

**Emerging infectious diseases of honey bees: within host interactions**

Dissertation

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

der

Naturwissenschaftlichen Fakultät I – Biowissenschaften –

der Martin-Luther-Universität  
Halle-Wittenberg,

vorgelegt

von

Frau Myrsini Eirini Natsopoulou

geb. am 19. März 1985 in Kavala, Griechenland

Gutachter:

1. Prof. Dr. R. J. Paxton
2. PD Dr. H. Michael G. Lattorff
3. Prof. Dr. Mark J. Brown

Promotionsgesuch eingereicht am: 01. February 2016

Tag der Tag der öffentlichen Verteidigung: 02. June 2016

# Table of Contents

General Introduction .....	1
I. European isolates of the Microsporidia <i>Nosema apis</i> and <i>Nosema ceranae</i> have similar virulence in laboratory tests on European worker honey bees .....	15
II. Interspecific competition in honey bee intracellular gut parasites is asymmetric and favours the spread of an emerging infectious disease .....	16
III. Within-host competition among the honey bees pathogens <i>Nosema ceranae</i> and Deformed wing virus is asymmetric and to the disadvantage of the virus .....	17
IV. Parasites modulate within-colony activity and accelerate the temporal polyethism schedule of a social insect, the honey bee .....	18
Synthesis.....	19
Acknowledgements .....	25
Curriculum Vitae .....	26
Education.....	26
Research and work experience .....	26
Awards and Grants .....	26
Oral contributions in Conferences.....	26
Publication List.....	27
Declaration of own contribution to the original articles .....	28
Eidesstattliche Erklärung .....	29

## **General Introduction**

The production of about 75% of all crop plant species used by humans for food consumption depends directly on insect pollination, which is carried out mostly by bees (Klein et al. 2007). Pollination services for global agricultural crop production have been estimated at €153 billion while in Europe they exceed €20 billion (Gallai et al. 2009). The western honey bee (*Apis mellifera*) is by far the most important commercial insect pollinator due to its ability to increase yield in more than 90% of insect pollinated crops (Klein et al. 2007). In addition to the economic contribution of managed honey bee colonies as crop pollinators, their contribution in sustaining plant biodiversity through pollination of wild plants and to ecosystem services (as providers of the service of pollination) exceeds any conceivable monetary value (Potts et al. 2010a). Thus it is not surprising that honey bees are considered keystone components for ecosystem functioning.

However, over the last decades severe declines of honey bee populations across some parts of the globe (Stokstad 2007; Neumann and Carreck 2010; Ratnieks and Carreck 2010; Potts et al. 2010b) have raised concerns about food security and maintenance of biodiversity (Morse and Calderone 2000; Moritz et al. 2010; Potts et al. 2010a). Even if overwinter colony mortality can be extremely variable within a geographic region and across years, there is an evident increase in the number of colonies that are not able to survive during winters (Dukas 2008; vanEngelsdorp et al. 2009; Genersch et al. 2010). Reoccurring high (> 20-30%), overwinter colony losses are of particular concern in temperate zones both in Europe and North America (vanEngelsdorp et al. 2010; van der Zee et al. 2012; Spleen et al. 2013).

A combination of several stressors is suspected to be involved in the elevated incidence of overwinter colony losses, including anthropogenic pressures such as habitat alteration and degradation, beekeeping practices, use of pesticides but also pathogen spread (Goulson et al. 2015). The latter factor seems to be one of the most prominent ones as the emergence of several infectious diseases (EIDs) appeared to coincide with the observed elevated overwinter colony losses (Stokstad 2007; Schroeder and Martin 2012; González-Varo et al. 2013; Goulson et al. 2015).

Emerging infectious diseases (EIDs) refer to any pathogen that is the causative agent of an infectious disease and has been recently introduced or has existed in a host population but

whose incidence or geographic range is increasing due to changes in its epidemiology (Daszak et al. 2000; Woolhouse and Dye 2001). Emerging pathogens can trigger devastating epidemics that could lead to severe declines and even extinction of host populations (Daszak et al. 2000). Recent examples include lethal chytridiomycosis, which is a cause of recent amphibian species declines globally (Daszak et al. 1999; Fisher et al. 2009; Blaustein and Johnson 2010), Ebola and the HIV/AIDS pandemic in humans (Daszak et al. 2000; Morens et al. 2004; Morens and Fauci 2013; WHOER Team 2014). There is a general consensus that EIDs threaten both wild and domestic animal health and thus they are tightly linked to the current biodiversity crisis (Daszak et al. 2000; Smith et al. 2006). In the USA EIDs actually featured in the top five causes of species extinction (Wilcove et al. 1998).

Pathogen emergence has been associated with a wide range of environmental and ecological factors that ultimately affect transmission opportunities (Morse 1995; Daszak et al. 2001; Taylor et al. 2001; Jones et al. 2008). A major driver includes anthropogenic interference. Translocation of animals into new regions, e.g. for commercial or conservation reasons, increases the probability of a parasite jumping from one host species to another (Cunningham 1996; Daszak et al. 2001). Indeed, movement of honey bee colonies between continents has resulted in the “pollution” of *A. mellifera* populations with pathogens and pests that had never been detected before in the host (Genersch and Aubert 2010).

One prominent example is the ectoparasitic mite *Varroa destructor*, which was originally associated with the Asian honey bee *Apis ceranae* (Oudemans 1904). The mite has managed not only to successfully jump onto the European honey bee, *A. mellifera*, but also to become established and increase its prevalence globally (Rosenkranz et al. 2010). Within 50 years *Varroa* spread from Asia to Europe, America and recently to New Zealand due to honey bee importations (Ruttner and Ritter 1980; Oldroyd 1999; Zhang 2000). Due to the low resistance of the European honey bee to this exotic invasive pest species, *Varroa* has been accused of being responsible for the death of millions of *A. mellifera* colonies (Shimanuki et al. 1994; Le Conte et al. 2010; Rosenkranz et al. 2010). The mite’s profound negative effect on honey bee colony health is probably caused indirectly by the viruses that the mite vectors. *Varroa*’s ability to act as a viral reservoir and potential incubator of several honey bee RNA viruses gave rise to a new viral transmission route, thereby aiding the spread and re-emergence of several bee viruses (Tentcheva et al. 2004; Shen et al. 2005; Chen and Siede 2007; Boecking and Genersch 2008; Genersch and Aubert 2010). In particular one RNA virus, Deformed

wing virus (DWV), has been tightly linked to Varroa infestation (de Miranda and Genersch 2010; Martin et al. 2012).

DWV is a positive single-stranded RNA virus (Lanzi et al. 2006) that affects several tissues of a bee's body, including the midgut and the brain (Yue and Genersch 2005; Fievet et al. 2006; Shah et al. 2009; Gauthier et al. 2011; Möckel et al. 2011). Clinical symptoms during overt infections include deformed wings and shortened abdomens (de Miranda and Genersch 2010; Möckel et al. 2011). Though DWV was present in *A. mellifera* before the arrival of Varroa, mainly causing covert infections with no detectable impact on colony health (Bailey and Ball 1991; Genersch and Aubert 2010), its prevalence has dramatically increased after the introduction of the mite (Carreck et al. 2010; Martin et al. 2012). Additionally, it seems that the mite has induced changes in the epidemiology of the virus, possibly favouring the predominance of more virulent variants of DWV accompanied by an increase in viral titer and the generation of overt infections (Martin et al. 2012).

Viral transmission via Varroa occurs when the mite that feeds on the haemolymph of honey bee pupae or adults is infected (de Miranda and Genersch 2010). However, once found in a colony, DWV can be also transmitted between individuals within a colony or between colonies by a variety of other routes. That permits the virus to persist in the host population. Examples of transmission routes include trophallaxis between colony members, contact with contaminated material, venereal transmission during mating and vertical transmission from mother to offspring (Chen et al. 2005; Chen and Siede 2007; Yue et al. 2007; de Miranda and Fries 2008). It is generally accepted that DWV serves as a predictor of overwinter colony decline as several studies have shown an association with colony mortality (Highfield et al. 2009; Guzmán et al. 2010; Genersch 2010; Dainat et al. 2012; Nazzi et al. 2012; van Dooremalen et al. 2012). However, all evidence is correlational and a causal link between DWV exposure and overwinter honey bee losses is lacking.

We find a quite similar story to the introduction of Varroa mites in European honey bees in the case of the microsporidan *Nosema ceranae*. *Nosema ceranae* was originally isolated in East Asia, where it was initially believed to be restricted to the Asian honey bee, *A. cerana* (Fries et al. 1996). However, studies have demonstrated that *N. ceranae* recently switched to the western honey bee *A. mellifera* prior to 1997 (Paxton et al. 2007), probably as a result of anthropogenic influences (i.e. managed apiculture), as for *V. destructor* (Higes et al. 2013).

The microsporidian has not only successfully jumped to its new host but it has also greatly expanded its distribution globally (Klee et al. 2007).

As for all microsporidia species, *N. ceranae* is a unicellular, obligate intracellular pathogen that depends completely on host resources (Texier et al. 2010; Fries 2010; Fries et al. 2013). According to a recent study, it seems that *N. ceranae* does not differ genetically across the range of *A. mellifera* subspecies and exists as a single clonal variant across host populations (Pelin et al. 2015). It is transmitted horizontally *per os* when adult honey bees come into contact with contaminated faeces or via trophalaxis with infected nestmates (Fries 1988; Fries et al. 1996). After ingestion, spores travel through the food canal and germinate in the host midgut, attacking the epithelial cells of the ventriculus of adult bees. There they replicate and produce new spores, a process that takes approximately four days (Fries et al. 1996; Gisder et al. 2011). Despite the general belief that *N. ceranae* exhibits tissue tropism and that it is restricted to the host's gut tissue (Huang and Solter 2013), a recent study suggests that *N. ceranae* can also be found in the semen of infected drones (Roberts et al. 2015). Although infected honey bees do not exhibit any obvious, external disease symptoms, *N. ceranae* damages the gut tissue and has been shown to evoke a variety of physiological changes, including energetic stress and immune suppression (Mayack and Naug 2009; Martín-Hernández et al. 2011; Dussaubat et al. 2012; Aufauvre et al. 2014). Earlier behavioural maturation (i.e. precocious foraging) and reduced lifespan of adult bees have also been attributed to *N. ceranae* infection (Higes et al. 2007; Goblirsch et al. 2013).

One of the most controversial issues regarding this exotic parasite has to do with its effects on *A. mellifera* colony health. Although it is well documented that *N. ceranae* can affect negatively the health of individual bees, studies are quite contradictory when it comes to impact at the colony level (Higes et al. 2013). Data from Spain suggest that the presence of *N. ceranae* is highly correlated with colony losses (Martín-Hernández et al. 2007; Higes et al. 2008; Higes et al. 2009; Higes et al. 2010; Botías et al. 2013). However, most published data from around the globe fail to find any link and thus its role as a predictor of colony failure has been ruled out (Cox-Foster et al. 2007; VanEngelsdorp et al. 2009; Gisder et al. 2010). It seems that, at the colony level, *N. ceranae* is a benign parasite and the dramatic effects described in Spain probably reflect local idiosyncrasies. Nonetheless, the ubiquity of this invasive parasite, coupled with its ability to spread rapidly and cause disease, have turned this

microsporidian into a pathogen of high interest among the bee-pathology research community.

As disease emergence is a multifactorial process, understanding the various determinants that lead to pathogen spread and the subsequent consequences of their introduction into a host population means that several parameters have to be taken into consideration. For example, the epidemiological dynamics of a parasite depend not only on its interaction with its host but also on its interaction with the other pathogens that it may encounter within the same host environment. Parasite-parasite interactions have the potential to influence the likelihood and the outcome of disease emergence as they affect disease transmission and virulence (Cox 2001; Brown et al. 2002; Mideo 2009; Alizon and Lion 2011). Thus, interactions between novel and existing pathogens can have a major influence on each other's fitness, ultimately affecting their epidemiological patterns and distribution in a host population (Poulin 2001; Lawn et al. 2006; Telfer et al. 2010). Positive, synergistic, within-host associations between pathogens can facilitate the spread of a disease, as has been shown in the case of HIV and malaria in Africa (Abu-Raddad et al. 2006). Additionally, recombination events between newly introduced and existing strains of pathogens may result in the generation and emergence of new highly virulent variants (Farrer et al. 2011). On the other hand, negative associations can also affect the distribution of novel pathogens in a host population. Existing infections could prevent secondary infections from establishing through, for example, competitive exclusion, as has been found between *Salmonella* spp. (Rabsch et al. 2000). This phenomenon in general has proven to be a useful control measure to contain epidemic outbreaks (Mead 2000; La Ragione and Woodward 2003).

As honey bees are susceptible to a wide range of pathogens and pests (Evans and Schwarz 2011), co-infections are commonly observed in nature, both at the colony and individual levels (Cox-Foster et al. 2007; Chen et al. 2009; Runckel et al. 2011). However, studies exploring interactions between pathogens and how these affect their epidemiological dynamics and subsequently shaping their community structure and distribution are lacking. An important observation after *N. ceranae* emergence in *A. mellifera* population was that it seems to be gradually replacing, at least in some regions of the world, *Nosema apis* (Klee et al. 2007; Higes et al. 2013), which is the native microsporidian infecting the western honey bee (Zander 1909; Bailey 1955). Thus the invasive pathogen did not only manage to become widespread but it is also becoming gradually the major microsporidian of the honey bee in



many regions of the world (Chen et al. 2008; Jara et al. 2012; Botías et al. 2012; Fernández et al. 2012; Meixner et al. 2014). This apparent gradual dominance of *N. ceranae* over *N. apis* had been initially attributed to a higher virulence exhibited by *N. ceranae* (Paxton et al. 2007; Martín-Hernández et al. 2011; Williams et al. 2014). However, recent studies from USA addressing the comparative virulence of the two microsporidia show little difference in virulence between the two pathogens (Milbrath et al. 2015; Huang et al. 2015). Whether these discrepancies in results reflect continental differences between European (former studies) and American (recent studies) host-pathogen strains needs to be investigated. Nevertheless, these contradictory results highlight the need for future research that must focus on other mechanisms besides host mortality when trying to explain the infection pattern of these two microsporidia, such as differences in parasite growth or transmission.

As a step towards understanding this apparent replacement of the native *N. apis* by the invasive *N. ceranae*, the first two chapters (chapter I and II) of this thesis try to identify factors that are driving their epidemiological dynamics and distribution. More specifically, because of the still open question of the possibly greater virulence of *N. ceranae* over *N. apis*, the first (I) chapter of this thesis tests the hypothesis proposed by Huang et al. (2015) that *N. ceranae* is more virulent than *N. apis* for honey bees in Europe by performing mortality bioassays using a European honey bee strain and European isolates of *Nosema* spp.

The second (II) chapter addresses the role of co-infections in shaping pathogen distribution patterns in a host community. Previous studies have shown that when these two pathogens are introduced simultaneously to a host, *N. apis* and *N. ceranae* growth trajectories proceed independently, even when introduced at unequal frequencies (Forsgren and Fries 2010). However, little is known about the growth response of these parasites under alternative infection regimes, particularly during sequential infection. My study focuses on how differences in exposure sequence affect interaction outcomes, with implications of interspecific interactions on shaping patterns of parasite prevalence in nature.

Additionally, as interactions between infectious agents are not restricted between taxonomically closely related species (Cox 2001) and given that both *N. ceranae* and DWV are frequently found within the same host (Fürst et al. 2014), occupying similar niches (i.e. gut cells) (Fries 2010; Möckel et al. 2011), I further explored the interaction dynamics between these two emergent pathogens (Chapter III). Previous field studies have linked

*Nosema* spp. infection with increased susceptibility to other pathogens, including DWV (Cornman et al. 2012), while a recent correlational study by Costa et al. (2011) showed a negative association between *N. ceranae* and DWV loads, suggesting antagonistic interactions. However, there is a lack of studies examining the outcome of concomitant DWV/*N. ceranae* infections under control laboratory conditions despite their importance since they can potentially affect disease dynamics of both emerging pathogens. This is addressed in chapter III of the thesis, where, using sequential infections under a reciprocally crossed experimental design, I explore the nature of their between-species interactions.

Disease dynamics is not only affected by within-host interactions but also by between host-interactions. In order to established and spread in a (new) population, a pathogen has to transmit to susceptible hosts (Woolhouse et al. 2005). Hence factors that enhance transmission opportunities also represent important parameters in disease emergence. Changes in host behaviour can directly affect contact rates and exposure risk. Thus the ability of a novel pathogen to modify a host's behaviour, such as its mobility or habitat preference, could be an effective mean to increase its chances to spread in a host population (Poulin 2006; Schmid-Hempel 2011). On the other hand the ability of a host to modify its behaviour in response to an infection could be proven beneficial for containing the spread of the disease in the population (Poulin 2006; Schmid-Hempel 2011). Therefore, modifications in host behaviour following the introduction of a pathogen to a new host species could be an important predictor of its establishment in the host population.

In eusocial insects such as honey bees where highly related individuals live in densely packed colonies and exhibit complex social organisation, behaviour plays an important role in disease dynamics. The high density of individuals within a colony results in high rates of contacts among nestmates, increasing exposure and transmission relative to solitary animals (Schmid-Hempel 1998). Yet they have also evolved a suit of behavioural patterns that depend on cooperation between individuals in order to counteract this increased exposure risk (Cremer et al. 2007). These anti-parasite behaviours are part of a wider complex of physiological and behavioural collective defence mechanism exhibited by social insects and known as "social immunity" (Cremer et al. 2007; Evans and Spivak 2010). Examples include hygienic practices such as allo- or self-grooming and removal of infected brood or nestmates (Cremer et al. 2007).

However, these behavioural defences could also work to the benefit of the pathogen by increasing its transmission. If, for example, an infected individual becomes the target of increased grooming, this can increase the likelihood of healthy nestmates becoming exposed to a disease (e.g. through licking). In addition to sanitary measures, the spatial and behavioural compartmentalisation observed in colonies of many eusocial insects seems to serve as an important mechanism affecting disease dynamics (Cremer et al. 2007; Evans and Spivak 2010). Honey bee workers, exhibit age-related biases in task performance, known as temporal polyethism (Winston 1987). This task specialisation affects directly disease dynamics as members of the same age group interact more frequent with each other than with members of other age groups, creating a heterogeneous interaction network within the colony (Naug and Smith 2007; Naug 2008; Stroeymeyt et al. 2014). Young bees performing nurse tasks are found mainly in the centre of the colony, where the brood is located, while foragers, who are the oldest members of the colony, operate mainly outside or in the peripheral of the nest (Seeley 1982). Hence, age-structure seems to serve as a kind of barrier to pathogen transmission while at the same time maximises colony efficiency (Naug 2008; Evans and Spivak 2010). Furthermore, the system seems also to be highly flexible in response to stressors, including diseases (Oster and Wilson 1978; Huang and Robinson 1992; Woyciechowski and Moroń 2009). It has been shown that *Nosema* spp. infected individuals become foragers to an earlier age than their healthy nestmates and thus they spend less time performing nurse tasks and minimizing infection risk of the young members of the nest (Woyciechowski and Kozłowski 1998; Goblirsch et al. 2013).

As any behavioural response due to, or directed against, a pathogen can impact the course of a disease within a honey bee population, it is becoming apparent that investigations tackling the issue of disease spread of emerging pathogens such as *N. ceranae* and DWV should take into account alterations in host behaviour. This was addressed in the fourth (IV) chapter of this thesis, where I recorded behavioural modifications in honey bee workers after infection with DWV and *N. ceranae*. The aim was to identify which behavioural responses are triggered under the pressure of these two emerging pathogens, focusing both on social and temporal polyethism patterns.

## References

- Abu-Raddad LJ, Patnaik P, Kublin JG (2006) Dual infection with HIV and Malaria fuels the spread of both diseases in Sub-Saharan Africa. *Science* 314:1603–1606. doi: 10.1126/science.1132338
- Alizon S, Lion S (2011) Within-host parasite cooperation and the evolution of virulence. *Proc R Soc B Biol Sci* 278:3738–3747. doi: 10.1098/rspb.2011.0471

- Aufauvre J, Misme-Aucouturier B, Viguès B, et al. (2014) Transcriptome analyses of the honeybee response to *Nosema ceranae* and insecticides. PLoS One 9:e91686. doi: 10.1371/journal.pone.0091686
- Bailey L (1955) The epidemiology and control of *Nosema* disease of the honey-bee. Ann Appl Biol 43:379–389. doi: 10.1111/j.1744-7348.1955.tb02488.x
- Bailey L, Ball BV (1991) Honey Bee Pathology, second edi. Academic Press, London
- Blaustein AR, Johnson PTJ (2010) When an infection turns lethal. Nature 465:881–882. doi: 10.1038/465881a
- Boecking O, Genersch E (2008) Varroosis – the ongoing crisis in bee keeping. J Consum Prot Food Saf 3:221–228. doi: 10.1007/s00003-008-0331-y
- Botías C, Martín-hernández R, Barrios L, et al. (2013) *Nosema* spp . infection and its negative effects on honey bees (*Apis mellifera iberiensis*) at the colony level. Vet Res. doi: doi:10.1186/1297-9716-44-25
- Botías C, Martín-Hernández R, Garrido-Bailón E, et al. (2012) The growing prevalence of *Nosema ceranae* in honey bees in Spain, an emerging problem for the last decade. Res Vet Sci 93:150–155. doi: 10.1016/j.rvsc.2011.08.002
- Brown SP, Hochberg ME, Grenfell BT (2002) Does multiple infection select for raised virulence? Trends Microbiol 10:401–405.
- Carreck NL, Ball B V, Martin S (2010) Honey bee colony collapse and changes in viral prevalence associated with *Varroa destructor*. J Apic Res 49:93–94. doi: 10.3896/IBRA.1.49.1.13
- Chen Y, Evans JD, Smith IB, Pettis JS (2008) *Nosema ceranae* is a long-present and wide-spread microsporidian infection of the European honey bee (*Apis mellifera*) in the United States. J Invertebr Pathol 97:186–8. doi: 10.1016/j.jip.2007.07.010
- Chen Y, Evans JD, Zhou L, et al. (2009) Asymmetrical coexistence of *Nosema ceranae* and *Nosema apis* in honey bees. J Invertebr Pathol 101:204–209. doi: 10.1016/j.jip.2009.05.012
- Chen Y, Pettis JVS, Feldlaufer MF (2005) Detection of multiple viruses in queens of the honey bee *Apis mellifera* L . J Invertebr Pathol 90:118–121. doi: 10.1016/j.jip.2005.08.005
- Chen YP, Siede R (2007) Honey bee viruses. Adv Virus Res 70:33–80. doi: 10.1016/S0065-3527(07)70002-7
- Cornman RS, Tarpy DR, Chen Y, et al. (2012) Pathogen webs in collapsing honey bee colonies. PLoS One 7:e43562.
- Costa C, Tanner G, Lodesani M, et al. (2011) Negative correlation between *Nosema ceranae* spore loads and deformed wing virus infection levels in adult honey bee workers. J Invertebr Pathol 108:224–225. doi: 10.1016/j.jip.2011.08.012
- Cox FEG (2001) Concomitant infections, parasites and immune responses. Parasitology 122:S23–S38.
- Cox-Foster DL, Conlan S, Holmes EC, et al. (2007) A metagenomic survey of microbes in honey bee colony collapse disorder. Science 318:283–287. doi: 10.1126/science.1146498
- Cremer S, Armitage SAO, Schmid-Hempel P (2007) Social immunity. Curr Biol 17:R693–R702. doi: 10.1016/j.cub.2007.06.008
- Cunningham AA (1996) Disease risks of wildlife translocations. Soc Conserv Biol 10:349–353.
- Dainat B, Evans JD, Chen YP, et al. (2012) Dead or alive: Deformed wing virus and *Varroa destructor* reduce the life span of winter honeybees. Appl Environ Microbiol 78:981–987. doi: 10.1128/AEM.06537-11
- Daszak P, Berger L, Cunningham AA, et al. (1999) Emerging infectious diseases and amphibian population declines. Emerg Infect Dis 5:735–748. doi: 10.3201/eid0506.990601
- Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife--threats to biodiversity and human health. Science 287:443–449. doi: 8209 [pii]
- Daszak P, Cunningham AA, Hyatt AD (2001) Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Trop 78:103–116.
- de Miranda JR, Fries I (2008) Venereal and vertical transmission of deformed wing virus in honeybees (*Apis mellifera* L.). J Invertebr Pathol 98:184–189. doi: 10.1016/j.jip.2008.02.004
- de Miranda JR, Genersch E (2010) Deformed wing virus. J Invertebr Pathol 103:S48–S61. doi: 10.1016/j.jip.2009.06.012
- Dukas R (2008) Mortality rates of honey bees in the wild. Insectes Soc 55:252–255. doi: 10.1007/s00040-008-0995-4

- Dussaubat C, Brunet J-L, Higes M, et al. (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 JD, Schwarz RS (2011) Bees brought to their knees: microbes affecting honey bee health. Trends Microbiol 19:614–620. doi: 10.1016/j.tim.2011.09.003
- Evans JD, Spivak M (2010) Socialized medicine: Individual and communal disease barriers in honey bees. J Invertebr Pathol 103:S62–S72. doi: 10.1016/j.jip.2009.06.019
- Farrer RA, Weinert LA, Bielby J, et al. (2011) Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. Proc Natl Acad Sci U S A 108:18732–18736. doi: 10.1073/pnas-1111915108
- Fernández JM, Puerta F, Cousinou M, et al. (2012) Asymptomatic presence of *Nosema* spp. in Spanish commercial apiaries. J Invertebr Pathol 111:106–110. doi: 10.1016/j.jip.2012.06.008
- Fievet J, Tentcheva D, Gauthier L, et al. (2006) Localization of deformed wing virus infection in queen and drone *Apis mellifera* L. Virol J 3:16. doi: 10.1186/1743-422X-3-16
- Fisher MC, Garner TWJ, Walker SF (2009) Global emergence of *Batrachochytrium dendrobatidis* and amphibian Chytridiomycosis in space, time, and host. Annu Rev Microbiol 63:291–310. doi: 10.1146/annurev.micro.091208.073435
- Forsgren E, Fries I (2010) Comparative virulence of *Nosema ceranae* and *Nosema apis* in individual European honey bees. Vet Parasitol 170:212–217. doi: 10.1016/j.vetpar.2010.02.010
- 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
- Fries I (1988) Infectivity and multiplication of *Nosema apis* Z. in the ventriculus of the honey bee. Apidologie 19:319–328.
- Fries I, Chauzat, M-P, Chen Y-PP, et al. (2013) Standard methods for *Nosema* research. J Apic Res 52:1–28. doi: 10.3896/IBRA.1.52.1.14
- Fries I, Feng F, da Silva A, et al. (1996) *Nosema ceranae* n. sp. (Microspora, Nosematidae), morphological and molecular characterization of a microsporidian parasite of the Asian honey bee *Apis cerana* (Hymenoptera, Apidae). Eur J Protistol 32:356–365. doi: 10.1016/S0932-4739(96)80059-9
- Fürst MA, McMahon DP, Osborne JL, et al. (2014) Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature 506:364–366. doi: 10.1038/nature12977
- Gallai N, Salles J-M, Settele J, Vaissière BE (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol Econ 68:810–821. doi: 10.1016/j.ecolecon.2008.06.014
- Gauthier L, Ravallec M, Tournaire M, et al. (2011) Viruses associated with ovarian degeneration in *Apis mellifera* L. queens. PLoS One 6:e16217. doi: 10.1371/journal.pone.0016217
- Genersch E (2010) Honey bee pathology: current threats to honey bees and beekeeping. Appl Microbiol Biotechnol 87:87–97. doi: 10.1007/s00253-010-2573-8
- Genersch E, Aubert M (2010) Emerging and re-emerging viruses of the honey bee (*Apis mellifera* L.). Vet Res 41:54. doi: 10.1051/vetres/2010027
- Genersch E, von der Ohe W, Kaatz H, et al. (2010) The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie 41:332–352. doi: 10.1051/apido/2010014
- Gisder S, Hedtke K, Möckel N, et al. (2010) Five-year cohort study of *Nosema* spp. in Germany: does climate shape virulence and assertiveness of *Nosema ceranae*? Appl Environ Microbiol 76:3032–3038. doi: 10.1128/aem.03097-09
- Gisder S, Möckel N, Linde A, Genersch E (2011) A cell culture model for *Nosema ceranae* and *Nosema apis* allows new insights into the life cycle of these important honey bee-pathogenic microsporidia. Environ Microbiol 13:404–413. doi: 10.1111/j.1462-2920.2010.02346.x
- Goblirsch M, Huang ZY, Spivak M (2013) Physiological and behavioral changes in honey bees (*Apis mellifera*) induced by *Nosema ceranae* infection. PLoS One 8:e58165. doi: 10.1371/journal.pone.0058165
- González-Varo JP, Biesmeijer JC, Bommarco R, et al. (2013) Combined effects of global change pressures on animal-mediated pollination. Trends Ecol Evol 28:524–30. doi: 10.1016/j.tree.2013.05.008

- Goulson D, Nicholls E, Botías C, Rotheray EL (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* (347). doi: 10.1126/science.1255957
- Guzmán E, Eccles L, Calvete Y, et al. (2010) *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie* 41:443–450.
- 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, Martín-Hernández R, Botías C, et al. (2008) How natural infection by *Nosema ceranae* causes honeybee colony collapse. *Environ Microbiol* 10:2659–2669. doi: 10.1111/j.1462-2920.2008.01687.x
- Higes M, Martín-Hernández R, Garrido-Bailón E, et al. (2009) Honeybee colony collapse due to *Nosema ceranae* in professional apiaries. *Environ Microbiol Rep* 1:110–113. doi: 10.1111/j.1758-2229.2009.00014.x
- Higes M, Martín-Hernández R, Meana A (2010) *Nosema ceranae* in Europe: an emergent type C nosemosis\*. *Apidologie* 41:375–392.
- Higes M, Meana A, Bartolomé C, et al. (2013) *Nosema ceranae* (Microsporidia), a controversial 21st century honey bee pathogen. *Environ Microbiol Rep* 5:17–29. doi: 10.1111/1758-2229.12024
- Highfield AC, Nagar A El, Mackinder LCM, et al. (2009) Deformed wing virus implicated in overwintering honeybee colony losses. *Appl Environ Microbiol* 75:7212–7220. doi: 10.1128/AEM.02227-09
- Huang W, Solter L, Aronstein K, Huang Z (2015) Infectivity and virulence of *Nosema ceranae* and *Nosema apis* in commercially available North American honey bees. *J Invertebr Pathol* 124:107–113. doi: 10.1016/j.jip.2014.10.006
- Huang W-F, Solter LF (2013) Comparative development and tissue tropism of *Nosema apis* and *Nosema ceranae*. *J Invertebr Pathol* 113:35–41. doi: 10.1016/j.jip.2013.01.001
- Huang ZY, Robinson GE (1992) Honeybee colony integration: worker-worker interactions mediate hormonally regulated plasticity in division of labor. *Proc Natl Acad Sci U S A* 89:11726–11729. doi: 10.1073/pnas.89.24.11726
- Jara L, Cepero A, Garrido-Bailón E, et al. (2012) Linking evolutionary lineage with parasite and pathogen prevalence in the Iberian honey bee. *J Invertebr Pathol* 110:8–13. doi: 10.1016/j.jip.2012.01.007
- Jones KE, Patel NG, Levy MA, et al. (2008) Global trends in emerging infectious diseases. *Nature* 451:990–994. doi: 10.1038/nature06536
- Klee J, Besana A, Genersch E, et al. (2007) Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. *J Invertebr Pathol* 96:1–10. doi: 10.1016/j.jip.2007.02.014
- Klein A-M, Vaissière BE, Cane JH, et al. (2007) Importance of pollinators in changing landscapes for world crops. *Proc Biol Sci* 274:303–13. doi: 10.1098/rspb.2006.3721
- La Ragione RM, Woodward MJ (2003) Competitive exclusion by *Bacillus subtilis* spores of *Salmonella enterica* serotype Enteritidis and *Clostridium perfringens* in young chickens. *Vet Microbiol* 94:245–256. doi: 10.1016/S0378-1135(03)00077-4
- Lanzi G, Miranda JR De, Boniotti MB, et al. (2006) Molecular and biological characterization of Deformed Wing Virus of honeybees (*Apis mellifera* L.). *J Virol* 80:4998–5009. doi: 10.1128/JVI.80.10.4998
- Lawn SD, Bekker L-G, Middelkoop K, et al. (2006) Impact of HIV infection on the epidemiology of tuberculosis in a peri-urban community in South Africa: the need for age-specific interventions. *Clin Infect Dis* 42:1040–1047. doi: 10.1086/501018
- Le Conte Y, Ellis M, Ritter W (2010) Varroa mites and honey bee health: can Varroa explain part of the colony losses? *Apidologie* 41:353–363.
- Martin SJ, Highfield AC, Brettell L, et al. (2012) Global honey bee viral landscape altered by a parasitic mite. *Science* 336:1304–1306. doi: 10.1126/science.1220941
- Martín-Hernández R, Botías C, Barrios L, et al (2011) Comparison of the energetic stress associated with experimental *Nosema ceranae* and *Nosema apis* infection of honeybees (*Apis mellifera*). *Parasitol Res* 109:605–612. doi: 10.1007/s00436-011-2292-9

- Martín-Hernández R, Meana A, Prieto L, et al. (2007) Outcome of colonization of *Apis mellifera* by *Nosema ceranae*. *Appl Environ Microbiol* 73:6331–8. doi: 10.1128/AEM.00270-07
- 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
- Mead GC (2000) Prospects for “competitive exclusion” treatment to control *Salmonellas* and other foodborne pathogens in poultry. *Vet J* 159:111–123. doi: 10.1053/tvj.1999.0423
- Meixner MD, Francis RM, Gajda A, et al. (2014) Occurrence of parasites and pathogens in honey bee colonies used in a European genotype-environment interactions experiment. *J Apic Res* 53:215–219. doi: 10.3896/IBRA.1.53.2.04
- Mideo N (2009) Parasite adaptations to within-host competition. *Trends Parasitol* 25:261–268. doi: 10.1016/j.pt.2009.03.001
- Milbrath MO, van Tran T, Huang W-F, et al. (2015) Comparative virulence and competition between *Nosema apis* and *Nosema ceranae* in honey bees (*Apis mellifera*). *J Invertebr Pathol* 125:9–15. doi: 10.1016/j.jip.2014.12.006
- Möckel N, Gisder S, Genersch E (2011) Horizontal transmission of deformed wing virus : pathological consequences in adult bees (*Apis mellifera*) depend on the transmission route. *J Gen Virol* 92:370–377. doi: 10.1099/vir.0.025940-0
- Morens DM, Fauci AS (2013) Emerging infectious diseases: threats to human health and global stability. *PLoS Pathog* 9:e1003467. doi: 10.1371/journal.ppat.1003467
- Morens DM, Folkers GK, Fauci AS (2004) The challenge of emerging and re-emerging infectious diseases. *Nature* 430:242–249. doi: 10.1038/nature02759
- Moritz RFA, de Miranda JR, Fries I, et al. (2010) Research strategies to improve honeybee health in Europe. *Apidologie* 41:227–242.
- Morse RA, Calderone NW (2000) The value of honey bees as pollinators of U. S. crops in 2000. *Bee Cult* 1–15.
- Morse SS (1995) Factors in the emergence of infectious diseases. *Emerg Infect Dis* 1:7–15. doi: 10.3201/eid0101.950102
- Naug D (2008) Structure of the social network and its influence on transmission dynamics in a honeybee colony. *Behav Ecol Sociobiol* 62:1719–1725. doi: 10.1007/s00265-008-0600-x
- Naug D, Smith B (2007) Experimentally induced change in infectious period affects transmission dynamics in a social group. *Proc R Soc B Biol Sci* 274:61–65. doi: 10.1098/rspb.2006.3695
- Nazzi F, Brown SP, Annoscia D, et al. (2012) Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. *PLoS Pathog* 8:e1002735. doi: 10.1371/journal.ppat.1002735
- Neumann P, Carreck NL (2010) Honey bee colony losses. *J Apic Res* 49:1–6. doi: 10.3896/IBRA.1.49.1.01
- Oldroyd BP (1999) Coevolution while you wait: *Varroa jacobsoni*, a new parasite of western honeybees. *Trends Ecol Evol* 14:312–315. doi: 10.1016/S0169-5347(99)01613-4
- Oster GF, Wilson EO (1978) *Caste and ecology in the social insects*. Princeton University Press, Princeton, New Jersey
- Oudemans A. (1904) On a new genus and species of parasitic acari. *Notes from Leyden Museum* 24:216–222.
- Paxton R, Klee J, Korpela S, 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.
- Pelin A, Selman M, Aris-Brosou S, et al. (2015) Genome analyses suggest the presence of polyploidy and recent human-driven expansions in eight global populations of the honeybee pathogen *Nosema ceranae*. *Environ Microbiol* 17:4443–4458. doi: 10.1111/1462-2920.12883
- Potts SG, Biesmeijer JC, Kremen C, et al. (2010a) Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol* 25:345–53. doi: 10.1016/j.tree.2010.01.007
- Potts SG, Roberts SPM, Dean R, et al. (2010b) Declines of managed honey bees and beekeepers in Europe. *J Apic Res* 49:15–22. doi: 10.3896/ibra.1.49.1.02
- Poulin R (2001) Interactions between species and the structure of helminth communities. *Parasitology* 122:S3–S11.
- Poulin R (2006) *Evolutionary ecology of parasites*. Smithsonian Institution Press, Washington, D. C.
- Rabsch W, Hargis BM, Tsolis RM, et al. (2000) Competitive exclusion of *Salmonella Enteritidis* by *Salmonella Gallinarum* in poultry. *Emerg Infect Dis* 6:443–448. doi: 10.3201/eid0605.000501

- Ratnieks FLW, Carreck NL (2010) Clarity on honey bee collapse? *Science* 327:152–153. doi: 10.1126/science.1185563
- Roberts KE, Evison SEF, Baer B, Hughes WOH (2015) The cost of promiscuity: sexual transmission of *Nosema* microsporidian parasites in polyandrous honey bees. *Sci Rep* 5:10982. doi: 10.1038/srep10982
- Rosenkranz P, Aumeier P, Ziegelmann B (2010) Biology and control of *Varroa destructor*. *J Invertebr Pathol* 103:S96–S119. doi: 10.1016/j.jip.2009.07.016
- Runckel C, Flenniken ML, Engel JC, et al. (2011) Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, *Nosema*, and *Crithidia*. *PLoS One* 6:e20656. doi: 10.1371/journal.pone.0020656
- Ruttner F, Ritter W (1980) Das Eindringen von *Varroa jacobsoni* nach Europa im Rückblick. *Allg Deut Imkerz* 14:130–134.
- Schmid-Hempel P (2011) *Evolutionary parasitology: The Integrated Study of Infections, Immunology, Ecology, and Genetics*. Oxford Univ Press, Oxford
- Schmid-Hempel P (1998) *Parasites in social insects*. Princeton University Press, Princeton, New Jersey
- Schroeder DC, Martin SJ (2012) Deformed wing virus: the main suspect in unexplained honeybee deaths. *Virulence* 3:589–598. doi: 10.4161/viru.22219
- Seeley TD (1982) Adaptive significance of the age polyethism schedule in honeybee colonies. *Behav Ecol Sociobiol* 11:287–293. doi: 10.1007/BF00299306
- Shah KS, Evans EC, Pizzorno MC (2009) Localization of deformed wing virus (DWV) in the brains of the honeybee, *Apis mellifera* Linnaeus. *Virol J* 6:182. doi: 10.1186/1743-422X-6-182
- Shen M, Yang X, Cox-Foster D, Cui L (2005) The role of varroa mites in infections of Kashmir bee virus (KBV) and deformed wing virus (DWV) in honey bees. *Virology* 342:141–149. doi: 10.1016/j.virol.2005.07.012
- Shimanuki H, Calderone NW, Knox DA (1994) Parasitic mite syndrome: The symptoms. *Am Bee J* 134:827–828.
- Smith KF, Sax DF, Lafferty KD (2006) Evidence for the role of infectious disease in species extinction and endangerment. *Conserv Biol* 20:1349–1357. doi: 10.1111/j.1523-1739.2006.00524.x
- Spleen AM, Lengerich EJ, Rennich K, et al. (2013) A national survey of managed honey bee 2011–12 winter colony losses in the United States : results from the Bee Informed Partnership. *J Apic Res* 52:44–53. doi: 10.3896/IBRA.1.52.2.07
- Stokstad E (2007) The Case of the empty hives. *Science* 316:970–972.
- Stroeymeyt N, Casillas-Pérez B, Cremer S (2014) Organisational immunity in social insects. *Curr Opin Insect Sci* 5:1–15. doi: 10.1016/j.cois.2014.09.001
- Taylor LH, Latham SM, Woolhouse MEJ (2001) Risk factors for human disease emergence. *Philos Trans R Soc B Biol Sci* 356:983–989. doi: 10.1098/rstb.2001.0888
- Telfer S, Lambin X, Birtles R, et al. (2010) Species interactions in a parasite community drive infection risk in a wildlife population. *Science* 330:243–6. doi: 10.1126/science.1190333
- Tentcheva D, Gauthier L, Zappulla N, et al. (2004) Prevalence and seasonal variations of six bee viruses in *Apis mellifera* L. and *Varroa destructor* mite populations in France. *Appl Environ Microbiol* 70:7185–7191. doi: 10.1128/AEM.70.12.7185
- Texier C, Vidau C, Viguès B, et al. (2010) Microsporidia: a model for minimal parasite–host interactions. *Curr Opin Microbiol* 13:443–449. doi: 10.1016/j.mib.2010.05.005
- van der Zee R, Pisa L, Andonov S, et al. (2012) Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008–9 and 2009–10. *J Apic Res* 51:100–114. doi: 10.3896/IBRA.1.51.1.12
- van Dooremalen C, Gerritsen L, Cornelissen B, et al. (2012) Winter survival of individual honey bees and honey bee colonies depends on level of *Varroa destructor* infestation. *PLoS One* 7:e36285. doi: 10.1371/journal.pone.0036285
- vanEngelsdorp D, Evans JD, Saegerman C, et al. (2009) Colony collapse disorder: a descriptive study. *PLoS One* 4:e6481. doi: 10.1371/journal.pone.0006481
- vanEngelsdorp D, Hayes JJ, Underwood RM, Pettis JS (2010) A survey of honey bee colony losses in the United States , fall 2008 to spring 2009. *J Apic Res* 49:7–14. doi: 10.3896/IBRA.1.49.1.03



- WHOER Team (2014) Ebola virus disease in West Africa- The first 9 months of the epidemic and forward projections. *N Engl J Med* 371:1481–1495. doi: 10.1056/NEJMoa1411100
- Wilcove DS, Rothstein D, Dubow J, et al. (1998) Quantifying threats to imperiled species in the United States. *Bioscience* 48:607–615. doi: 10.2307/1313420
- Williams GR, Shutler D, Burgher-MacLellan KL, Rogers REL (2014) Infra-population and -community dynamics of the parasites *Nosema apis* and *Nosema ceranae*, and consequences for honey bee (*Apis mellifera*) hosts. *PLoS One* 9:e99465.
- Winston M (1987) The biology of the honey bee. Harvard University Press, Cambridge, Massachusetts and London, England
- Woolhouse MEJ, Dye C (2001) Population biology of emerging and re-emerging pathogens. *PhilosTrans R Soc London Ser B* 356:981–1106.
- Woolhouse MEJ, Haydon DT, Antia R (2005) Emerging pathogens: the epidemiology and evolution of species jumps. *Trends Ecol Evol* 20:238–244. doi: 10.1016/j.tree.2005.02.009
- Woyciechowski M, Kozłowski J (1998) Division of labor by division of risk according to worker life expectancy in the honey bee (*Apis mellifera* L.). *Apidologie* 29:191–205. doi: 10.1051/apido:19980111
- Woyciechowski M, Moroń D (2009) Life expectancy and onset of foraging in the honeybee (*Apis mellifera*). *Insectes Soc* 56:193–201. doi: 10.1007/s00040-009-0012-6
- Yue C, Genersch E (2005) RT-PCR analysis of Deformed wing virus in honeybees (*Apis mellifera*) and mites (*Varroa destructor*). *J Gen Virol* 86:3419–3424. doi: 10.1099/vir.0.81401-0
- Yue C, Schröder M, Gisder S, Genersch E (2007) Vertical-transmission routes for deformed wing virus of honeybees (*Apis mellifera*). *J Gen Virol* 88:2329–2336. doi: 10.1099/vir.0.83101-0
- Zander E (1909) Tierische Parasiten als Krankheitserreger bei der Biene. *Münchener Bienenzeitung* 31:196–204.
- Zhang Z-Q (2000) Notes on *Varroa destructor* (Acari : Varroidae) parasitic on honeybees in New Zealand. *Syst Appl Acarol Spec Publ* 5:9–14.

## **I. European isolates of the Microsporidia *Nosema apis* and *Nosema ceranae* have similar virulence in laboratory tests on European worker honey bees**

Myrsini E. Natsopoulou<sup>1\*</sup>, Vincent Doublet<sup>1,2</sup>, Robert J. Paxton<sup>1,2</sup>

<sup>1</sup> Institut für Biologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 8, 06120 Halle (Saale), Germany

<sup>2</sup> German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

\*Correspondence: Myrsini E. Natsopoulou; Email: myrsini.natsopoulou@zoologie.uni-halle.de

### **Abstract**

*Nosema apis* and *Nosema ceranae* are gut parasites that infect western honey bees (*Apis mellifera*) worldwide. *N. ceranae* is an exotic infectious disease agent of *A. mellifera*, having been originally described in the Asian honey bee (*Apis cerana*), while *N. apis* is native to the western honey bee. To better understand the dynamics and epidemiology of the two pathogens, we examined the impact of European isolates of both Microsporidia on the longevity of European *A. mellifera* in a controlled laboratory experiment. *N. ceranae* caused slightly higher host mortality compared to *N. apis*, but differences in virulence were subtle and non-significant. Variation across published studies may reflect geographic differences in the coadaptation of hosts and parasites and seasonal differences in host susceptibility.

**Keywords:** *Apis mellifera*, Microsporidia, host, parasite, survival

**Apidologie (2015), doi: 10.1007/s13592-015-0375-9**

(Accepted 9 June 2015)

## II. Interspecific competition in honey bee intracellular gut parasites is asymmetric and favours the spread of an emerging infectious disease

Myrsini E. Natsopoulou<sup>1\*</sup>¶, Dino P. McMahon<sup>1,2,3,4\*</sup>, Vincent Doublet<sup>1,5</sup>, John Bryden<sup>6</sup>, Robert J. Paxton<sup>1,2,5</sup>

<sup>1</sup> Institut für Biologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 8, 06120 Halle (Saale), Germany

<sup>2</sup> School of Biological Sciences, MBC, Queen's University Belfast, Belfast BT9 7BL, UK

<sup>3</sup> Institute of Biology, Freie Universität Berlin, Schwendenerstr. 1, 14195 Berlin, Germany

<sup>4</sup> Department for Materials and Environment, BAM Federal Institute for Materials Research and Testing, Unter den Eichen 87, 12205 Berlin, Germany

<sup>5</sup> German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

<sup>6</sup> School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey, TW20 0EX, UK

\*These authors contributed equally

¶ Correspondence: Myrsini E. Natsopoulou; Email: myrsini.natsopoulou@zoologie.uni-halle.de

### Abstract

There is increasing appreciation that hosts in natural populations are subject to infection by multiple parasite species. Yet the epidemiological and ecological processes determining the outcome of mixed infections are poorly understood. Here, we use two intracellular gut parasites (Microsporidia), one exotic and one co-evolved in the western honey bees (*Apis mellifera*), in an experiment in which either one or both parasites were administered either simultaneously or sequentially. We provide clear evidence of within host competition; order of infection was an important determinant of the competitive outcome between parasites, with the first parasite significantly inhibiting the growth of the second, regardless of species. However the strength of this 'priority effect' was highly asymmetric, with the exotic *Nosema ceranae* exhibiting stronger inhibition of *Nosema apis* than vice versa. Our results reveal an unusual asymmetry in parasite competition that is dependent on order of infection. When incorporated into a mathematical model of disease prevalence, we find asymmetric competition to be an important predictor of the patterns of parasite prevalence found in nature. Our findings demonstrate the wider significance of complex multi-host multi-parasite interactions as drivers of host-pathogen community structure.

**Keywords:** *Apis mellifera*, Microsporidia, host, parasite, co-infection, priority effect

**Proceedings of the Royal Society B: Biological Sciences (2015); 282:1798, doi: 10.1098/rspb.2014.1896**

(Accepted 24 October 2014)

### III. Within-host competition among the honey bees pathogens *Nosema ceranae* and Deformed wing virus is asymmetric and to the disadvantage of the virus

Vincent Doublet<sup>1,\*</sup>, Myrsini E. Natsopoulou<sup>1</sup>, Luise Zschiesche<sup>1</sup>, Robert J. Paxton<sup>1,2,3</sup>

<sup>1</sup> Institut für Biologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 8, 06120 Halle (Saale), Germany

<sup>2</sup> German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

<sup>3</sup>School of Biological Sciences, Queen's University Belfast, UK

\*Corresponding author: Vincent Doublet, Vincent.doublet@zoologie.uni-halle.de

#### Abstract

Two pathogens co-infecting a common host can either interact positively (facilitation), negatively (competition) or act independently. A correlative study has suggested that two pathogens of the honey bee, *Nosema ceranae* and Deformed wing virus (DWV), interact negatively within a host (Costa et al. 2011). To test this hypothesis, we sequentially co-infected honey bees with these pathogens in a reciprocally crossed experimental design. Prior establishment in the host ventriculus by *N. ceranae* inhibited DWV while prior infection by DWV did not impact *N. ceranae*, highlighting an asymmetry in the competitive interaction between these emerging pathogens.

**Keywords:** DWV, Microsporidian, *Apis mellifera*, Priority effect, Interaction

**Journal of Invertebrate Pathology (2015); 124: 31-34,**

**doi: <http://dx.doi.org/10.1016/j.jip.2014.10.007>**

(Accepted 22 October 2014)

## IV. Parasites modulate within-colony activity and accelerate the temporal polyethism schedule of a social insect, the honey bee

Myrsini E. Natsopoulou<sup>1\*</sup>, Dino P. McMahon<sup>2,3</sup>, Robert J. Paxton<sup>1</sup>

<sup>1</sup> Institut für Biologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 8, 06120 Halle (Saale), Germany

<sup>2</sup> Institute of Biology, Free University Berlin, Schwendenerstr.1, 14195 Berlin, Germany

<sup>3</sup> Department for Materials and Environment, BAM Federal Institute for Materials Research and Testing, Unter den Eichen 87, 12205 Berlin, Germany

\*Correspondence: Myrsini E. Natsopoulou; Email: myrsini.natsopoulou@zoologie.uni-halle.de

### Abstract

Task allocation in social insect colonies is generally organised into an age-related division of labour, termed the temporal polyethism schedule, which may in part have evolved to reduce infection of the colony's brood by pests and pathogens. The temporal polyethism schedule is sensitive to colony perturbations that may lead to adaptive changes in task allocation, maintaining colony homeostasis. Though social insects can be infected by a range of parasites, little is known of how these parasites impact within-colony behaviour and the temporal polyethism schedule. We use honey bees (*Apis mellifera*) experimentally infected by two of their emerging pathogens, Deformed wing virus (DWV), which is relatively understudied concerning its behavioural impact on its host, and the exotic microsporidian *Nosema ceranae*. We examined parasite effects on host temporal polyethism and patterns of activity within the colony. We found that pathogens accelerated the temporal polyethism schedule, but without reducing host behavioural repertoire. Infected hosts exhibited increased hyperactivity, allocating more time to self-grooming and foraging-related tasks. The strength of behavioural alterations we observed was found to be pathogen specific; behavioural modifications were more pronounced in virus-treated hosts versus *N. ceranae*-treated hosts, with potential benefits for the colony in terms of reducing within-colony transmission. Investigating the effects of multiple pathogens on behavioural patterns of social insects could play a crucial role in understanding pathogen spread within a colony and their effects on colony social organisation.

**Keywords:** Host, Pathogen, Multiple infection, *Apis mellifera*, *Nosema ceranae*, Deformed wing virus

**Behavioral Ecology and Sociobiology (2015), doi: 10.1007/s00265-015-2019-5**

(Accepted 29 September 2015)

## **Synthesis**

Apiculture has a long history, with honey and wax being important trade products in human societies since the ancient years (Roffet-Salque et al. 2015). Thus it is not surprising that honey bee biology and health has been the focus of many studies for centuries (Bloch et al. 2010). Until a few decades ago, most infections of the honey bee (other than the bacterial ‘foulbroods’) were considered harmless and easy to treat by beekeepers (Bailey et al. 1981; Chen and Siede 2007). Currently honey bee pathogens are the subject of renewed interest.

This has happened in part because new tools in biology led to the identification of existing and novel pathogens and provided a new appreciation of their uses in addressing a wide range of fundamental questions in the evolutionary biology of social insects (Schmid-Hempel 1994; Cremer et al. 2007; Singh et al. 2010; Runckel et al. 2011), and in part because there is a pressing need to find out more about their role in the global pollinator biodiversity crisis at a time when outbreaks of (re)emerging infections have been associated with unusual high mortality of honey bee colonies (Cox-Foster et al. 2007; Paxton 2010; Schroeder and Martin 2012). Nonetheless, within this new and growing field of bee pathology, knowledge gaps remain substantial with regard to concurrent multi-parasite infections.

Whether and to what extent the coexistence of multi-parasite infections influences not only parasite infracommunity structure and dynamics but also the health of their host is still largely unexplored in honey bees. The work presented in the current thesis, pursued in close collaboration with several colleagues, provides new insights on the interactions that occur when a bee host is infected with more than one infectious agent. In particular, it focused on two pathogens that recently emerged in honey bees: the microsporidan *N. ceranae* and the virus DWV. I used a series of experimental infections coupled with survival and behavioural experiments which allowed me to specify the nature of the relationship between co-infecting microsporidia and/or viruses and to investigate their consequences on within-colony honey bee behaviour. The implications of my PhD work relate to the understanding of epidemiological factors that shape parasite communities within a host population and that will help to re-evaluate the role of diseases in honey bees as tractable experimental models in biology.

First I show a lack of significant difference in virulence in terms of induced host mortality between *N. ceranae* and *N. apis* (Chapter I), which is in agreement with two recent studies from the US (Milbrath et al. 2015; Huang et al. 2015). My study expands their results as I used a European honey bee strain and European isolates of *N. ceranae*. At the same time my paper rejects the hypothesis of Huang et al. (2015) of “continental differences”. My results highlight the idea that the suggested competitive advantage of *N. ceranae* over *N. apis* and the apparent replacement of *N. apis* by *N. ceranae* in some regions of the world cannot be explained by increased host mortality induced by *N. ceranae*, as has commonly been reported in recent years. Thus future research must focus on other mechanisms besides host mortality.

This provided the basis for an experimental investigation of the within-host interaction dynamics between these two gut parasites (chapter II). I demonstrated that parasite competition critically depends on order of infection (priority effect), and that the exotic microsporidian species is a better competitor than the native species. An important aspect of the research was the implementation of a mathematical modelling approach. By incorporating empirical findings into a mathematical model of disease prevalence I found that asymmetric competition contributes significantly to the observed patterns of prevalence of these two pathogens in nature. The study highlights the importance of taking within-host interactions into account when investigating distribution patterns of emerging infectious diseases. It also emphasises that knowledge of complex multi-species interaction is critical for gaining a better understanding of host-parasite community structure.

Focusing again on multiple infections within adult worker bees, I extended our studies by examining the role of priority effects in interactions between pathogens that are not taxonomically related, namely the microsporidian *N. ceranae* and the virus DWV (Chapter III). My results revealed once again an asymmetric competitive interaction that depends strongly on order of infection. Interestingly, competitive suppression was found to affect only the virus while the microsporidian was unaffected. These results provide important information as they suggest that the presence of *N. ceranae* may play an important role in structuring the dynamics of DWV loads in honey bee colonies.

Negative associations between pathogens may arise due to one of three well described mechanisms of competition i.e. exploitation competition for host resources, apparent competition by stimulation of host immune responses, and interference competition through

e.g. secretion of toxic molecules (Read and Taylor 2001). Although in the current work I do not explicitly explore the processes behind the observed competitive asymmetry, I have attempted to identify and discuss the possible key mechanisms that may be responsible (Chapter II, Chapter III).

Future research attention should be given on unravelling the resource or mechanism that limits the replication success of competitors. The availability of new tools in biology, including next-generation sequencing, proteomics, cell cultures models and improved microscopy will enable more focused experiments to understand the mechanisms in operation under the presented study system. As both *Nosema* spp. and DWV are obligate intracellular pathogens (Fries et al. 1996; de Miranda and Genersch 2010), the unit of infection is the cell. Tracking the real time the course of mixed infections and visualising what is happening at the within-cell level will give a more complete picture of how *Nosema* spp. and DWV interact within the host. For example, exploring whether a pathogen is able to infect a cell that has already been infected by another pathogen will help determine if the two pathogens compete over space (cell pool) within the host or inside co-infected cells. Flow cytometry methods could also be valuable to determine the number of cells infected by the two pathogens while the infections spread within the host and reveal whether the abundance of host resources represents a limiting factor (e.g. see Tromas et al. 2014). Finally as the honey bee genome has now been sequenced and annotated (Honey bee genome sequencing consortium, 2006; Elisk et al. 2014), identifying genome-wide gene expression patterns under single and co-infection conditions will give critical insight into the role of the host-immune system in shaping the observed competitive outcomes.

An important question that derives from the presented chapters is whether the antagonistic relationship among *Nosema* spp. or between *N. ceranae* and DWV translates into competition for transmission between the co-infecting pathogens. The transmission rate is an important epidemiological parameter that determines community infrastructure, pathogen ecology and evolution (Poulin 2006; Schmid-Hempel 2011). Hence, determining whether within-host dynamics alter the between-host transmission of *Nosema* spp. and DWV under co-infection conditions is urgently needed. Studies across different host-parasite systems have shown that within-host competition and pathogen transmission are tightly linked and co-infection has the potential to change rates of pathogen transmission (e.g. de Roode et al. 2005; Karvonen et al. 2011). However, a recent study of Abkallo et al. (2015) focusing on different strains of the



rodent malaria parasite *Plasmodium yoelli* found that, even if different strains compete within the same host, there was no relationship between parasite growth rate and transmission.

Finally, disease dynamics in a community are affected not only by within-host but also by between-host interactions. For that reason, in the last presented study (Chapter IV), I examined how exposure to *N. ceranae* and DWV, two emerging honey bee pathogens with distinct pathologies, administered singly or combined, affect the honey bee's temporal polyethism schedule. In addition, I explored how these two prominent pathogens can shape social behaviours and activity patterns within a colony, traits that represent important mechanisms for maintaining colony homeostasis (Seeley 1982; Winston 1987; Johnson 2010) but have been largely ignored in bee pathology research.

My results reveal behavioural modulations that could potentially benefit the colony in terms of reduced within-colony transmission. These insights pave the way for further studies on the role of host behavioural modifications in pathogen spread within a colony. Incorporating new technologies, such as automated observation technologies that track continuously a large number of individuals (e.g. Mersch et al. 2013), into future studies would be beneficial for constructing networks of interactions between infected and healthy nestmates. This information could improve our understanding of the strategies employed by both the host and the parasite in relation to disease transmission and generate novel insights into the factors that affect parasite community structure and assembly. Theoretical epidemiological modelling could also supplement empirical studies to obtain predictions regarding the observed behavioural modification on exposure and maintenance of pathogens in the population (e.g. Theis et al. 2015).

Overall in the presented work I tried to link within-host and behavioural dynamics so as to bring new insights to the epidemiology of emerging pathogens, using honey bees as a model system. As a next step, research should focus on how these within-host interactions and behavioural alterations affect the between-host transmission of both emerging pathogens so we can understand and maybe predict their evolutionary trajectories.

## References

Abkallo HM, Tangena J-A, Tang J, et al. (2015) Within-host competition does not select for virulence in malaria parasites; studies with *Plasmodium yoelii*. PLoS Pathog 11:e1004628. doi: 10.1371/journal.ppat.1004628

- Bailey L, Ball B V, Perry JN (1981) The prevalence of viruses of honey bees in Britain. *Ann Appl Biol* 97:109–118. doi: 10.1111/j.1744-7348.1981.tb02999.x
- Bloch G, Francoy TM, Wachtel I, et al. (2010) Industrial apiculture in the Jordan valley during Biblical times with Anatolian honeybees. *Proc Natl Acad Sci U S A* 107:11240–11244. doi: 10.1073/pnas.1003265107
- Chen YP, Siede R (2007) Honey bee viruses. *Adv Virus Res* 70:33–80. doi: 10.1016/S0065-3527(07)70002-7
- Cox-Foster DL, Conlan S, Holmes EC, et al. (2007) A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* 318:283–287. doi: 10.1126/science.1146498
- Cremer S, Armitage SAO, Schmid-Hempel P (2007) Social immunity. *Curr Biol* 17:R693–R702. doi: 10.1016/j.cub.2007.06.008
- de Miranda JR, Genersch E (2010) Deformed wing virus. *J Invertebr Pathol* 103:S48–S61. doi: 10.1016/j.jip.2009.06.012
- de Roode JC, Pansini R, Cheesman SJ, et al. (2005) Virulence and competitive ability in genetically diverse malaria infections. *Proc Natl Acad Sci U S A* 102:7624–7628. doi: 10.1073/pnas.0500078102
- Elsik CG, Worley KC, Bennett AK, et al. (2014) Finding the missing honey bee genes: lessons learned from a genome upgrade. *BMC Genomics* 15:1–29.
- Fries I, Feng F, da Silva A, et al. (1996) *Nosema ceranae* n. sp. (Microspora, Nosematidae), morphological and molecular characterization of a microsporidian parasite of the Asian honey bee *Apis cerana* (Hymenoptera, Apidae). *Eur J Protistol* 32:356–365. doi: 10.1016/S0932-4739(96)80059-9
- Honey bee genome sequencing consortium (2006) Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature* 443:931–949.
- Huang W, Solter L, Aronstein K, Huang Z (2015) Infectivity and virulence of *Nosema ceranae* and *Nosema apis* in commercially available North American honey bees. *J Invertebr Pathol* 124:107–113. doi: 10.1016/j.jip.2014.10.006
- Johnson BR (2010) Division of labor in honeybees: form, function, and proximate mechanisms. *Behav Ecol Sociobiol* 64:305–316. doi: 10.1007/s00265-009-0874-7
- Karvonen A, Rellstab C, Louhi K-R, Jokela J (2011) Synchronous attack is advantageous: mixed genotype infections lead to higher infection success in trematode parasites. *Proc R Soc London B Biol Sci* 279:171–176.
- Mersch DP, Crespi A, Keller L (2013) Tracking individuals shows spatial fidelity is a key regulator of ant social organization. *Science* 340:1090–1093. doi: 10.1126/science.1234316
- Milbrath MO, van Tran T, Huang W-F, et al. (2015) Comparative virulence and competition between *Nosema apis* and *Nosema ceranae* in honey bees (*Apis mellifera*). *J Invertebr Pathol* 125:9–15. doi: 10.1016/j.jip.2014.12.006
- Paxton R (2010) Does infection by *Nosema ceranae* cause “Colony Collapse Disorder” in honey bees (*Apis mellifera*)? *J Apic Res* 49:80. doi: 10.3896/IBRA.1.49.1.11
- Poulin R (2006) Evolutionary ecology of parasites. Smithsonian Institution Press, Washington, D. C.
- Roffet-Salque M, Regert M, Evershed RP, et al. (2015) Widespread exploitation of the honeybee by early Neolithic farmers. *Nature* 527:226–230. doi.org/10.1038/nature15757
- Read AF, Taylor LH (2001) The Ecology of Genetically Diverse Infections. *Science* 292:1099–1102.
- Runckel C, Flenniken ML, Engel JC, et al. (2011) Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, *Nosema*, and *Crithidia*. *PLoS One* 6:e20656.
- Schmid-Hempel P (1994) Infection and colony variability in social insects. *Philos Trans R Soc London B Biol Sci* 346:313–321.
- Schmid-Hempel P (2011) Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics. Oxford Univ Press, Oxford
- Schroeder DC, Martin SJ (2012) Deformed wing virus: the main suspect in unexplained honeybee deaths. *Virulence* 3:589–598. doi: 10.4161/viru.22219
- Seeley TD (1982) Adaptive significance of the age polyethism schedule in honeybee colonies. *Behav Ecol Sociobiol* 11:287–293. doi: 10.1007/BF00299306

- Singh R, Levitt AL, Rajotte EG, et al. (2010) RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-*Apis* Hymenopteran species. PLoS One 5:e14357.
- Theis FJ, Ugelvig L V, Marr C, Cremer S (2015) Opposing effects of allogrooming on disease transmission in ant societies. Philos Trans R Soc London B Biol Sci 370:20140108.
- Tromas N, Zwart MP, Lafforgue G, Elena SF (2014) Within-host spatiotemporal dynamics of plant virus infection at the cellular level. PLoS Genet 10:e1004186. doi: 10.1371/journal.pgen.1004186
- Winston M (1987) The biology of the honey bee. Harvard University Press, Cambridge, Massachusetts and London, England

**Acknowledgements**

I would like to thank my supervisor, Robert J. Paxton, for giving me the opportunity to work in his group and for providing an excellent research environment that made possible the successful accomplishment of my doctorate. I am very grateful for all the opportunities I was given to follow new ideas and new experiments.

My gratitude is extended to Dino McMahon and Vincent Doublet. This work will have never been possible without their help. I strongly enjoyed the collaboration with them. They have provided me with invaluable guidance. I thank them for discussions and constant support. Vincent Doublet deserves another special thank for being a constant source of encouragement and support ever since I met him in both scientific and non-scientific terms.

I want to thank the entire working group of Allgemeine Zoologie of MLU Halle-Wittenberg. I especially thank Christopher Mayack for the help provided and also as a friend. Also special thanks to Lorraine McKendrick for all the moments we shared inside but also outside the lab. I would like to thank all the current and former lab members with whom I share one or the other memory. I also thank Anja Miertsch and Kerstin Goessel for solving many technical issues and keeping the lab running. Also I thank the entire working group of Molecular Ecology of MLU for all the fruitful discussions.

Finally a special thank goes to Panas (Panagiotis T.) who is always there for me, supporting and encouraging me to do my best. Talking with him is always a source of inspiration.

Thanks to all the friends I met in Halle and Leipzig who made my experience so unique and exciting.

## Curriculum Vitae

Myrsini E. Natsopoulou  
myrsini.natsopoulou@zoologie.uni-halle.de

Born on March 19<sup>th</sup>, 1985

Nationality: Greek

---

### **Education**

2011-present	<b>Ph.D candidate</b> , department of Zoology, Martin-Luther-University (MLU) Halle-Wittenberg, Halle/Saale, Germany
2008-2010	<b>M.Sc. in Biology</b> , University of Iceland, Faculty of Life and Environmental Sciences, Department of Biology, Reykjavik, Iceland
2002-2007	<b>B.Sc. in Biology</b> , Major: Molecular Biology, Genetics and Biotechnology, Aristotle University of Thessaloniki, Faculty of Science, School of Biology, Thessaloniki, Greece

---

### **Research and work experience**

2011- present	<b>Ph.D. candidate</b> at MLU Halle-Wittenberg, Germany, group of Insect evolutionary ecology. Ph.D. thesis to be submitted: <i>Emerging infectious diseases of honey bees: within host interactions</i> . Supervisor: Prof. Dr. Robert Paxton
2009-2010	<b>M.Sc. Research project</b> at the Research Centre of the Westfjords, University of Iceland. <i>MHC allelic richness between sympatric populations in Icelandic threespine stickleback morphs</i> . Supervisors: Dr. Gudbjorg Asta Olafsdottir and Prof. Dr. Snaebjorn Palsson
2008-2009	<b>M.Sc. Research project</b> at the Research Centre of the Westfjords, University of Iceland. <i>Morphological variability and sexual dimorphism related to ecological opportunity in Icelandic threespine stickleback (<i>Gasterosteus aculeatus</i> L.)</i> . Supervisors: Dr. Gudbjorg Asta Olafsdottir and Prof. Dr. Snaebjorn Palsson
2007	<b>Bachelor thesis</b> at University of Iceland, Faculty of Life and Environmental Sciences, Department of Biology, Reykjavik, Iceland (Exchange student, ERASMUS-Scholarship). <i>Expression of antibacterial activity in <i>Chlamydomonas reinhardtii</i></i> . Supervisors: Prof. Dr. Gudmundur Gudmundsson and Prof. Dr. Zophonias Jonsson
Jun.-Aug.2006	Internship at the “424 General Military Hospital”, Department of Microbiology, Thessaloniki, Greece

---

### **Awards and Grants**

2015	<b>Support grant of UK 750 £</b> from the Genetics Society (UK), for attending the ESEB 2015 meeting, August 10-14, 2015, in Lausanne, Switzerland (poster presentation)
2008-2010	<b>Stipend award for M.Sc. research</b> , University of Iceland, Iceland
2007	<b>6 months Socrates-Erasmus-Scholarship</b> to visit University of Iceland, awarded from the Greek State Scholarship Foundation (IKY)

---

### **Oral contributions in Conferences**

- ❖ 2015: *Natsopoulou ME*. et al., Varroa destructor virus (VDV-1) causes elevated rates of overwinter decline of honeybees (*Apis mellifera*), IUSSI: IV central European meeting Lichtenfels, Germany
- ❖ 2014: *Natsopoulou ME*. et al., Congeneric competition among Microsporidia of the honey bee is asymmetric and favours the spread of an emerging infectious disease. 61.AG-Tagung der Institute für Bienenforschung 19-21st March 2013, Marburg, Germany
- ❖ 2013 *Natsopoulou*: Competition between the intracellular pathogens *Nosema apis* and *Nosema ceranae* in the midgut of the honey bee (*Apis mellifera*), 3rd SPP 1399 Symposium and RCNE meeting, Blossin, Berlin, Germany
- ❖ 2013 *Natsopoulou ME* et al., DWV/VDV-1, overwinter colony losses, honeybee. 60. AG-Tagung der Institute für Bienenforschung 19-21st March 2013, Würzburg, Germany
- ❖ 2012 *Natsopoulou ME* et al.: Worker and drone drifting in relation to parasite transmission: a component of the FitBee project, 59. Jahrestagung der Arbeitsgemeinschaft der Bieneninstitute Bonn 28-30th March 2012, Bonn, Germany.

**Publication List**

10. **Natsopoulou ME**, McMahon DP, Paxton RJ (2015). Parasites modulate within-colony activity and accelerate the temporal polyethism schedule of a social insect, the honey bee. *Behavioral Ecology and Sociobiology*. doi: [10.1007/s00265-015-2019-5](https://doi.org/10.1007/s00265-015-2019-5)
9. Forfert N\*, **Natsopoulou ME\***, Frey E, Rosenkranz P, Paxton RJ, Moritz RFA (2015). Parasites and pathogens of the honeybee (*Apis mellifera*) and their influence on inter-colonial transmission. *PLoS ONE*, **10**: e0140337, doi: [10.1371/journal.pone.0140337](https://doi.org/10.1371/journal.pone.0140337)  
[\* co-first authorship]
8. Mayack C, **Natsopoulou ME**, McMahon (2015). *Nosema ceranae* alters a highly conserved hormonal stress pathway in honeybees. *Insect Molecular Biology*. doi: [10.1111/imb.12190](https://doi.org/10.1111/imb.12190)
7. **Natsopoulou ME**, Doublet V, Paxton RJ. (2015) European isolates of the Microsporidia *Nosema apis* and *Nosema ceranae* have similar virulence in laboratory tests on European worker honey bees. *Apidologie*. doi: [10.1007/s13592-015-0375-9](https://doi.org/10.1007/s13592-015-0375-9)
6. **Natsopoulou ME\***, McMahon DP\*, Doublet V, Bryden J, Paxton RJ (2015) Interspecific competition in honeybee intracellular gut parasites is asymmetric and favours the spread of an emerging infectious disease. *Proceedings of the Royal Society B*, **282**: 20141896. doi:[10.1098/rspb.2014.1896](https://doi.org/10.1098/rspb.2014.1896)  
[\* co-first authorship]
5. Doublet V, **Natsopoulou ME**, Zschieche L, Paxton RJ (2015) Within-host competition among the honey bees pathogens *Nosema ceranae* and Deformed wing virus is asymmetric and to the disadvantage of the virus. *Journal of Invertebrate Pathology*, **124**: 31-34. doi:[10.1016/j.jip.2014.10.007](https://doi.org/10.1016/j.jip.2014.10.007)
4. Suraporn S, **Natsopoulou ME**, Doublet V, McMahon DP, Paxton RJ (2013) *Nosema ceranae* is not detected in honey bees (*Apis* spp.) of northeast Thailand. *Journal of Apicultural Research*, **52**(5): 259-261. doi:[10.3896/IBRA.1.52.5.13](https://doi.org/10.3896/IBRA.1.52.5.13)
3. Fries I, Chauzat M-P, Chen YP, Doublet V, Genersch E, Gisder S, Higes M, McMahon DP, Martín-Hernández R, **Natsopoulou ME**, Paxton RJ, Tanner G, Webster TC, Williams GR (2013) Standard methods for *Nosema* research. In V Dietemann, JD Ellis, P Neumann (Eds) *The COLOSS BEEBOOK: Volume II: Standard methods for Apis mellifera pest and pathogen research*. *Journal of Apicultural Research*, **52**(1). doi:[10.3896/IBRA.1.52.1.14](https://doi.org/10.3896/IBRA.1.52.1.14)
2. Williams GR, Alaux C, Costa C, Csáki T, Doublet V, Eisenhardt D, Fries I, Kuhn R, McMahon DP, Medrzycki P, Murray TE, **Natsopoulou ME**, Neumann P, Oliver R, Paxton RJ, Pernal SF, Shutler D, Tanner G, Van der Steen JJM, Brodschneider R (2013) Standard methods for maintaining adult *Apis mellifera* in cages under in vitro laboratory conditions. In V Dietemann; JD Ellis; P Neumann (Eds) *The COLOSS BEEBOOK, Volume I: standard methods for Apis mellifera research*. *Journal of Apicultural Research*, **52**(1). doi:[10.3896/IBRA.1.52.1.04](https://doi.org/10.3896/IBRA.1.52.1.04)
1. **Natsopoulou ME**, Pálsson S, Ólafsdóttir, G. Á. (2012) Parasites and parallel divergence of the number of individual MHC alleles between sympatric three-spined stickleback *Gasterosteus aculeatus* morphs in Iceland, *Journal of Fish Biology*, **81**(5): 1696-1714. doi: [10.1111/j.1095-8649.2012.03430.x](https://doi.org/10.1111/j.1095-8649.2012.03430.x)

## **Declaration of own contribution to the original articles**

I. Natsopoulou ME, Doublet V, Paxton RJ. (2015) European isolates of the Microsporidia *Nosema apis* and *Nosema ceranae* have similar virulence in laboratory tests on European worker honey bees. *Apidologie*. doi: 10.1007/s13592-015-0375-9

Design of the project: 80%  
Experimental work: 90%  
Data analysis: 100%  
Wrote the paper: 80%

II. Natsopoulou ME, McMahon DP, Doublet V, Bryden J, Paxton RJ (2015) Interspecific competition in honeybee intracellular gut parasites is asymmetric and favours the spread of an emerging infectious disease. *Proceedings of the Royal Society B*, 282: 20141896. doi:10.1098/rspb.2014.1896

Design of the project: 50%  
Experimental work: 70%  
Data analysis: 80%  
Wrote the paper: 70%

III. Doublet V, Natsopoulou ME, Zschieche L, Paxton RJ (2015) Within-host competition among the honey bees pathogens *Nosema ceranae* and Deformed wing virus is asymmetric and to the disadvantage of the virus. *Journal of Invertebrate Pathology*, **124**: 31-34. doi:10.1016/j.jip.2014.10.007

Design of the project: 50%  
Experimental work: 50%  
Data analysis: 50%  
Wrote the paper: 20%

IV. Natsopoulou ME, McMahon DP, Paxton RJ (2015). Parasites modulate within-colony activity and accelerate the temporal polyethism schedule of a social insect, the honey bee. *Behavioral Ecology and Sociobiology*. doi: 10.1007/s00265-015-2019-5

Design of the project: 80%  
Experimental work: 90%  
Data analysis: 100%  
Wrote the paper: 80%

### **Eidesstattliche Erklärung**

Halle, den 28.01.2016

Hiermit erkläre ich, dass diese Arbeit von mir bisher weder bei der Naturwissenschaftlichen Fakultät I 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 an Eides statt, dass ich dies Arbeit selbstständig und nur unter Zuhilfenahme der angegebenen Hilfsmittel und Literatur angefertigt habe.

Myrsini E. Natsopoulou