016/j.jinsphys.2010.05.016

Mocarski, E.S., Upton, J.W., Kaiser, W.J., 2012. Viral infection and the evolution of caspase 8-regulated apoptotic and necrotic death pathways. Nat. Rev. Immunol. 12, 79-88. doi:10.1038/nri3131

Moffett, J.O., Lawson, F.A., 1975. Effect of *Nosema*-infection on O2 consumption by honey bees. . J. Econ. Entomol. 68, 627-629.

Molestina, R.E., Payne, T.M., Coppens, I., Sinai, A.P., 2003. Activation of NF-kappa B by *Toxoplasmal gondii* correlates with increased expression of antiapoptotic genes and localization of phosphorylated I kappa B to the parasitophorous vacuole membrane. J. Cell Sci. 116, 4359-4371. doi:10.1242/jcs.00683

Moore, J., 2002. Parasites and the behavior of animals. Oxford University Press.

Pedron, T., Thibault, C., Sansonetti, P.J., 2003. The invasive phenotype of *Shigella flexneri* directs a distinct gene expression pattern in the human intestinal epithelial cell line Caco-2. J. Biol. Chem. 278, 33878-33886. doi:10.1074/jbc.M303749200

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25, 345-353. doi:10.1016/j.tree.2010.01.007

Råberg, L., 2014. How to live with the enemy: understanding tolerance to parasites. Plos Biol. 12, e1001989. doi:10.1371/journal.pbio.1001989

Råberg, L., Graham, A.L., Read, A.F., 2009. Decomposing health: tolerance and resistance to parasites in animals. Philos. T. Roy. Soc. B. 364, 37-49. doi:10.1098/rstb.2008.0184

Råberg, L., Sim, D., Read, A.F., 2007. Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. Science 318, 812-814. doi:10.1126/science.1148526

Rosenkranz, P., Aumeier, P., Ziegelmann, B., 2010. Biology and control of *Varroa destructor*. J. Invertebr. Pathol. 103 Suppl 1, S96-119. doi:10.1016/j.jip.2009.07.016

Schmid-Hempel, P., 2005. Evolutionary ecology of insect immune defenses. Annu Rev Entomol 50, 529-551. doi:10.1146/annurev.ento.50.071803.130420

Schmid-Hempel, P., 2011. Evolutionary parasitology: The integrated study of infections, immunology, ecology, and genetics. Oxford University Press, United States.

Thomas, F., Poulin, R., Brodeur, J., 2010. Host manipulation by parasites: a multidimensional phenomenon. Oikos 119, 1217-1223. doi:10.1111/j.1600-0706.2009.18077.x

Thompson, S.N., 2003. Trehalose – The insect 'blood' sugar, in: Simpson, S.J. (Ed.), Adv Insect Physiol, Vol 31, pp. 205-285.

Vidau, C., Panek, J., Texier, C., Biron, D.G., Belzunces, L.P., Le Gall, M., Broussard, C., Delbac, F., El Alaoui, H., 2014. Differential proteomic analysis of midguts from *Nosema ceranae*-infected honeybees reveals manipulation of key host functions. J. Invertebr. Pathol. 121, 89-96. doi:10.1016/j.jip.2014.07.002

Williams, B.A.P., 2009. Unique physiology of host-parasite interactions in Microsporidia infections. Cell Microbiol 11, 1551-1560. doi:10.1111/j.1462-5822.2009.01362.x

General Acknowledgements

First of all, I would like to thank Prof. Dr. Dr. h.c. Robin F.A. Moritz for giving me the opportunity of working in his Molecular Ecology group. His advice, support, liberal leading style and inspiring discussions with him made it possible to develop and conduct my own research for the accomplishment of doctorate.

I am also very grateful to all my co-authors for their support and invaluable guidance in conducting my experiments and the analysis of my samples. Their contribution was essential for my scientific achievements during my PhD. In particular, my gratitude is extended to Dr. Yves Le Conte (INRA Avignon) for providing me with *Nosema* sensitive honeybees and for hosting me and two Danish colonies in Avignon; Dr. Per Kryger (Aarhus University) for providing me with *Nosema* tolerant honeybees from Denmark; Dr. Thomas Müller for hosting me in his Oncology group (Department of Internal Medicine IV, MLU Halle) and his advice on immunohistochemistry; Prof. Dr. Gabriele Stangl and Dr. Frank Hirche for hosting me in the Institute of Agricultural and Nutritional Sciences (MLU Halle) and technical support in conducting HPLC analyses; Dr. Christopher Mayack, Dr. Claudia Dussaubat, Dr. Silvio Erler and Oleg Lewkowski for their experimental help provided; Prof. Dr. Boris Baer, Dr. Ryan Dosselli and Dr. Julia Grassl for kindly hosting me at the Centre for Integrative Bee Research (CIBER) and ARC Centre of Excellence in Plant Energy Biology (The University of Western Australia) and also for their excellent technical advice and help on proteomics.

Furthermore I want to thank the entire Molecular Ecology group of Prof. Dr. Dr. h.c. Robin Moritz and the General Zoology group of Prof. Dr. Robert Paxton for providing an ideal research environment for fruitful scientific discussions and support. They have been a constant source of encouragement throughout my PhD. Especially, I am thankful to Alexis Beaurepaire, Jonathan Kidner, Panagiotis Theodorous, Myrsini Natsopoulou, Christopher Mayack, Oleg Lewkowski, Nadege Forfert, Bertrand Fouks, Vincent Doublet, Sophie Helbing, Silvio Erler and Jarkko Routtu for scientific and non-scientific discussions, all the humorous moments and also as good friends.

Finally, I want to thank all my friends and family for their encouragement in general. In particular, I am grateful to Karin, who is always there for me, for her understanding, patience and love.

APPENDIX

A. Curriculum vitae

Personal details

Full name: Christoph Kurze
Date of birth: 14/05/1986
Place of birth: Greifswald, Germany

Education

2012/11-present	**PhD candidate**, Martin-Luther-Universität Halle-Wittenberg, Germany Advisor: Prof Dr Dr h.c. Robin F.A. Moritz.
2010/10-2012/06	**MSc Biodiversity, Evolution & Ecology** (bilingual English/German), Freie Universität Berlin, Germany Main focus: Animal Evolution and Ecology **MSc Thesis** at Leibniz Institute for Zoo and Wildlife Research (IZW) Berlin: "Identity and prevalence of *Dipylidium* spp. in Serengeti spotted hyenas using a non-invasive approach: do host's life history traits matter?" Advisors: Dr Marion L. East and Prof Dr Heribert Hofer
2009/09-2010/06	**ERASMUS**, University of Aberdeen, United Kingdom Main focus: Animal Evolution, Population Ecology and Animal Physiology **BSc Thesis:** "Energetic consequences of infection with African trypanosomes" Advisors: Dr Jeremy M. Sternberg and Prof Dr John R. Speakman
2007/10-2010/08	**BSc Biology**, Justus-Liebig-Universität Gießen, Germany Main focus: Zoology, Animal Ecology and Animal Physiology
2006/07-2007/06	**Civil service** (ADiA), FASCA (Fundación Acción Social Caritas), Ecuador
2004/07-2006/06	**Abitur** (*A-levels*), Maxim-Gorki-Gymnasium Heringsdorf, Germany

Scientific awards & scholarships

- **IGSS student award,** invited speaker at the *International Congress of Entomology (ICE) 2016*
- **DAAD fellowship**, *German academic exchange service*, supporting my research visit at The University of Western Australia in 2015
- **ABF's student award 2015**, *Foundation for the Preservation of Honey Bees, Inc.*
- **Evenius Award 2014**, *German society of bee research (AG Institute für Bienenforschung)*
- **MLU student travel grant 2014**, *Martin-Luther-Universität Halle-Wittenberg*
- **IUSSI student travel award 2014**, *International Union for the Study of Social Insects*
- Second prize for student oral contribution at the Central European IUSSI conference 2013
- **ERASMUS scholarship 2009**

Conference presentations

2015

- North American Beekeeping Conference & Tradeshow of the American Beekeeping Federation (ABF) in Anaheim, California, USA (6th –11th Jan), *oral & poster contribution (invited)*

2014

- 4th Symposium of the DFG Priority Programme 1399 Host-Parasite Coevolution near Kiel (29th Sep –2nd Oct), *oral contribution*
- 6th Congress of the European Association for Bee Research (EurBee) in Murcia, Spain (9th–11th Sep), *oral contribution*
- International Congress on Invertebrate Pathology and Microbial Control & 47th Annual Meeting of the Society for Invertebrate Pathology (SIP) in Mainz, Germany (3rd–7th Aug), *oral contribution*
- 17th congress of the “International Union for the Study of Social Insects” (IUSSI) in Cairns, Australia (13th–18th Jul), *oral contribution*
- 61st conference of the “Arbeitsgemeinschaft der Institute für Bienenforschung e.V. “ in Marburg, Germany (25th –27th Mar), *oral contribution*

2013

- 3rd Symposium of the DFG Priority Programme 1399 Host-Parasite Coevolution & RCNE meeting near Berlin, Germany (29th Aug–2nd Sep), *oral contribution*
- XIV Congress of the European Society for Evolutionary Biology in Lisbon, Portugal (19th –24th Aug), *poster presentation*
- 60th conference of the “Arbeitsgemeinschaft der Institute für Bienenforschung e.V.“, Würzburg, Germany (19th–21st Mar), *oral contribution*

- conference of "Central European section of the International Union for the Study of Social Insects (IUSSI)", Cluj-Napoca, Romania (14th–18th Mar), *oral contribution*

Scientific internships, research visits & workshops

2015/02-2015/06	Centre for Integrative Bee Research (CIBER) & Plant Energy Biology (PEB) Australian Research Council (ARC) centre of excellence, University of Western Australia, Perth, Australia (four months of research visit)
2014/07	Centre for Integrative Bee Research (CIBER), University of Western Australia, Perth, Australia (research visit: 7th–12th Jul)
2014/05	Institut national de la recherche agronomique (INRA), Avignon, France
2013/07-2013/12	Dept. Internal Medicine IV, Oncology research group, Universitätsklinikum Halle
2013/10	sTRANS-BEE workshop, German Centre for Integrative Biodiversity Research (iDiv), Leipzig (10th–11th Oct)
2013/05	Institut national de la recherche agronomique (INRA), Avignon, France (research visit)
2013/01	DFG SPP 1399 Nosema workshop, Münster (29th–30th Jan)
2012/06-2012/07	Institute of Experimental Ecology, University of Ulm Field assistant in bat monitoring, *'Biodiversity Exploratories'* (DFG SPP 1374) in Schorfheide-Chorin.
2010/11-2011/02	Applied Zoology & Animal Ecology, Freie Universität Berlin Distinction of the odour signature in Chinese cabbage plant (GC-MS) used for mate-choice experiments and pilot study on odour perception (EAG) in two *Phaedon cochleariae* populations.
2010/06-2010/09	Institute of Biological and Environmental Sciences (IBES), University of Aberdeen, United Kingdom Field assistant of Dr Fredrik Christiansen studying the effect of whale watching boats on Minke whales in Iceland.

Teaching & Mentoring

2015/07	Animal Ecology course (BSc level)
2014/04-2014/08	supervision of an intern (Oleg Lewkowski)
2013/12-2014/06	co-supervision of a MSc thesis (Sarah Biganski)
2013/09-2013/11	project module for MSc students
2013/06	Molecular Ecology course (MSc level)

B. Publication list

Kurze C, Routtu J, and Moritz RFA (*in press*). Parasite resistance and tolerance in honeybees at the individual and social level. ***Zoology*** doi:10.1016/j.zool.2016.03.007

Kurze C, Mayack C, Hirche F, Stangl GI, Le Conte Y, Kryger P, and Moritz RFA (2016). *Nosema* spp. infection causes no energetic stress in tolerant honeybees. ***Parasitol Res*** 1-8. doi:10.1007/s00436-016-4988-3

Kurze C, Le Conte Y, Dussaubat C, Erler S, Kryger P, Lewkowski O, Müller T, Widder M, and Moritz RFA (2015). *Nosema* tolerant honeybees (*Apis mellifera*) escape parasitic manipulation of apoptosis. ***Plos One*** 10, e0140174. doi:10.1371/journal.pone.0140174

East ML, **Kurze C**, Wilhelm K, Benhaiem S, and Hofer H (2013). Factors influencing *Dipylidium* sp. infection in a free-ranging social carnivore, the spotted hyaena (*Crocuta crocuta*). ***Int J Parasitol Parasites Wildl*** 2, 257-265. doi:10.1016/j.ijppaw.2013.09.003

In review

Kurze C, Dosselli R, Baer B, Grassl J, Le Conte Y, Kryger P, and Moritz RFA (*submitted*). Mechanisms of parasitic manipulation and how to escape them: differential proteomics of *Nosema*–honeybee interactions. ***Scientific Reports***

C. Declaration of own contribution to the original articles

E. Eidesstattliche Erklärung

Halle, den 29.03.2016

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

Ich erkläre, dass ich mich bisher noch nicht um den Doktorgrad beworben habe. Ferner erkläre ich, dass ich diese Arbeit selbstständig und nur unter Zuhilfenahme der angegebenen Quellen und Hilfsmittel angefertigt habe. Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen sind als solche kenntlich gemacht worden.

Christoph Kurze