Sustainable agriculture through protection of wild bee health: Investigation of transmission risk of the honey bee pathogen *Nosema ceranae*

Inaugural-Dissertation

to obtain the academic degree

Doctor rerum naturalium (Dr. rer. nat.)

submitted to the Department of Biology, Chemistry and Pharmacy

of Freie Universität Berlin

by

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2019

This thesis was conducted between January 2015 and December 2018, under the supervision of Prof. Dr. Jens Rolff (Freie Universität Berlin) and Prof. Dr. Dino McMahon (Bundesanstalt für Materialforschung Berlin & Freie Universität Berlin). The work was carried out at Freie Universität Berlin.

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Date of defense: 02.05.2019

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Acknowledgements

I am thankful for the Deutsche Bundesstiftung Umwelt for providing funding and a valuable network for my thesis. I very much thank Prof. Jens Rolff and Prof. Dino McMahon for their engaged support and supervision of the thesis. I am very greatful to the Bachelorstudents Julia Lorenz and Lorin Schellenberg and the Masterstudent Kathrin Bramke for their contribution. I thank Dr. Christoph Saure for identification of collected bee sample and valuable professional advice, Dr. Erhard Strohm on sharing expertise on *Osmia bicornis*, the *Deutsche Bieneninstitut* for providing data on distribution of *N. ceranae* in honey bees and laboratory training, Dr. Lena Wilfert for exchange of information and skills in virus detection, Marika Harz and Benedickt Polacez for providing honey bees for laboratory experiments. I appreciate the cooperativeness of numerous beekeepers in providing honey bee samples and field sides for collection, the Landschaftspflegeverein Nuthe-Nieplitz for allowing the establishment of nesting resources on their orchards. I thank the Departments for Environment of Berlin and Brandenburg for granting their permission to sample wild bees.

I thoroughly thank my working group members for all their support, in professional questions and discussions, motivation, laughing and the great office atmosphere. Many, many thanks go to my friends and family who have always been there with great encouragement.

Summary

Pollinators are declining globally and emerging pathogens are among the causative factors. Pathogens are shared between domesticated honey bees and wild pollinators with flowers as transmission hubs. In contrast to honey bees, pathogen impacts in wild bees remain mostly unstudied. This thesis aimed to investigate associations between honey bees infected with *Nosema ceranae* and wild bees in the field as well as direct effects in a solitary wild bee experimentally inoculated with *N. ceranae*.

Chapter 1 used a field survey approach to test how the presence of honey bees infected with *N. ceranae* affects the community composition of wild bees sharing the habitat. The results demonstrate a negative correlation between functional diversity of wild bees and the presence of infected honey bees. However, this relationship differs between functional groups. The occurrence of the rare parasitic, the cavity-nesting ones and also the Red List species is negatively associated with pathogen presence in the surrounding honey bee hives whereas the opposite is shown for common social-polylectic species.

Furthermore, a habitat analysis revealed that both the highly specialized solitary-oligolectic and the threatened Red List species were particularly dependent on high coverage of suitable foraging and nesting habitat. In conclusion, sensitivity to *N. ceranae* associated stressors has to be considered differentially for particular functional groups which argue for the support of a functionally diverse pollinator community and special arrangements for the most severely affected groups.

In Chapter 2, the agriculturally managed wild bee *Osmia bicornis* was used as model for solitary bees in an inoculation experiment with *N. ceranae*. The results revealed that inoculation had negligible impacts only on male survival. Several different species of wild bees that shared floral resources with infected honey bees in the environment were screened for the pathogen and were shown to be widely positive for *N. ceranae* whereas the pathogen was not detected in wild bees collected at another field site with uninfected honey bees. *O. bicornis* could therefore be acting as a pathogen reservoir and vector enhancing the circulation of *N. ceranae* within the pollinator network which affects both managed and wild bees.

Larvae of *O. bicornis* were exposed to spores of *N. ceranae* during development in Chapter 3. Exposed individuals were characterized by a greater mortality and a delay in the onset of pupation even though very few spores were detected in a few individuals. Exposure was also associated with a reduction in body size in males as well as an increase in head capsule width in both sexes. The individuals which were exposed to the pathogen as larvae

did not show any fitness costs after hatching. The results indicate that even without proliferation, *N. ceranae* can have sublethal effects on particular life history stages of *O. bicornis* and affect development in this solitary bee model.

Collectively, the results suggest indirect and direct impacts of *N. ceranae* on solitary wild bees but have to be extrapolated with caution regarding species-specific differences in pathogen impacts. Furthermore, in the environment, multiple stressors are afflicting wild bees and can interact synergistically. The present thesis delivers useful starting points for further inoculation experiments with a suitable solitary wild bee model and important information regarding pathogen reservoir species within the pollinator community. In conservation management, special support for particularly sensitive functional groups and life stages is required.

Zusammenfassung

Bestäuber sind weltweit von Rückgängen betroffen wobei neue Krankheitserreger eine der Ursachen sind. An Blüten als Übertragungspunkten treffen sowohl domestizierte Honigbienen als auch auf wildlebende Bestäuber auf die Erreger. Im Gegensatz zu Honigbienen sind die Auswirkungen der Pathogene in Wildbienen bisher kaum erforscht. Diese Arbeit untersucht Zusammenhänge zwischen Honigbienen, die mit *Nosema ceranae* infiziert sind, und Wildbienen im Freiland. Außerdem werden direkte Effekte einer experimentellen Infizierung mit *N. ceranae* in einer solitären Wildbienenart betrachtet.

Im ersten Kapitel wurde mittels einer Feldstudie untersucht, wie sich das Vorhandensein von Honigbienen, die mit *N. ceranae* infiziert sind, auf die Gemeinschaft der Wildbienen im geteilten Habitat auswirkt. Die Ergebnisse zeigen, dass ein negativer Zusammenhang zwischen der funktionellen Diversität der Wildbienen und der Präsenz von infizierten Honigbienen besteht. Allerdings unterschied sich dieser Zusammenhang zwischen verschiedenen funktionellen Gruppen. Das Vorkommen von seltenen, von parasitischen und von in Hohlräumen nistenden sowie der Rote-Liste Arten stand in einem negativen Zusammenhang mit Pathogenbefall in umliegenden Bienenstöcken. In Hinblick auf die häufigen, die sozial lebenden und die polylektischen Arten zeigte sich eine gegenläufige Beziehung. Weiterhin legte eine Habitatanalyse offen, dass sowohl hochspezialisierte solitärlebende und oligolektische wie auch bedrohte Rote-Liste Arten besonders abhängig von einer hohen Deckung von Nahrungs- und Nisthabitaten sind. Schlussfolgernd muss die Anfälligkeit gegenüber mit *N. ceranae* assoziierten Stressfaktoren für verschiedene funktionelle Gruppen unterschiedlich bewertet werden. Dies spricht für die Unterstützung einer diversen Bestäubergesellschaft mit besonderen Maßnahmen für die am stärksten beeinträchtigten Gruppen.

In Kapitel 2 wurde die landwirtschaftlich eingesetzte Wildbiene *Osmia bicornis* als ein Modell für Solitärbienen in einem Infektionsexperiment mit *N. ceranae* untersucht. Die Ergebnisse der Infizierung zeigten eine geringfügig negative Auswirkung ausschließlich auf das Überleben der behandelten Männchen. Verschiedene Wildbienenarten, die sich in ihrer natürlichen Umgebung Blüten mit infizierten Honigbienen teilten, wurden gefangen und auf *N. ceranae* untersucht. Dabei erwiesen sich viele von ihnen als Träger des Pathogens wohingegen es in Wildbienen, von einer anderen Probestelle mit uninfizierten Honigbienen, nicht gefunden wurde. Demnach kann *O. bicornis* ein Pathogenreservoir sowie einen Übertragungsvektor darstellen und die Zirkulation von *N. ceranae* im Bestäubernetzwerk verstärken wovon sowohl domestizierte als auch wildlebende Bienen betroffen sind.

Im dritten Kapitel wurden Larven von *O. bicornis* während ihrer Entwicklung Sporen von *N. ceranae* ausgesetzt. Die behandelten Individuen zeichneten sich durch erhöhte Mortalität sowie einen verspäteten Verpuppungsbeginn aus. Jedoch konnten nur sehr wenige Sporen in einer geringen Anzahl an Individuen gefunden werden. Dennoch wiesen eine geringere Körpergröße in behandelten Männchen sowie eine erhöhte Kopfkapselweite in beiden Geschlechtern auf weitere sublethale Effekte hin. In den behandelten Individuen wurden keine Fitnesskosten nach dem Schlüpfen festgestellt. Das Ergebnis impliziert, dass *N. ceranae* auch ohne Ausbreitung sublethale Auswirkungen auf *O. bicornis* haben kann und die Entwicklung dieses Modellorganismus für solitäre Wildbienen beeinflusst.

Zusammengefasst suggerieren die Ergebnisse indirekte und direkte Auswirkungen von *N. ceranae* in solitären Wildbienen wobei die Generalisierung innerhalb der Artengruppe aufgrund von artspezifischen Unterschieden in der Pathogenauswirkung mit Vorsicht zu betrachten ist. Weiterhin sind Wildbienen in ihrer natürlichen Umgebung mehreren Stressfaktoren ausgesetzt, welche zusammenwirken können. Die vorliegende Arbeit liefert nützliche Ansatzpunkte für weitere Infektionsexperimente mit geeigneten Solitärbienenmodellen sowie wichtige Informationen hinsichtlich von Pathogenreservoiren innerhalb der Bestäubergesellschaft. Im Naturschutzmanagement ist die spezielle Unterstützung von besonders anfälligen funktionalen Gruppen und Lebensstadien von Wildbienen vonnöten.

Thesis outline

This thesis is a monograph consisting of three chapters. Chapter 2 has been submitted for publication and is currently under reconsideration after a major review. Chapter 3 will be submitted for publication. The co-authors and their contributions are stated below. References are outlined below each chapter. The general introduction includes a literature review and the general discussion refers to the three chapters coherently. The references for the general introduction and discussion are placed at the end.

Chapter 1: Müller, U., Hausmann, S. L., McMahon, D. P., Rolff, J.

Associations between wild bee communities and *Nosema ceranae* in honey bees

Author contributions: U.M. designed the study, U.M. conducted field and laboratory work, UM analyzed the data except the landscape analysis, S.H. analysed the landscape data, U. M. interpreted the data, all authors contributed to the manuscript.

Chapter 2: Müller, U., McMahon, D. P., Rolff, J.

Susceptibility of the wild bee *Osmia bicornis* to the honey bee pathogen *Nosema ceranae*

Author contributions: U. M. designed the study and conducted field and laboratory work, U. M. analyzed and interpreted the data, all authors contributed to the manuscript.

Published:

Müller, U., McMahon, D. P., Rolff, J. 2019. Exposure of the wild bee *Osmia bicornis* to the honey bee pathogen *Nosema ceranae*. Agricultural and Forest Entomology 21 (4): 363-371.

Chapter 3: Bramke, K., Müller, U., McMahon, D. P., Rolff, J.

Life history effects in the solitary bee *Osmia bicornis* after larval exposure to the honey bee pathogen *Nosema ceranae*

Author contributions: K. B. and U. M. are shared first authors in the study. K. B. and U. M. designed the study and conducted field and laboratory work, K. B. and U. M. analyzed and interpreted the data, all authors all authors contributed to the manuscript.

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General Introduction

Importance of wild pollinators

Pollination of managed crops and wild plant species by insects is an essential ecosystem service and a key component of biodiversity (Ghazoul 2005, Steffan-Dewenter et al. 2005, Klein et al. 2007, Allsopp et al. 2008, Potts et al. 2010, IPBES 2016). On a global scale 35% of global crops (Klein et al. 2007) and 85% of wild flowers rely at least to some extent on insects for pollination (Ollerton et al. 2011). A variety of species provide these services, including over 20.000 species of bees, as well as butterflies, flies and even some vertebrates (IPBES 2016). However, public concern is focused predominantly on the honey bee *Apis mellifera* (Geldmann and González-Varo 2018), even though managed pollinators are just one component within the diverse pollinator community (Hoehn et al. 2008, Blüthgen and Klein 2011). In Germany, for example, 561 different species of wild bees have been recorded (Amiet and Krebs 2014). High species richness in pollinators implies an enhanced pollination success via supplementary features of the different species (Hoehn et al. 2008, Blüthgen and Klein 2011). However, *A. mellifera* is used intensively for managed crop pollination. It provides 90% of commercial pollination services and demands are increasing continuously (Aizen et al. 2008). The current dependence on predominantly one species for agricultural pollination is risky as it entails reduced resilience against predators, parasites and pathogens and other stressors (Tscharntke et al. 2005, Winfree et al. 2007, Hillebrand et al. 2008, Potts et al. 2010). Low pollinator species diversity is associated with a reduced fruit set independent of honey bee pollination events or species evenness (Garibaldi et al. 2013). Pollination effectiveness in apple trees increases with wild bee diversity and abundance (Blitzer et al. 2016). Consequently, conservation of honey bees alone will not adequately address the loss of pollination services caused by the current pollinator decline (Geldmann and González-Varo 2018).

Wild bees as alternative –also commercial pollinators- to honey bees

Currently, wild bees are increasing in popularity as alternative or supplemental commercial crop pollinators (Schoonvaere et al. 2016). Bumble bees are widely used in cultures such as raspberries and tomatoes for which they are more effective pollinators than honey bees (Greenleaf and Kremen 2006, Velthuis and van Doorn 2006) due to their ability to buzz pollinate and better performance in colder temperatures and lower light intensities (Velthuis and van Doorn 2006, Goulson 2009, de Luca et al. 2013). *Megachile rotundata*, the alfalfa leafcutting bee, is intensively used for pollination of alfalfa and blueberries in the USA (Pitts-Singer and Cane 2011). The alkali bee *Nomia melandri* and the grey-haired alfalfa bee

Rhophitoides canus are both used in alfalfa pollination the USA as well as in eastern Europe (Stephen 1959, Johansen et al. 1982, Ptacek 1989, Dobrynin 1998). Moreover, species from the genera *Osmia* are effective pollinators of agricultural relevance. They are able to forage at lower temperatures than honey bees making them ideally adapted to unpredictable weather conditions in spring when important fruit tree species flower (Vicens and Bosch 2000). Furthermore they visit a large number of flowers per time and contact flower stigmas during each visit (Vicens and Bosch 2000) and as they prefer Rosaceae pollen they are reliable fruit tree pollinators (Márquez et al. 1984, Bosch et al. 2000, Schindler and Peters 2011). *Osmia cornifrons* is used for over 75% of total managed apple tree pollination in Japan (Bosch and Kemp 2001). *O. lignaria* is an established fruit tree pollinator in North America (Bosch and Kemp 2001). *O. ribifloris* is used in blueberry pollination in the southern USA (Sampson et al. 2004, 2009). *O. aglaia* is a potential pollinator of red raspberries and blackberries in California and Western Oregon (Cane 2005); *O. excavate*, *O. pedicornis* and *O. taurus* are auspicious fruit tree pollinators (Maeta 1978, Wei et al. 2002, Sheffield et al. 2013). *Osmia cornuta* is used in southern European orchards (Bosch and Kemp 2001) as is *O. bicornis* for fruit trees, rape and oil-seeds (Kornmilch 2010). In contrast to honey bees they are active in lower temperatures, show a higher visitation rate of flowers per time and can transport more pollen (Kornmilch 2010). So far, *Osmia* species are the only solitary species used in pollination management in European agriculture (Bosch et al. 2002).

Bee declines

Loss of insect diversity and abundance is a highly important current environmental issue as it could result in dramatic cascading effects on food webs and ecosystem services (Hallmann et al. 2017). Reductions in insect biomass of 75% within the last 27 years were detected in a North-West-German nature reserve (Hallmann et al. 2017). For bees, population declines have been reported globally (Fitzpatrick et al. 2007, Oldroyd 2007, Stokstad 2007, Brown and Paxton 2009, Neumann and Carreck 2010, Potts et al. 2010, Cameron et al. 2011, Bartomeus et al. 2013, Vanbergen and Garratt 2013). Some pollinator species are extinct already, and more are becoming vulnerable (Biesmeijer et al. 2006, Potts et al. 2010). In Europe, the conservation status of most bee species is currently unknown (Nieto et al. 2015) but in the Red List of Germany, 7.0 % of all species checked were extinct or disappeared, 5.6 % were critically endangered (Category I), 14.0 % were endangered (Category II) and 15.3 % were classified as vulnerable (Category III) (Westrich et al. 2011). Concomitant decline in plant species dependent on these pollinators show a further dimension of the situation (Biesmeijer et al. 2006). A drastic decline in pollinators would definitely reduce the variety and quality of human diet as various plants delivering important

nutrients like vitamins, proteins and minerals are dependent on insect pollination (Steffan-Dewenter et al. 2005). To prevent these losses, we have to understand what causes them.

Declines in honey bees as well as wild bees cannot be attributed to one single cause but rather to an interaction of multiple factors (Di Pasquale et al. 2013, Brunner et al. 2014, Brown et al. 2016, Goulson et al. 2015, Renzi et al. 2016, Sánchez-Bayo et al. 2016, Genersch et al. 2018). These include abiotic factors such as habitat loss and homogenization (Potts et al. 2010, Kennedy et al. 2013), pesticide applications (Godfray et al. 2015) and climate change (Kerr et al. 2015). Biotic stressors include parasites and pathogens (Graystock et al. 2013a, Graystock et al. 2013b, Fürst et al. 2014, McMahon et al. 2015, Wilfert et al. 2016) and invasive species (Stout and Morales 2009). Among the various stressors, pathogens are known to have severe impacts (Potts et al. 2010, Schroeder and Martin 2012, Fürst et al. 2014) which can be exacerbated by the presence of other stressors: For example, *N. ceranae* infection increases honey bees' sensitivity to pesticides due to synergistic effects (Alaux et al. 2010, Aufavre et al. 2012, Pettis et al. 2012, Vidau et al. 2014, Retschnig et al. 2015, Kairo et al. 2017). For this reason, it is imperative that we better understand how pathogens interact with other abiotic and biotic stressors to affect bee health and survival .

Bee pathogens and situation of pathogens in wild bees

In the case of *A.mellifera*, pathogens and parasites are considered key drivers of colony losses (Genersch et al. 2018). Of particular importance are parasitic mites and the viruses that they transfer (Shen et al. 2005, Cox-Foster et al. 2007, Forsgren et al. 2009, vanEngelsdorp et al. 2009, Bacandritsos et al. 2010, Genersch 2010, Anderson and Roberts 2013, Meixner et al. 2014). For wild bees however, the impact of pathogens remains elusive. Whereas a number of pathogens, ranging from bacteria (Fünfhaus et al. 2018), viruses (McMenamin and Flenniken 2018), fungi (Evison and Jensen 2018) and microsporidia (Paris et al. 2018), are known for the honey bee, the pathogen landscape of wild bees has received little research attention. However, pathogens can have a broad host range among related species (Engelstädter and Hurst 2006) and pathogens previously classified as honey bee pathogens are shared with wild bee taxa. One example of such a pathogen is the microsporidium *Nosema ceranae* which was found in social wild bees (Genersch et al. 2006, Graystock et al. 2013a, Graystock et al. 2013b, Fürst et al. 2014, Graystock et al. 2014) and, after only one study (Ravoet et al. 2014), remains to be studied in solitary wild bees. Bees naturally host a variety of parasites, parasitoids and pathogens (Goulson et al. 2015). Wild bees are a highly diverse taxon and should therefore maintain diverse pathogens. However, contemporary research focused mainly on the pathogens they share with honey bees.

Literature research on micropathogens in European wild bees

In order to obtain an extensive overview of the current state of knowledge on microparasites in wild bees, I conducted a systematic search using the Thomas Reuters Web of Knowledge (http://apps.webofknowledge.com/WOS_GeneralSearch_input.do?product=WOS&search_mode=GeneralSearch&SID=Q2bgHcYu2XQ26AG5kSQ&preferencesSaved=). The following search strings were employed: Topic = wild bees + pathogen* / wild bees + disease* / name of wild bee genus + pathogen* / name of wild bee genus + disease*. The bee genus was based on the system of middle European bee species by Westrich (2013). Information about bee ecology was obtained from Westrich (1989, 2013) and http://www.wildbienen.de/ (15.02.2015). In total, 41 genera from 6 subfamilies were searched. Studies that are included had to fulfill the following selection criteria: (I) the investigation of European wild bee species, (II) the focus on microparasites identified at least to the genus, (III) the detection of pathogens in wild bees in vivo by microscopy and/or PCR as opposed to exclusively in vitro infection experiments. Furthermore I only included already classified pathogens. Only if all criteria were met the study was included. Reports of infections from one individual of a host species were regarded as sufficient. The classification of an agent as a pathogen was made according to the general literature nomenclature. Controversial commensals were considered as well. Species identification of hosts and pathogens could comprise genera as well as species level. Additionally, references in studies obtained from the Web of Knowledge using the above search strings were checked for further studies fulfilling the criteria. Studies selected with expert knowledge not encompassed by the literature search were also included.

Table 1 presents the results obtained until from the literature research until September 9th 2018.

Viruses

Concerning viruses, the 11 honey bee viruses Deformed wing virus (DWV), Black queen cell virus (BQCV), Israel acute paralysis virus (IAPV), Slow bee paralysis virus (SBPV), Acute bee paralysis virus (ABPV), Sacbrood virus (SBV), Kashmir bee virus (KBV), Lake Sinai Virus (LSV), Chronic bee paralysis virus (CBPV), *Apis mellifera* filamentous virus (AmFV) and *Varroa destructor* macula-like Virus (VdMLV) have been detected in a total of 24 species of the wild bee genera Andrenidae, Halictidae, Apidae and Megachilidae (Tehel et al. 2016). As 24 honey bee viruses are currently known (Tehel et al. 2016) more than 11 can expected to be actually shared with wild bees. Low levels of the honey bee viruses BQCV, DWV; IAPV; LSV and SBV were detected in 4 bee families and 8 genera of wild bees in the USA (Dolezal et al. 2016). However, the study pointed out that viral loads were not

replicating or associated with increasing mortality in an inoculation experiment with two wild bee species, in contrast to honey bees. The two solitary bee viruses Scaldi River Bee Virus (SRBV1) and Ganda Bee Virus (GABV) were discovered in *Osmia cornuta* (Schoonvaere et al. 2016). Both viruses were subsequently re-confirmed in the same species (Schoonvaere et al. 2018). A metatranscriptomic study of eight wild bee species in Belgium revealed four new viruses with homology to insect pathogens as well as 11 sequences for yet unclassified arthropod viruses. RNA viruses were also found in other hymenopteran taxa in the USA (Singh et al. 2010) as well as in diptera (Bailes et al. 2018) and a range of other non-hymenopteran honey bee-associated arthropods (Levitt et al. 2013). Viruses that were considered as honey bee pathogens might actually have a wider host range and transmission might be commonplace (McMahon et al. 2015). More studies about potential pollinator hosts are likely to identify additional hosts (Gisder and Genersch 2017). Moreover, viruses can be considered to exists as quasispecies comprised of swarms of mutational variants rather than as one genetically homogeneous species (Domingo and Holland 1997, Lauring and Andino 2010). Genetic detection of viruses will only work for the variants in which the primer binding site did not mutate yet even if they are a minority in the aggregates. Thus primer biases towards specific variants in PCR-based virus detection may lead to underestimates of virus abundance and prevalence (Gisder and Genersch 2017). This might particularly hold true for current species-specific honey bee detection protocols, which do possibly not target the dominant virus mutants in other bee species (Gisder and Genersch 2017). Studies about the actual pathogenicity of viruses are still rare. So far it has been shown for DWV (Genersch et al. 2006, Evison et al. 2012, Fürst et al. 2014, Graystock et al. 2015), IAPV (Meeus et al. 2014, Piot et al. 2015), KBV (Meeus et al. 2014) in *B. terrestris* as well as ABPV in *B. agrorum* and *B. ruderarius* (Graystock et al. 2015) and *B. hortorum*, *B. lucorum*, *B. terrestris* (Graystock et al. 2015, McMahon et al. 2015).

Bacteria

Concerning bacteria, *Wolbachia* spp. were most commonly detected and found in the wild bee genera *Andrena*, *Halictus*, *Lasioglossum* and *Bombus* (Evison et al. 2012). However, their pathogenic impact remains controversial (Werren et al. 2008). Potential bacterial pathogens in *Osmia* sp. comprise seven different genera but are not identified to the species level in most cases (Keller et al. 2013). Two species of spiroplamas known to occur in honey bees were found in *Osmia* sp. (Skou 1975).

Fungi

Fungal pathogens of the genera Ascosphaera, have been detected in *Bombus* sp.,(Maxfield-Taylor et al. 2015), *Megachile* sp. (Skou 1975, Skou and Holm 1989, Goerzen et al. 1990, 1992, Bissett et al. 1996, Anderson et al. 1998, Wynns et al. 2013, McFrederick et al. 2014, Klinger et al. 2015), *Osmia* sp. (Skou 1975, Anderson et al. 1998), *Chelostoma florisome* (Wynns et al. 2013) and *Coelioxys echinata* (Hermoso de Mendoza 1989). *Bettsia alvei* was found in *Melipona fascinata* (Gilliam et al. 1990) and *Osmia cornuta* (Ravoet et al. 2014). *Aspergillus flavus* and *A. tamari* were detected in *Nomia melandri* (Batra and Bohart 1969). *Crithidia bombi* is a common parasite in bumble bees (Shykoff and Schmid-Hempel 1991a) and results in various rather benign effects (Shykoff et al. 1991). However, the parasite is known to impair the founding of a colony when bumble bee queens are infected (Brown et al. 2003). The parasite has also been detected in *Andrena vaga* and *Osmia bicornis* with unknown consequences (Ravoet et al. 2014). New associations with *Crithidia pragensis*, a tubulinosematid, a neogregarine parasite and yeasts of the genus *Metschnikowia* were detected (Schoonvaere et al. 2018). Among the neogregarinida, *Apicystis bombi* is widely distributed in *Bombus* sp. (Lipa and Triggiani 1992, Colla et al. 2006, Plischuk et al. 2009, Graystock et al. 2014) and was also found in *O. cornuta* and O. bicornis as well as in *Andrena vaga*, *A. ventralis* and *Heriades truncorum* (Ravoet et al. 2014). In *B. terrestris A. bombi* results in increased mortality and sucrose sensitivity as well as in a lower lipid:body size ratio (Graystock et al. 2016).

The genus *Nosema* belongs to the fungi-related order Microsporidia, which is characterized by obligate intracellular parasitism (Vávra and Lukeš 2013). It includes more than 150 species and infects hosts from 12 insect orders (Becnel and Andreadis 1999). *Nosema bombi* is a widespread parasite in bumblebees (Durrer and Schmid-Hempel 1995). Effects are variable and not generally detrimental (Schmid-Hempel and Loosli 1998).

Nosema ceranae

N. ceranae switched from the Asian honey bee *Apis ceranae* to the western honey bee *A. mellifera* a few decades ago, possibly due to extensive global trade of managed honey bees (Paxton et al. 2007). The pathogen infects the ventriculus and damages the intestinal tissue and inhibits genes that would initiate renewal (Dussaubat et al. 2012). It utilizes its host's ATP, enabling its own proliferation (Hacker et al., 2014). *N. ceranae* infection interferes with other metabolic processes such as those for carbohydrates, amino acids and lipids and decreases levels of the respective components (Vidau et al. 2014, Badaoui et al. 2017). Moreover, *Nosema* could be responsible for host behavior manipulation via up-regulation of octopamine pathways involved in foraging in order to make its host acquire more energy for

its own usage (Mayack et al. 2015). *N. ceranae* was found to suppress host immune responses (Antúnez et al. 2009, Chaimanee et al. 2012, Huang et al. 2012, Aufauvre et al. 2014, Badaoui et al. 2017). It downregulates gene expression for antimicrobial peptides and cuticle (Antúnez et al. 2009, Chaimanee et al. 2012, Badaoui et al. 2017) and initiates up-regulation of immune-supression genes (Li et al. 2016). Furthermore, *N. ceranae* prevents the self-defense mechanism of apoptosis (Higes et al. 2013). Honey bees that tolerate the pathogen are not affected by the apoptosis manipulation of the pathogen (Kurze et al. 2015). Infected honey bees were found to have a shorter life-span than non-infected individuals (Vidau et al. 2011, Goblirsch et al. 2013). Pathogen load was found to be higher in older (forager) honey bees than in younger house bees (Botías et al. 2013, Goblirsch et al. 2013).

N. ceranae is transmitted via the fecal-oral or oral-oral route (Fries et al. 1996). In honey bee colonies in temperate regions, the pathogen shows varying spore levels throughout the year (Runckel et al. 2011, Smart and Sheppard 2012, Stevanovic et al. 2013) with a seasonal peak in spring and early summer (Traver et al. 2012). However, the findings could not be corroborated for Spain (Higes et al. 2006, Martín-Hernández et al. 2007). Prevalence patterns might be determined by climatic conditions (Fries 2010) rather than *Nosema* species (Stevanovic et al. 2013). Climatic factors might play a role as colder temperatures result in lower infection rates (Retschnig et al. 2017). It is unclear whether or not *N. ceranae* can be considered a causative agent of colony collapse disorder in honey bees or how much it interacts with other influential factors such as habitat loss or diminishing floral resources to affect honey bee health and survival (Paxton 2010). No relationship between the occurrence of *N. ceranae* and CCD could be detected by Cox-Foster et al. (2007) and Chen et al. (2008). In contrast, an association between the colonization of *N. ceranae* and bee losses was supported by Martín-Hernández et al. (2007) and Higes et al. (2008).

In Europe, *N. ceranae* was also detected in the social bumble bees (Genersch et al. 2006, Graystock et al. 2013a, Graystock et al. 2013b, Fürst et al. 2014, Graystock et al. 2014) and a few solitary wild bees of the genera *Andrena* and *Osmia* (Ravoet et al. 2014). Impacts of the pathogen were so far only tested in *B. terrestris audax* with infections shown to spread from the midgut to other tissues, resulting in increased mortality as well as sub-lethal effects. Virulence was found to be even greater than in honey bees (Graystock et al. 2013a, Graystock et al. 2013b). No infection experiments for solitary bees exist so far.

Table 1: Known microparasites in European wild bees

Bee Genera	Bee species	Viruses	Bacteria	Fungi	Pollen collection	Lifeform	References
Andrena	*Andrena* spp.	DWV, BQVC, SBPV	*Wolbachia* spp.	*Ascosphaera* spp.	polylectic and oligolectic	solitary-communal	Evison et al. (2012)
Andrena	*Andrena haemorrhoa*	BeeIV-1, BeeMLV-2, DWV	-	*Nosema thomsoni*	polylectic	solitary	Radzevičiūtė et al. (2017), Schoonvaere et al. (2018)
Andrena	*Andrena helvola*	DWV	-	-	polylectic	solitary	Radzevičiūtė et al. (2017)
Andrena	*A. scotica*	-	-	*Microspora spp., Antonospora scoticae*	polylectic	communal	Fries et al. (1999), Paxton et al. (2007)
Andrena	*A. vaga*	AmFV, BQCV, *IAPV*, LSV, SBV	-	*Apicystis bombis, Crithidia bombi, Nosema thomsoni*	oligolectic (Salix)	solitary	Ravoet et al. (2014)
Andrena	*A. ventralis*	AmFV, LSV	-	*A. bombis, Nosema ceranae*	oligolectic (Salix), sometimes Rosaceae	solitary	Ravoet et al. (2014)
Halictus	*Halictus* spp.	-	*Wolbachia* spp.	*Ascosphaera* spp.	polylectic	social to different degrees	Evison et al. (2012)
Halictus	*Halictus scabiosae*	HsAV (strain D, E, H)	-	-	polylectic	social to different degrees	Bigot et al. (2017)
Lasioglossum	*Lasioglossum* spp.	-	*Wolbachia* spp.	*Ascosphaera* spp., *Microspora* spp.	polylectic and oligolectic	social to different degrees	Evison et al. (2012)

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Megachile	*Megachile centuncularis*	-	-	*Ascosphaera aggregata, Ascosphaera major, Ascosphaera proliperda*	polylectic	solitary	Holm and Skou (1972), Skou (1972), (1975)
Megachile	*Megachile rotundata*	-	-	*A. aggregata, A. proliperda*	polylectic	solitary	Holm and Skou (1972), Hermoso de Mendoza (1989), Youssef and McManus (1991), Jensen et al. (2012)
Megachile	*M. willughbiella*	-	-	*A. tenax, Microascus spp.*	polylectic	solitary	Holm and Skou (1972)
Coelioxys	*Coelioxys echinata*	-	-	*A. aggregata*	polylectic	solitary	Hermoso de Mendoza (1989)
Osmia	*Osmia bicornis*	ABPV, BQCV, CBPV, DWV, LSV, AmFV, VdMLV	*Spiroplasma melliferum, Spiroplasma apis, Bacillus spp., Paenibacillus spp., Photorhabdus luminescens, Xenorhabdus nematophila, Pseudomonas entomophila*	*Ascosphaera spp., A. aggregata, A. major, A. proliperda, A. fimicola, Apicystis bombis, C. bombi, N. ceranae*	polylectic	solitary	Skou (1975), Keller et al. (2013), Ravoet et al.(2014), Radzevičiūtė et al. (2017)

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Osmia	*O. cornuta*	AmFv, BeeMLV-2, BQCV, GABV, LSV, OcNV, SRBV, VdMLV, VdV-1	-	*Ascosphaera* spp., *A. bombis, C.bombi, Crithidia pragensis, N. ceranae*	polylectic	solitary	Ravoet et al. (2014), Schoonvaere et al. (2016, 2018)
Osmia	*Heriades truncorum*	BQCV	-	*A. bombis, N. ceranae*	oligolectic	solitary	Ravoet et al. (2014)
Osmia: Chelostoma	*Chelostoma florisomne*	-	-	*Ascosphaera callicarpa*	oligolectic	solitary	Wynns et al. (2013)
Anthophora	*Anthophora plumipes*	BQCV, SBPV, SBV	-	-	polylectic	solitary	Radzevičiūtė et al. (2017)
Melecta	*Melecta albifrons*	SBPV	-	-	polylectic	parasitic	Radzevičiūtė et al. (2017)
Bombus	*Bombus* spp.	ABPV	*Spiroplasma* sp., Aerobacter cloaca	*Acrostalagmus* spp., *Beauveria bassiana*, *Candida* spp., *Hirsutella* spp., *Metarhizium* sp., *Paecilomyces* spp., *Crithidia bombi*	polylectic	social	Erler et al. (2011, Salathé and Schmid-Hempel (2011), Tognazzo et al. (2012)
Bombus	*B. cryptarum*	BQCV	-	-	polylectic	social	(Schoonvaere et al. 2018)

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Bee Genera	Bee species	Viruses	Bacteria	Fungi	Pollen collection	Lifeform	References
Bombus	*B. hortorum*	BQCV, ABPV, SBPV	*Wolbachia* sp.	*Ascosphaera* spp., *Nosema bombi, N. ceranae, Microsporidia* spp.	polylectic	social	Fantham and Porter (1914), Shykoff and Schmid-Hempel (1991), Larsson et al. (2007), Evison et al. (2012), Graystock et al. (2013a), Fürst et al. (2014), McMahon et al. (2015), Manley et al. (2017)
Bombus	*B. humilis*			*C.bombi*	polylectic	social	Shykoff and Schmid-Hempel (1991)
Bombus	*B. hypnorum*	-	-	*N. bombi, N.ceranae*	polylectic	social	Shykoff and Schmid-Hempel (1991), Tay et al. (2005), Larsson et al. (2007), Graystock et al. (2013a), Graystock et al. (2014)
Bombus	*B. jonellus*	-	-	*N. bombi*	polylectic	social	Tay et al. (2005)
Bombus	*B. lapidarius*	ABPV, BQCV, SBPV, DWV, DWV complex, LSV, VdMLV	*Wolbachia* sp.	*Ascosphaera* spp., *C. bombi, N. bombi, N. ceranae, N.apis, Microsporidia* spp.	polylectic	social	Fantham and Porter (1914), Shykoff and Schmid-Hempel (1991), Tay et al. (2005), Larsson et al. (2007), Evison et al. (2012), Whitehorn et al. (2013), Graystock et al. (2013a), Fürst et al. (2014), Graystock et al. (2014), McMahon et al. (2015), Parmentier et al. (2016), Radzevičiūtė et al. (2017)
Bombus	*B. lucorum*	ABPV, BQCV, SBPV, DWV, DWV complex	-	*C. bombi, N. bombi, N.ceranae*	polylectic	social	Shykoff and Schmid-Hempel (1991), Durrer and Schmid-Hempel (1994), Tay et al. (2005), Yourth and Schmid-Hempel (2006), Larsson et al.(2007), Rutrecht et al. (2007), Rutrecht and Brown (2008, 2009), Ruiz-González et al. (2012), Graystock et al. (2013a), Fürst et al. (2014), Graystock et al. (2014), McMahon et al. (2015)
Bombus	*B. monticola*	DWV	-	-	polylectic	social	Fürst et al. (2014)
Bombus	*B.muscorum*	-	-	*C.bombi*	polylectic	social	Whitehorn et al. (2011)

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Bee Genera	**Bee species**	**Viruses**	**Bacteria**	**Fungi**	**Pollen collection**	**Lifeform**	**References**
Bombus	*B. pascuorum*	BeeMLV-2, DWV, BQCV, ABPV, SBPV	*Wolbachia* sp., *S. melliferum*	*Ascosphaera* spp., *C. bombi, Nosema* spp., *N. bombi, N. ceranae, Microsporidia* spp.	polylectic	social	Fantham and Porter (1914), Betts (1920), Shykoff and Schmid-Hempel (1991), Genersch et al. (2006), Klee et al. (2006), Larsson et al. (2007), Evison et al. (2012), Meeus et al. (2012), Goulson et al. (2012), Whitehorn et al. (2013), Graystock et al. (2013a), Graystock et al. (2014), McMahon et al. (2015), Parmentier et al. (2016), Schoonvaere et al. (2016, 2018), Manley et al. (2017), Radzevičiūtė et al. (2017), Jabal-Uriel et al. (2017)
Bombus	*B. pratorum*	-	*Wolbachia* sp., *S.apis*	*Ascosphaera* spp., *C. bombi, N. bombi, N. ceranae*	polylectic	social	Tay et al. (2005), Larsson et al. (2007), Evison et al. (2012), Meeus et al. (2012), Graystock et al. (2013a), Maharramov et al. (2013), Whitehorn et al. (2013), Graystock et al. (2014), Parmentier et al. (2016)
Bombus	*B. ruderaris*	ABPV	-	*N. bombi*	polylectic	social	Larsson et al. (2007)
Bombus	*B. rupestris*	BQCV	-	-	polylectic	parasitic	Radzevičiūtė et al. (2017)
Bombus	*B. subterraneus/B. latereillelus*	-	-	*N. bombi*	polylectic	social	Fantham and Porter (1914, Tay et al. (2005), Larsson et al. (2007)
Bombus	*B. sylvarum*	-	-	*C.bombi, N. bombi*	polylectic	social	Fantham and Porter (1914), Shykoff and Schmid-Hempel (1991), Schmid-Hempel and Schmid-Hempel (1993)
Bombus	*B. sylvarum*	-	-	*C.bombi, N. bombi*	polylectic	social	Fantham and Porter (1914), Shykoff and Schmid-Hempel (1991), Schmid-Hempel and Schmid-Hempel (1993)

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Bee Genera	Bee species	Viruses	Bacteria	Fungi	Pollen collection	Lifeform	References
Bombus	*B. terrestris*	ABPV, BQCV, IAPV, DWV, DWV complex, KBV, SBPV, SBV, VdV-1	*Wolbachia* sp.,	*Ascosphaera* spp., *A. bombis, Candida bombiphila, C. bombi, Crithidia expoekii, N. bombi, N. ceranae, N. apis,Wickerhamiella sp.*	polylectic	social	(Fantham and Porter 1914, Lipa and Triggiani 1980, Shykoff and Schmid-hempel 1991, Shykoff and Schmid-Hempel 1991, Shykoff et al. 1991, Schmid-Hempel and Schmid-Hempel 1993, Wu 1994, Durrer, Stephan, Schmid-Hempel 1994, Imhoof and Schmid-Hempel 1998, 1999, Liersch, Stephan, Schmid-Hempel 1998, Schmid-Hempel and Loosli 1998, Baer and Schmid-Hempel 1999, 2001, Schmid-Hempel et al. 1999, 2011, Brown et al. 2000, Mallon and Schmid-Hempel 2004, Schmid-Hempel and Funk 2004, Genersch et al. 2006, Klee et al. 2006, Ruiz-González and Brown 2006a, Yourth and Schmid-Hempel 2006, Larsson et al. 2007, Otti and Schmid-Hempel 2007, Rutrecht et al. 2007, Otti, O., Schmid-Hempel 2008, Van der Steen 2008, Yourth et al. 2008, Schmid-Hempel and Tognazzo 2010, Fouks and Lattorff 2011, Koch and Schmid-Hempel 2011, 2012, Goulson et al. 2012, Huth-Schwarz et al. 2012, Ruiz-González, Bryden, Moret, Reber-Funk, et al. 2012, Cisarovsky et al. 2012, Erler et al. 2012, Evison et al. 2012, Graystock, Yates, Darvill, et al. 2013a, Graystock et al. 2013b, Maharramov, Meeus, Maebe, Arbetman, Morales, Graystock, Hughes, Plischuk, Lange, Graaf, et al. 2013, Murray et al. 2013, Whitehorn et al. 2013, Fürst et al. 2014, Graystock et al. 2014, Cisarovsky and Schmid-Hempel 2014, Deshwal and Mallon 2014, McMahon et al. 2015b, Schoonvaere et al. 2016, 2018, Jabal-Uriel et al. 2017b, Radzevičiūtė et al. 2017)
Bombus	*B. terrestris audax*	DWV		*A. bombis, C. bombi, N. ceranae*	polylectic	social	Ruiz-González and Brown (2006), Graystock et al. (2013b)
Bombus	*B. terrestris dalmatinus*	DWV		*A. bombis, C. bombi*	polylectic	social	Ruiz-González and Brown (2006), Graystock et al. (2013)
Bombus	*B. vestalis*	BQCV, SBV	-	-	polylectic	parasitic	Radzevičiūtė et al. (2017)

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Host-pathogen interactions

Detection of a pathogen alone does not provide sufficient information about an infection. The parasite must also replicate. Detection may only reflect the ingestion of the pathogen, for example from food resources (Manley et al. 2015). For example, endogenous viral elements from RNA viruses sharing similarity to plant viruses were found to be integrated into numerous insect genomes (Cui and Holmes 2012). Besides contamination even mutualism or symbiosis are possible (Roossinck 2011). However, even if exposure to a pathogen does not result in an infection, more transmission hubs will enhance its circulation within the pollinator network. The pathogen *Crithidia bombi* can use larvae of *B. terrestris* as a transmission hub even though no infection is taking place (Folly et al. 2017).

As has been shown for vertebrates (Fisher et al. 2012), disease control in domesticated species is essential for the conservation of wild species as spillover of pathogens can be detrimental to the wild hosts (Daszak et al. 2000, Power and Mitchell 2004). Transmission of pathogens between species can result in higher virulence in a new host (Daszak et al. 2000, Power and Mitchell 2004) which has been shown in bumble bees (Graystock et al. 2013a). Closely related species to the original host are particularly vulnerable as potential novel targets hosts (Engelstädter and Hurst 2006, Longdon et al. 2018). Among pollinators, transmission happens via shared flowers and pollen (Durrer and Schmid-Hempel 1994, Graystock et al. 2014). The long-distance spread of infections is enabled by trading of domesticated species (Vitousek et al. 1996). Honey bees are traded worldwide (Wilfert et al. 2016). Consequently, they potentially present important pathogen reservoirs for other pollinators (Meeus et al. 2014). The same applies to commercial bumble bee pollinators (Otterstatter and Thomson 2008, Graystock et al. 2013a, Murray et al. 2013). However, many parasites naturally have a broad host range and multi-host agents outnumber specialists (Musselman and Press 1995, Cleaveland et al. 2001, Taylor et al. 2001, Pedersen et al. 2005). For example, there are several field and laboratory studies highlighting honey bees and wild bees as common hosts to the previously considered “honey bee pathogen” *N. ceranae* (Singh et al. 2010, Graystock et al. 2013a, Graystock et al. 2013b, Ravoet et al. 2014, Fürst et al. 2014, McMahon et al. 2015). We must improve our understanding of host-pathogen-relationships in pollinators to assess if a transmission to a new host has actually occurred, or if the assumption is skewed by the fact that further hosts get investigated for the first time (Goulson and Hughes 2015). The complexity of multiple host-parasite interactions might influence parasite abundances in host populations and virulence on the individual level (Brown 2013).

Evolution of virulence

In the case of *N. ceranae*, virulence of the pathogen in honey bees was found to be characterized by high variance in genetics of involved pathogen and host species (Roudel et al. 2013, Gómez-Moracho et al. 2014, Branchiccela et al. 2017). Strains of *N. ceranae* from different geographic origins differed in their virulence (Fenoy et al. 2009, Gisder et al. 2010, Fernández et al. 2012). To understand pathogen virulence, we should therefore apply the pathobiome concept, which suggests that the virulence of particular pathogens depend on its biotic environment, which will differ between host species (Vayssier-Taussat et al. 2014).

The environment as well as the host and parasite system, can influence virulence (Wolinska and King 2009).The level of genotype-by-genotype-by-environment-interactions (Thomas and Blanford 2003) is of current concern in the light of the multi-stressor environment of pollinators. Environmental stress can increase the impact of non-specific diseases (Lafferty and Holt 2003). These could concern new hosts of previously honey bee associated pathogens in the pollinator network.

Conquering new hosts with differing ecology

Pathogenicity of the same agents is known to differ depending on host species (Graystock et al. 2013a, Graystock et al. 2013b). Wild bees have a diverse ecology with clear differences in social behavior and food habits in comparison to honey bees (Westrich 1989). The social organization of bees can be classified in five categories ranging from solitary to highly social (Westrich 1989). In solitary bees, no physical contact between the female and the hatching larvae exists. However, brood cells are provided with nectar and pollen to nourish the larvae (Westrich 1989). Additionally, contacts between adults via common nests, sharing food and caring for the queen as in the social way of life are absent (Westrich 1989). Communal living refers to several females sharing a nest together (Westrich 1989). Social bees are characterized by life in communities; division of labor, a queen specialized on laying eggs and female worker bees responsible for collecting food and taking care of the nest as well as laying eggs in some species. Food sharing occurs in some social species (Westrich 1989). In the current knowledge about bee pathology, social species are represented by bumble bees and honey bees. Life in a colony, brood care and division of labor are found in both *Apis* and *Bombus* (Goulson 2009). Bumble bees are also the currently most intensively studied wild bee species (Tehel et al. 2016). However, the solitary bees comprise the majority of all wild bee species (Amiet and Krebs 2014). Another life form are parasitic bee species exploiting their hosts in nest-building, brood care and food resources (Westrich 1989).

The modes of pathogen transmission differ according to social structure. Horizontal refers to the transmission of pathogens among individuals of the same generation. If pathogens are

transmitted vertically, they are passed from the parents to their offspring (Chen et al. 2006). Due to the different life styles, this can occur in social but not in solitary species. Pathogen transmission via pollen and nectar was established in previous studies (Singh et al. 2010) and is likely to occur in solitary species as well. Horizontal transmission between adult solitary bees can be assumed to occur in a much lesser extent than it is the case in social bees. However, food-borne infections via shared flowers (Durrer and Schmid-Hempel 1994) as well as sexual transmission in mating are possible. In contrast to the solitary way of life, in communal and social bees horizontal transmission between adults in the nest is possible (Westrich 1989). The characteristics of the communal and social life style are beneficial to fitness on the one hand but providing various opportunities for pathogen transmission on the other hand (Manley et al. 2015). No studies on shared pathogens between these host-parasite affiliations of parasitic wild bee species exist so far.

Osmia bicornis

Osmia bicornis is a common wild bee species in Germany. It is characterized by a solitary, univoltine lifestyle and a flight period from March and June and nests in wood cavities or crevices in buildings (Kornmilch 2010, Strohm 2011). The linear nest consists of compartmentalized brood cells built and closed with soil which are endowed with a mix of pollen and nectar as food on which the egg is deposited (Westrich 1989). The eggs laid first are fertilized and will develop into females. They are provided with a higher amount of pollen as food resource than the later-laid unfertilized eggs from which males emerge (Seidelmann et al. 2010). About 30 days after hatching the larvae moult four times and then starts to spin a cocoon (Raw 1972). The hatching time is influenced by temperature (Radmacher and Strohm 2011). Female cocoons are bigger and heavier than those of males (Seidelmann et al. 2016). After two weeks in the cocoon the individuals enter the pre-pupae stage also known as summer diapause period lasting for a few months and finally enter pupation before eclosing as adults. They overwinter as a fully developed imago eclosing from the pupae and remaining in the cocoon. Wintering is terminated by exposure to warm temperatures in the following spring after which bees emerge. Males eclose before females. (Kornmilch 2010, Sedivy and Dorn 2014). Concerning pollen collection the species is polylectic and pollen resources close to the nest site are favoured (Westrich 1989, Radmacher and Strohm 2010). *Osmia bicornis* is native to the Berlin-Brandenburg area with no other subspecies present (Saure 2016, personal communication). Its high importance as well as representative features of wild bees makes it an ideal model species for the addressed research question of pathogen spillover from honey bees.

Aims of the thesis

The aim of this thesis was to investigate the transmission of the honey bee derived pathogen *N. ceranae* from honey bees to wild bees and characterize the impacts of this pathogen on the solitary bee model species *O. bicornis*.

The taxon of wild bees is characterized by high biodiversity and live a range of habitats (Westrich 1989). So far, no broad screening of a diverse set of wild bees in an environment in which pathogens are encountered has been undertaken. Chapter 1 includes a field survey in Berlin-Brandenburg in a variety of habitats over a whole season to investigate the association of wild bee communities and the pathogen in honey bees with which they share habitat and floral resources. Field sites were selected based on screening of *N. ceranae* in honey bees and expert recommendations. Sites with infected as well as those with uninfected honey bees were sampled to enable comparison. The approach was directed at providing information on community composition of wild bees in an environment where they encounter the pathogen *N. ceranae*. The communities were classified according to functional ecology attributes. In order to consider conservation status, Red List categorization was also incorporated. The chapter considers an indirect impact of the pathogen representing one of the current threats for wild bees.

Chapter 2 refers to the critical point that detection of a pathogen alone does not allow to distinguish between contamination and actual establishment. The question is particularly relevant to evaluate the role of wild bees as pathogen reservoirs in the pollinator network.

An effective method to investigate for the actual establishment of a pathogen as well as its fitness effects are infection experiments. The chapter comprises the first experimental approach to test the susceptibility of a solitary wild bee to *N. ceranae.* The model species *O. bicornis* is highly relevant to agriculture as it can be well managed for pollination (Bosch et al. 2002). Individuals of *O. bicornis* were fed with spores of the pathogen collected freshly from an infected honey bee hive in order to be able to assess the establishment of the pathogen. Additionally, mortality of infected versus control bees was monitored as a measure for survival impact of the pathogen. Males and females were tested separately and honey bees served as a control to confirm the infectivity of the spores. In a first experiment, survival was monitored during a period of three weeks. A second approach tested detection of the pathogen after three and six days for the purpose of detecting differences in establishment. As an indicator for field conditions, wild bees sharing floral resources with honey bees from the infected hive serving as spore donators, were sampled and screened for pathogen presence.

For infection studies it is of interest to investigate juveniles as infection at these stages of life history can be more detrimental than in later stages (Ashby and Bruns 2018). In Chapter 3, a semi-field experiment tested the susceptibility of larvae of *O. bicornis* to *N. ceranae.* Susceptibility was assessed by recording pathogen presence and mortality during development. Furthermore, we measured fitness correlates like growth, pupation start, fat body and wing muscle mass as indicator for costs of a potential infection due to a physiological trade-off between these traits and investment in immunity. This is the first study to provide information of pathogen impact and immune response costs during the development of a solitary bee after experimental infection with a honey bee derived pathogen. Results are crucial to better assess the actual significance of the pathogen for *O. bicornis* and consider them in pollination management particularly on sites where both *Osmia* and *A. mellifera* are used.

The findings of the thesis aim to elucidate the potential of wild bees, particularly *O. bicornis*, as a host and pathogen reservoir of *N. ceranae*. They can furthermore be applied to evaluate the actual threat of the pathogen for this solitary bee more profoundly. Management practices which monitor honey bee health and placements of hives near rich wild bee habitats will be able to incorporate the results to reduce pathogen spread in pollinators.

Chapter 1: Associations between wild bee communities and *Nosema ceranae* in honey bees

1.1 Abstract

1. Emerging pathogens are one of several stressors currently faced by pollinators, but the impact of pathogens on wild bee communities is not well understood. Honey bee pathogens have been recorded in some wild bee species and shared floral resources may act as an important transmission hub.

2. The present study investigates the association between wild bee community composition in different habitat types across Berlin-Brandenburg and honey bees infected with the emerging pathogen, the microsporidian, *Nosema ceranae*.

3. Throughout the summer season of 2015, 406 wild bee species were collected at 25 sites. Honey bees infected with *N. ceranae* in 2014 and/or 2015 were found at 13 sites, and wild bee species from each site were classified into functional groups.

4. Rare parasitic, cavity nesting and Red List species showed a negative association with *N. ceranae* infected honey bee populations but the pattern was reversed in common social-polylectic species. We detected a negative correlation between functional diversity and honey bee *N. ceranae* infection. A high percentage of foraging and nesting habitats was found to be particularly important for solitary-oligolectic and Red List species.

5. Our findings indicate that rarer and more specialized bees may be more sensitive to environmental factors that are associated with a higher disease burden in honey bees, although our data cannot reveal whether *N. ceranae* is causally responsible for the patterns observed. A functionally diverse wild bee community is required to ensure reliable pollination services and the mitigation of multiple stressors on wild bees should be prioritized.

1.2 Introduction

Pollination of managed crops and wild plant species by insects is an essential ecosystem service and a key component of biodiversity (Steffan-Dewenter et al. 2005, Klein et al. 2007, Potts et al. 2010, Blitzer et al. 2016). Wild insects play a very important role and are largely underappreciated in crop pollination (Garibaldi et al. 2013). Consequently, managed pollinators constitute only a single component of a much wider pollination service provided by different species (Hoehn et al. 2008, Blüthgen and Klein 2011). A dependence on largely one species for pollination represents a risk due to reduced resilience against predators, parasites and pathogens, in addition to other stressors (Tscharntke et al. 2005, Winfree et al. 2007, Hillebrand et al. 2008, Potts et al. 2010). Fields visited by a low diversity of pollinator species have a reduced fruit set independent of honey bee pollination events or species evenness suggesting that managed honey bees cannot replace wild bees (Garibaldi et al. 2013). Whereas some pollinator species are already extinct, many more are considered at risk (Biesmeijer et al. 2006, Brown and Paxton 2009, Potts et al. 2010). In Europe, most bee species are of unknown conservation status (Nieto et al. 2014). In the Red List of Germany, 7.0 % of all species checked were extinct or disappeared, 5.6 % are critically endangered (Category I), 14.0 % are endangered (Category II) and 15.3 % are classified as vulnerable (Category III) (Westrich et al. 2011). Particularly highly specialized species with oligolectic foraging behavior or specific host associations in parasitic lifestyles are affected (Westrich et al. 2011).

Wild bees are exposed to a number of stressors in the environment including habitat loss and –change (Potts et al. 2010), pesticides (Godfray et al. 2015) and pests and pathogens (Fürst et al. 2014, McMahon et al. 2015, Wilfert et al., 2016). Current bee losses cannot be attributed to one main causative agent (Vanbergen and Garratt 2013) but the combined effects of stressors are increasingly considered to play an important role in ongoing population decline (Brown et al. 2003; González-Varo et al. 2013).

The microsporidium *Nosema ceranae* was proposed to be a main driver of honey bee colony loss in Spain (Higes et al. 2008) although a direct causal link between *N. ceranae* and honey bee loss has not been universally detected (Cox-Foster et al. 2007, Invernizzi et al. 2009, vanEngelsdorp et al. 2009, Gisder et al. 2010, Fernández et al. 2012, Wilfert et al. 2016). The pathogen has also been found in some wild bee species and is thought to be capable of infecting bumblebees (Plischuk et al. 2009, Graystock et al. 2013a, Graystock 2013b; Ravoet et al. 2014). The present study aims to use information about the composition of wild bee communities to explore a potential association between *N. ceranae* infection in honey bees and pollinator network community. Wild bees are highly diverse with more than 500 species in Germany (Westrich et al. 1989). Species composition can provide data about diversity within communities and distribution of rare and threatened species thus presenting an

interesting field to investigate in the presence of potential stressors. While infections of wild bees by *N. ceranae* have rarely been reported, flowers are hubs for pathogen exposure (Koch et al. 2017). Bumble bee larvae without symptoms for example can still function as vectors of pathogens (Folly et al. 2017).

Our main questions are:
(a) Is there an association between the presence of *N. ceranae* in honey bees and wild bee community composition?

(b) Is there an association between the presence of *N. ceranae* in honey bees and Red List wild bees?

1.3 Material and Methods

Sample sites

Twenty-five different field sites in the area Berlin-Brandenburg were visited (Fig.1). The selection of 12 sites located close to hives with reported infection with *N. ceranae* in 2014 and the previous three seasons was based on information provided by the *Länderinstitut für Bienenkunde Hohen Neuendorf*. Only hives with continuous infection over the last 2 years were chosen. Infections were checked throughout the sampling period. The method consisted of investigating 10 living or 10 dead bees from 10 different colonies each. The 13 other sites were chosen to include more habitat types in Berlin-Brandenburg. Information about natural sites was

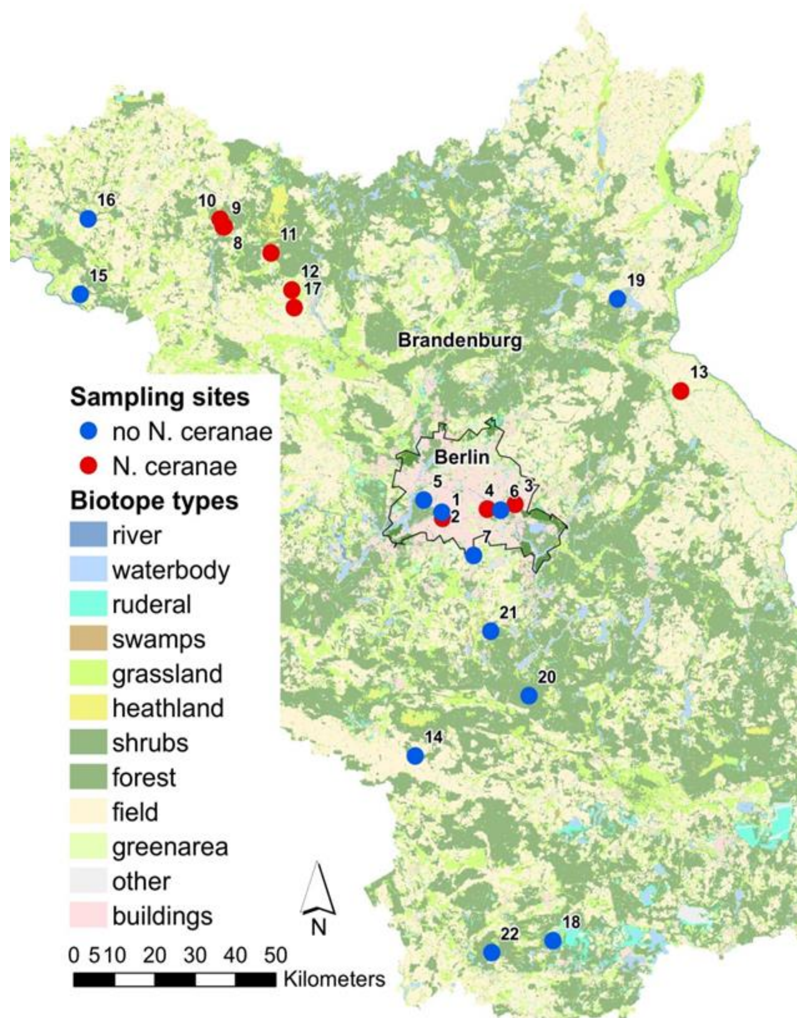

Figure 1: Overview of sampling sites and habitats in the area of Berlin-Brandenburg.

obtained from wild bee surveys from a local bee taxonomist, Dr. C. Saure. At each site, 10 honey bees were caught during the survey to be checked for *N. ceranae* spores. Forager bees were found to be a reliable source for sampling when assessing *N. ceranae* infectionin honey bee colonies (Mulholland et al. 2010). Table 2 presents an overview about all the honey bee infection data.

Sites included different biotopes representing a spectrum of urban, agricultural and natural landscapes. 12 sites were included in the habitat category "green", 4 in "urban" and 9 sites were attributed to "agricultural" habitats. Habitat types in the category "green" included grass- and parklands, dwarf shrubs and bushes, forest, swamps and parks. ''Urban'' referred to built area and "agricultural" to agricultural fields and open soil. Within the habitat type "green'' 50% of all sites were with and without *N. ceranae*. The same applied to urban habitats whereas 60% of all agricultural sites were *N. ceranae* free. *N. ceranae* was found on 66.6% of sites in the Berlin area (N=9) and in 43.75% of sites in Brandenburg (N=16).

Table 2: Screen for *N. ceranae* in selected honey bee hives.

Pathogen screen in honey bees

Site	10 flying bees	10 living bees from 10 colonies each	10 dead bees from 10 colonies each	Time of screen	% with *N.ceranae*	Category
1	x			April, July 2015	3	+
2	x			May 2015	0	-
3, 4		x		March 2015	10	+
			x	March 2015	10	
		x		July 2015	0	
5	x			May 2015	0	-
6		x		March 2015	10	+
			x	March 2015	0	
		x		July 2015	0	
7		x		March 2015	0	+
		x		July 2015	40	
8,9,10, 11,12		x		March 2015	0	+
			x	March 2015	0	
		x		July 2015	10	
13	x			May 2015	0	-
14	x			May 2015	0	-
15,16,17		x		March 2015	0	+
			x	March 2015	0	
		x		July 2015	0	
18	x			May 2015	0	-
19	x			July 2015	0	-
20	x			July 2015	0	-
21	x			August 2015	0	-
22	x			August 2015	0	-
23	x			August 2015	0	-
24	x			August 2015	0	-
25	x			September 2015	0	-

Sampling methodology

Sampling was carried out from April 23rd to September 5th 2015. Six sites were visited twice, in early (April-June) and late summer (July or August) in order to test for differences in species composition and associations with *N. ceranae* in honey bees at two points in time in one constant location. As many species are univoltine for a number of weeks species composition could be expected to differ. Sites visited twice included 4 sites with *N. ceranae* in honey bees and 2 sites where the pathogen was not detected in honey bees. Sampling took place within a time-frame of 0.5 and 3 hours between 9 am and 6pm and only on days with a minimum temperature of 15°C, low wind, no rain and dry vegetation. Due to the heterogeneity of the sites, species were sampled in variable transects including the most attractive foraging sites. Honey bees were collected at the same time and site as wild bees. Transect size and time required to collect a number of wild bees was recorded as a measure of pollinator abundance. Compositions of pollinator communites based on observations within a 15 minute window were recorded, in addition to the most visited flowers and the surrounding vegetation. Environmental data recorded were: weather conditions, vegetation favored by bees and surrounding habitats. Bees were cooled immediately on ice and stored at -20°C as soon as possible thereafter.

Identification and categorization

Identifications of defrosted and pinned bee samples followed Amiet & Krebs (2014), Gokcezade et al. (2010), Scheuchl (1995, 2006), Schmid- Egger (1997) and Amiet et al. (1999, 2001) and were confirmed by Dr. Christoph Saure. Species were classified into ecological groups based on sociality and pollen foraging behaviour. Furthermore, categories referring to nesting behaviour were compiled. Information was obtained from Saure (2005) and Westrich (1989). Classifications comprised social-polylectic, solitary-polylectic, solitary-oligolectic and parasitic species. Oligolecty was defined as collecting pollen only from one plant family (Westrich 1989). Nesting habits included below-ground, above-ground and species able to nest in both ways, as well as parasitic ones.

Investigation of *N. ceranae* in honey bees

The 10 honey bees sampled at the 13 sites for which no prior information about *N. ceranae* was available were dissected individually. The gut of each bee was homogenized in 200 ml of NaCl (0.9%). Fifty µl were investigated microscopically (400x). If spores were present, the solution was processed with the DNeasy Plant Mini Kit from Qiagen (Hilden, Germany) according to manufacturer's instructions to extract DNA from the spores.

PCR

In order to identify *N. ceranae* all samples with visible spores were investigated by molecular methods. For the PCR, 5 µl DNA of each sample was mixed with 5 µl RNAse free H_2O, 12.5

µl KAPA 2GFast ReadyMix with dye (Kapabiosystems) and 2.5 µl of the primer pair NoscRNAPol-F2/NoscRNAPol-R2 (Gisder and Genersch 2013). The PCR programme was set according to Gisder et al. (2013). The amplified products were analysed in a 1.5% agarose gel stained with ethidium bromide or SYBR GOLD Nucleic Acid Stain (Thermofisher) and run for 80 minutes at 80V. In each run a positive control with confirmed *N. ceranae* bands was included as well as a negative control with ddH_2O. Differentiation of the samples was based on the 662 bp-fragment of the 5'-end of the *N. ceranae* sequence of a fragment of the gene coding for the DNA-dependent RNA-polymerase II largest subunit (RPB1). All positive samples were confirmed by sequencing.

Because of the preservation method and identification of wild bees with specialist support, a comprehensive screening for *Nosema* infections was not feasible in wild bees.

Landscape analysis

The proportion of Biotope habitat classes within a radius of 250m from the sampling sites was calculated based on the data set 'Biotop Types, Map 05.08' from the Digital Environmental Atlas Berlin (DUDEB 2013) and BTLN Brandenburg (LUGV 2009) following Tonietto et al. (2011). The radius size was based on accessible area for also small species that cover only short foraging distances (Zurbuchen et al. 2010). Habitats were grouped in three habitat categories depending on the dominant land use in the 250m radius: "green", "urban" and "agricultural". Furthermore, the proportion of all foraging and nesting habitats was calculated (ruderal areas, swamps, grasslands, heathlands, shrubs, forests and green area). Geographical analyses were done with the software ArcMap 10.4.1 (Environmental Systems Research Institute Inc. 2017).

Statistical analysis

In order to explore associations between characteristics of wild bee communities and environmental predictors we applied general linear models (GLM) with either Poisson or a binomial error structure. Wild bee diversity (in species numbers/m^2 as well as Simpson index), density (number of pollinators observed in 15min per sampled transect), and the proportion of Red List species and different functional groups of wild bees were used as response variables to *N. ceranae* presence in honey bees, habitat type, diversity of surrounding habitats or the location of sampling sites in city and countryside.

Habitat type was derived from the GIS data analysis. ''Berlin'' referred to all sites within the postcode area of Berlin, while all other outside of this area were attributed as ''Brandenburg''. Only sites with at least five wild bees collected were included in the statistical analysis.

For proportional data the package 'nlme' was used. Models for each combination of dependent variable and predictor were compared using model averaging with the packages 'AICcmodavg' and 'MuMin'. The model with the lowest AIC was checked for homogeneity of variance by plotting the residuals. In the case of any remaining heterocesdasticity, models

with second and third lowest AIC were tested. If homogeneity of variance was still not met, data were log transformed or rectified with a Box-Cox transformation.

If heterocedascity occurred in proportional data models, they were analysed with rough standard error correction with the package ‘sandwich‘. Variance inflation factors (VIF) from the package ‘car’ were used to examine co-linearity among predictor variables, setting a threshold of 3. Variables with higher VIF were excluded referring to Zuur et al. (2009).

Response variable data from the six sites visited twice were checked for normality and for homogeneity of variance and were compared with either paired t-test or Wilcox-test for differences in response parameters. All analyses were performed in R 3.3.4.

1.4 Results

No significant differences were detected between the different response parameters between first and second visits (Table 3). Consequently, the following analysis was conducted only once with the results from the first visit of a site.

Table 3: Test for significant differences in response parameters comparing data from first and second visit of double sampled sites.

Test for differences in responses between two samplings of same sites

	Shapiro-Wilk	Levene	paired t-test	Wilcox-test
Honey bees+Wild bees /m^2	**0.01**			0.69
Simpson Index	0.39	0.22	0.24	
Species number /m^2	**0.02**			0.91
Percentage wild bees	0.19	0.29	0.17	
Percentage honey bees	0.08	0.58	0.55	
Parasitic bees	**>0.01**			0.88
Below-ground bees	0.22	0.71	0.60	
Above-ground bees	**>0.01**			0.06
Above-ground&below-ground nesting bees	**>0.01**			0.31
Red List species	**>0.01**			0.90
Social-polylectic	0.25	0.83	0.66	
Solitary-polylectic	0.09	0.54	0.66	
Solitary-oligolectic	**>0.01**			0.41

Species richness

In total, 406 bees from 19 genera were caught at 25 sites. Sample numbers varied between 1 and 20 bees. Genera with the highest diversity were *Andrena* (12) and *Bombus* (11). In all other genera less than 10 different species were sampled. Genera with the highest numbers of individuals were: *Bombus* (140), *Andrena* (88), *Anthophora* (40), *Colletes* (39), *Halictus* (19) and *Osmia* (16). All other genera were represented by less than 10 individuals.

The four species with the highest number of individuals were *Bombus pascuorum* (43), *Anthophora plumipes* (37), *Andrena flavipes* (36), and *Bombus terrestris* (35). Table 4 presents an overview of all collected wild bee species, their ecological features and the number of caught individuals. At every site up to 5 most visited plant species were recorded. In total, 86 plant species from 22 different families were assigned to this category. Families with the highest numbers of representatives were *Asteraceae* (32), *Fabaceae* and *Papaveraceae* (each 15) and *Rosaceae* (12). The three most visited plant species were *Taraxcum officinale*, *Echium vulgare* and *Trifolium repens*.

Table 4: Overview of collected wild bee species, ecological features and number of individuals.

Species	Sociality	Foraging behaviour	Nesting behaviour	Total individuals
Andrena barbilabris	solitary	polylectic	below-ground	18
Andrena c.f. ovatula	solitary	polylectic	below-ground	2
Andrena dorsata	solitary	polylectic	below-ground	1
Andrena flavipes	solitary	polylectic	below-ground	36
Andrena fuscipes	solitary	oligolectic	below-ground	14
Andrena haemorrhoa	solitary	polylectic	below-ground	3
Andrena humilis	solitary	oligolectic	below-ground	4
Andrena labialis	solitary	oligolectic	below-ground	4
Andrena nitida	solitary	polylectic	below-ground	1
Andrena tibialis	solitary	polylectic	below-ground	1
Andrena vaga	solitary	oligolectic	below-ground	1
Andrena wilkella	solitary	oligolectic	below-ground	3
Anthophora bimaculata	solitary	polylectic	below-ground	1
Anthophora plumipes	solitary	polylectic	below-ground	37
Anthophora pubescens	solitary	polylectic	below-ground	2
Bombus bohemicus	parasitic	parasitic	parasitic	1
Bombus c.f. lucorum/ lucorum agg.	social	polylectic	below-ground	11
Bombus hortorum	social	polylectic	below-&above-ground	2
Bombus hypnorum	social	polylectic	above-ground	3
Bombus lapidarius	social	polylectic	below-&above-ground	23
Bombus pascuorum	social	polylectic	below-&above-ground	42
Bombus pratorum	social	polylectic	below-&above-ground	3
Bombus rupestris	parasitic	parasitic	parasitic	1

Bombus soroeensis	social	polylectic	below-ground	16
Bombus sylvarum	social	polylectic	below-ground	1
Bombus terrestris	social	polylectic	below-ground	37
Coelioxys aurolimbata	parasitic	parasitic	parasitic	1
Coelioxys rufescens	parasitic	parasitic	parasitic	1
Colletes cunicularius	solitary	oligolectic	below-ground	2
Colletes daviesanus	solitary	oligolectic	below-ground	20
Colletes fodiens	solitary	oligolectic	below-ground	4
Colletes succinctus	solitary	oligolectic	below-ground	13
Dasypoda hirtipes	solitary	oligolectic	below-ground	5
Epeolus cruciger	parasitic	parasitic	parasitic	2
Eucera longifornis	parasitic	parasitic	parasitic	2
Halictus c.f. tumulorum	social	polylectic	below-ground	1
Halictus confusus	social	polylectic	below-ground	1
Halictus quadricinctus	solitary	polylectic	below-ground	7
Halictus rubicundus	social	polylectic	below-ground	2
Halictus sexcinctus	solitary	polylectic	below-ground	6
Halictus smaragdulus	social	polylectic	below-ground	1
Halictus subauratus	social	polylectic	below-ground	1
Heriades c.f. crenulatus	solitary	oligolectic	above-ground	1
Heriades truncorum	solitary	oligolectic	above-ground	7
Hoplites dentriventris	solitary	oligolectic	below-&above-ground	1
Hoplitis adunca	solitary	polylectic	above-ground	5
Hyaleus hyalinatus	solitary	polylectic	below-&above-ground	2
Hyaleus sp.	solitary	polylectic	below-&above-ground	3
Lasioglossum calceatum	social	polylectic	below-ground	5
Lasioglossum pauxillum	social	polylectic	below-ground	1
Megachile centuncularis	solitary	polylectic	below-&above-ground	1
Megachile maritima	solitary	polylectic	below-ground	1
Megachile willughbiella	solitary	polylectic	below-&above-ground	2
Melecta albifrons	parasitic	parasitic	parasitic	6
Melitta haemorrhoidiales	solitary	oligolectic	below-ground	2
Melitta leporina	solitary	oligolectic	below-ground	5
Nomada rufipes	parasitic	parasitic	parasitic	5
Osmia aurulenta	solitary	polylectic	above-ground	3
Osmia bicornis	solitary	polylectic	above-ground	13
Sphecodes c.f. ephippus	parasitic	parasitic	parasitic	1
Sphecodes gibbus	parasitic	parasitic	parasitic	3
Sphecodes miniatus	parasitic	parasitic	parasitic	1
Sphecodes sp.	parasitic	parasitic	parasitic	1

N. ceranae in honey bees and wild bee diversity

The 12 sites selected via the Bee Monitoring (Bundesweites Bienenmonitoring 2014, unpublished data) were all *N. ceranae* positive for tested honey bee hives. At each site samples from 5 hives were tested. Among the additional 13 sites at which free flying honey bees were caught and checked for the pathogen, it was present at only one site. On one site there were no honey bees present at all. On 11 sites, the honey bees that were caught were not infected. However, dynamics of *N. ceranae* infection in honey bees are highly labile. Furthermore, we acknowledge the assessment of free flying honey bees might not account for potential presence of *Nosema* within the hive. Consequently, the attributed status is based on a probability of spore occurrence rather than an unambiguous diagnosis of spore presence or absence on each site.

The percentage of wild bees in the pollinator community was lower when infected honey bees were present (*p*=3.3E-04, df=22). Total bee abundance was not associated with *N. ceranae* in honey bees (*p*=0.63, df=21), and no difference between species richness (species/m^2) or Simpson's Index and the presence of *N. ceranae* in honey bees could be detected (*p*=0.94, df=23, t-value=0,074 / *p*=0.18, df=21, t-value=-1,4). Full test statistics are shown in Table 5.

Functional groups

From all collected samples, the majority of wild bees (41.3%) were assigned to social-polylectic lifestyle. Of the remaining samples, 32.1% were solitary-polylectic, 19.3% solitary-oligolectic and 7.3% parasitic. 66.5% the of wild bees collected nest below-ground while, 19.5% were nest both below- and above-ground, 7.88% were above-ground and 6.16% parasitic (Fig. 2, Table 4).

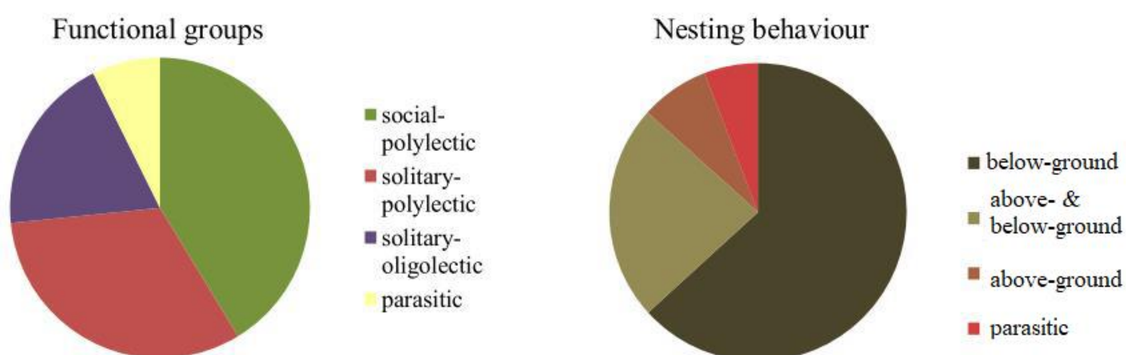

Figure 2: Percentages of the functional groups for sociality/foraging type and nesting behaviour in the wild bee community.

Common social-polylectic wild bees were found to have a positive association with *N. ceranae* infected honey bees (*p*=2.17E-07, df=22, t-value=5.148, Table 5, Fig.3a). Solitary bees of both foraging types were not related to *N. ceranae* infected honey bees (Table 5).

The rare parasitic bee species showed a negative association with *N. ceranae* in honey bees (*p*=0.05, df=23, t-value=-1.928, Table 5, Fig.3b). Also, above-ground nesting species responded negatively to honey bee populations infected with *N. ceranae* (*p*=0.00772, df=21, t-value=-2.664, Table 5). Species with below-ground and both nesting characteristics showed no association.

Altogether, 33,9% of all individuals caught are mentioned in the latest Red List of Germany (Westrich et al. 2011). Significantly fewer Red Listed species were detected in presence of *N. ceranae* in honey bees (*p*=2.87E-06, df=21, t-value=-4.680, Fig. 4a, Table 5). Among the Red List species, 33% of solitary-oligolectic as well as of solitary-polylectic lifestyle were found. 19% were parasitic and 14.3% social-polylectic.

Associations between wild bee community characteristics and habitat

Density of honey bees and wild bees was found to be higher in Berlin serving as a proxy for urban area than in Brandenburg serving as a proxy for rural environment (*p*=0.0291, df=21, t-value=-2.343, Table 5), irrespective of habitat type (*p*=0.627, df=21, t-value=0.829, Table 5). Diversity of wild bees was not associated with habitat type or location.

In the analysis of functional groups, solitary-oligolectic species showed a particularly strong association with green habitats (*p*=1.09E-08, df=22, t-value=-5.712, Table 5). Red List species were strongly connected to green habitats in areas outside of the city of Berlin (*p*=3.22E-10, df=21, t-value=-6.288/*p*=1.71E-12, df=21, t-value=7.056, Table 5).

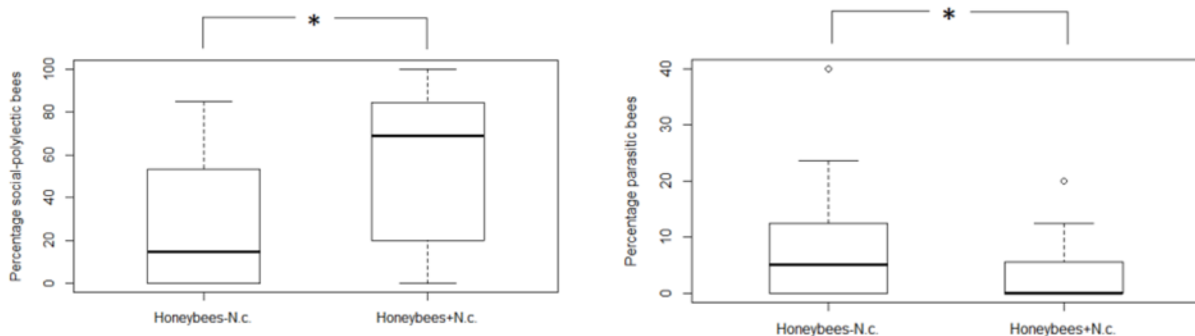

Figure 3 a, b: Associations between *N. ceranae* infected honey bees and wild bees. Social-polylectic (a) and parasitic (b) functional groups: The association between *N. ceranae* in honey bees and the proportion of social-polylectic bees or the proportion of parasitic bees is significantly increased or decreased, respectively.

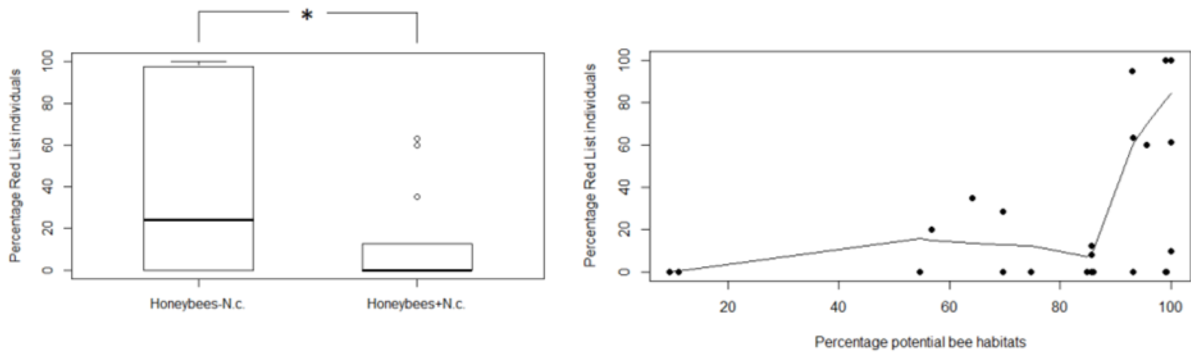

Figure 4a, b: Significant negative association between Red List individuals (%) and *N. ceranae* in honey bees (a), and positive association between Red List Individuals and proportion of foraging and nesting habitat (Lowess line) (b).

Regression analysis of response parameters revealed a positive relationship between the proportion of foraging and nesting habitats and number of Red List individuals (*p*=2,59E-10, df=23, residual deviance=181.69, Table 6, Fig.4b) and solitary-oligolectic species (*p*=8,71E-05, df=23, residual deviance=98.198, Table 6) although diversity and the proportions of other ecological groups did not display a significant association (Table 6). An overview about wild bee community composition in the areas of Berlin and Brandenburg is shown in Fig. 5.

Table 5: General linear models with lowest AIC explaining species diversity, proportion of wild bees in pollinator communities, ecological groups and Red List species as dependent on *N. ceranae* in honey bees, location in Berlin or Brandenburg and habitat type. Lowest AIC determined by model averaging. Significant variables are indicated in bold letters.

Results Model averaging 3 categories: (1) Location Berlin-Brandenburg, (2) Habitat type new, (3) *Nosema* in honey bees

Dependent variable	Independent variables	Model	Error structure	delta AIC	residual deviance	degrees of freedom	t-value/z-value	*p-value*
Honey bees+ Wild bees /m^2	Berlin-Brandenburg	glm with BoxCox Transformation	Poisson	0.62		21	-2.34	**0.03**
	Habitat						0.83	0.42
	Nosema						0.50	0.63
Simpson Index	Berlin-Brandenburg	Robust standard error correction	Poisson		30.05	21	-0.03	0.97
	Habitat						-1.84	0.08
	Nosema						-1.40	0.18
Species number /m^2	Habitat	glm with BoxCox Transformation	Poisson			23	0.07	0.94
Percentage wild bees	Berlin-Brandenburg	glm cbind	Binominal	0.34	76.21	22	0.21	0.83
	Nosema in Honey bees	glm cbind					-3.59	**>0.001**
Parasitic bees	Nosema	glm cbind	Binominal	0	35.49	23	-1.93	**0.05**
Endogeic bees	Berlin-Brandenburg	glm cbind Robust standard error correction	Binominal		82.43	22	0.28	0.78
	Nosema						0.14	0.89
Hypogeous bees	Berlin-Brandenburg	glm cbind	Binominal	0.25	74.86	21	-1.93	0.05
	Habitats						0.96	0.34
	Nosema						-2.66	**0.01**
Hypogeous& endogeous bees	Berlin-Brandenburg	glm cbind Robust standard error correction	Binominal		80.55	23	-0.24	0.81
Red List species	Berlin-Brandenburg	glm cbind	Binominal	0	102.04	21	7.06	**>0.001**
	Habitats						-6.29	**>0.001**
	Nosema						-4.68	**>0.001**
Social-polylectic	Berlin-Brandenburg	glm cbind	Binominal	0.33	197.57	22	2.10	0.04
	Nosema						5.18	**>0.001**
Solitary-polylectic	Berlin-Brandenburg	glm cbind	Binominal	0	191.96	22	-3.63	**>0.001**
	Habitat						6.29	**>0.001**
Solitary-oligolectic	Berlin-Brandenburg	glm cbind	Binominal	1	77.76	22	4.61	**>0.001**
	Habitat						-5.72	**>0.001**

Table 6: General linear models of wild bee community characteristics and percentage of foraging and nesting habitat within the study area. Significant variables are indicated in bold letters.

Regression Bee Habitats	model	residual deviance	df	response	p-value
Bee density/m^2	glm	1889	23		0.17
Simpson Index	glm	30.93	23		0.12
Species num/m^2	glm BoxCox transformation	1.72	23		0.11
Percentage wild bees	glm Robust standard error correction	89.62	23		0.98
Percentage parasitic bees	glm Robust standard error correction	32.37	23		0.94
Percentage endogeic bees	glm Robust standard error correction	124.18	23		0.80
Percentage hypogeous bees	glm Robust standard error correction	87.54	23		1.00
Percentage hypo&endo bees	glm Robust standard error correction	181.69	23		0.80
Percentage Red List Indiv.	glm	181.69	23	positive	**>0.001**
Percentage social-polylectic	glm Robust standard error correction	217.34	23		0.87
Percentage solitary-polylectic	glm Robust standard error correction	249.36	23		1.00
Percentage solitary-oligolectic	glm	98.20	23	positive	**>0.001**

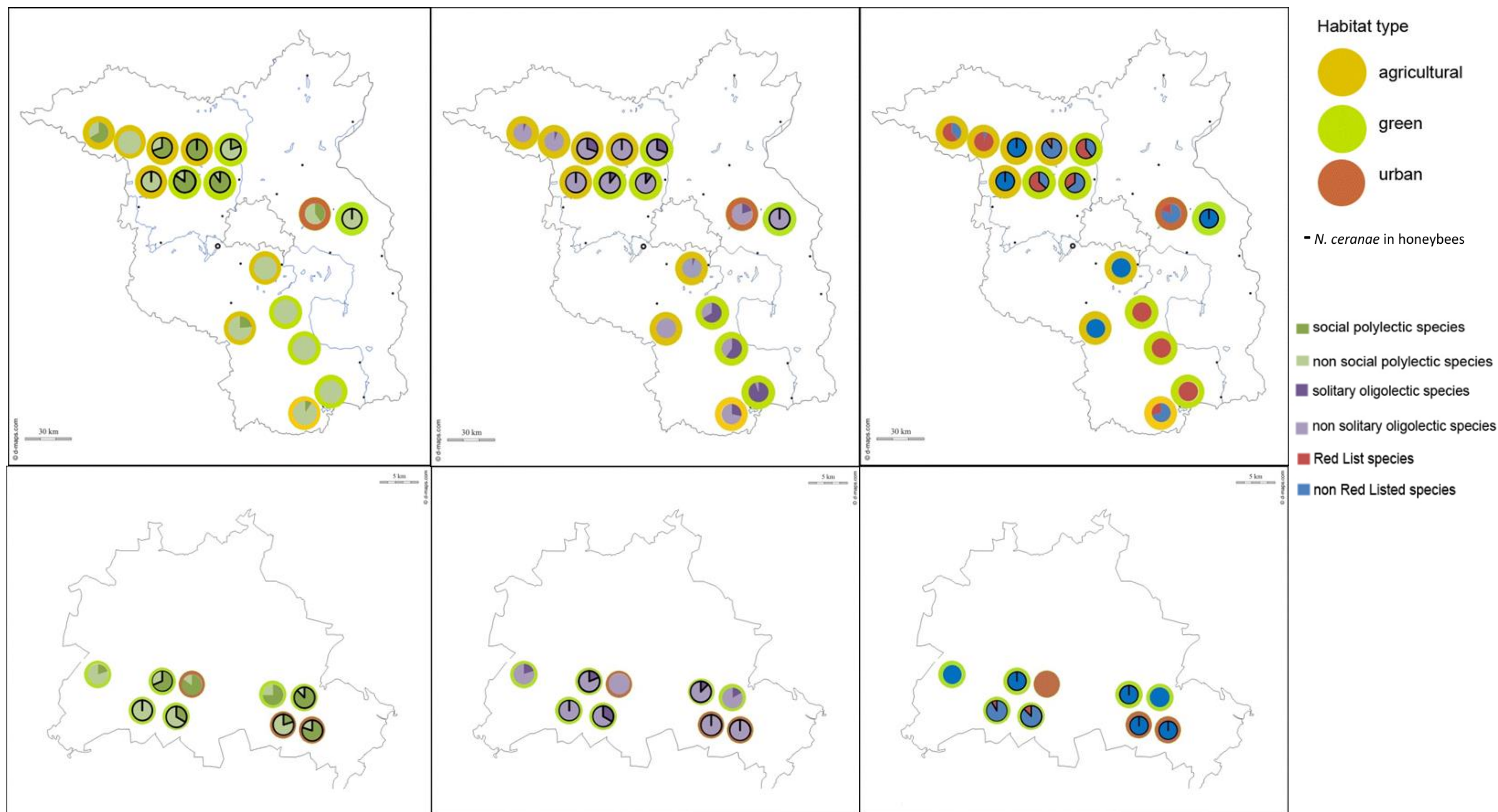

Fig. 5a-c: Sampling area (upper maps: state of Brandenburg, lower maps: Berlin), habitat types, *N. ceranae* in honey bees and proportions of (a) social-polylectic species, (b) solitary-oligolectic species , (c) RedList individuals

1.5 Discussion

Honey bees in high densities can compete with other pollinators for food resources (Torné-Noguera et al. 2016). Moreover, honey bee pathogens like *N. ceranae* can also spill over to wild bees (Graystock et al. 2013a, Graystock et al. 2013b, Fürst et al. 2014) and shared food resources provide a platform for pathogen exchange (Durrer and Schmid-Hempel 1994, Graystock et al. 2015). Here we focus on a putative indirect effect of an emerging pathogen on pollinator community composition. Our study shows an association between infected honey bees and functional diversity in wild bees. Furthermore, we detected a negative association of pathogen presence in honey bees and the abundance of Red List species.

Wild bee abundances

Our results show a lower proportion of wild pollinators in pollinator communities near honey bees infected with *N. ceranae*. According to the abundance-range size relationship the relative abundance of a species in a certain community correlates with its niche breadth (Gaston et al. 1997). In our study, the overall most abundant species were all characterized by a broad niche (Westrich 1989). Even though with exceptions, most of the rare species were restricted to narrower niches (Westrich 1989).

Wild bee abundances were already shown to be lower in the vicinity of apiaries (Forup and Memmot 2005, Torné-Noguera et al. 2016). However, other studies showed contradictory results. No decrease in native bee populations after invasion of honey bees was observed on an island (Roubik and Wolda 2001). Furthermore, decreased wild bee abundance and diversity due to food competition with present honey bees was found (Steffen-Dewenter and Tscharnkte 2000). However, none of the studies included information about pathogen presence in honey bees.

Diversity and Functional groups

The family Apoidea is highly diverse comprising 561 species in Germany alone varying in habitat preference and seasonality (Amiet and Krebs 2014). Our sampling intended to cover a broad spectrum of species by sampling from spring to late summer in a variety of habitats. Our findings indicate no association between wild bee species diversity and *N. ceranae* in honey bees.

However, species numbers alone are not sufficient to assess biodiversity. Both, diversity and relative abundance of functional traits have been shown to have a more profound effect on plant communities than the actual taxa (Ramírez et al. 2015), with functional diversity even being considered the most important component of biodiversity (Tilman et al. 1997, Hulot et al. 2000, Lavorel and Garnier 2002).

In contrast to taxon diversity, functional diversity differed with respect to the presence or absence of *N. ceranae* infected honey bees. Social-polylectic species displayed a positive association; parasitic species displayed a negative association while no association was detected for solitary bees.

Social lifestyle could lead to compensatory effects on individual restraints. Weak members of a colony could be displaced by more resistant individuals. Another reason might be the higher overlap of habitats between the social wild bees and honey bees than solitary species, because both are generalist social species (Westrich 1989). Exposure to pathogens could result in compromised foraging, nesting and propagation as immunity requires physiological resources (König and Schmid-Hempel 1995, Nordling et al. 1998, Fellowes et al. 1999, Moret and Schmid-Hempel 2000). As solitary bees comprise the majority of all wild bee species (Amiet and Krebs 2014) a continual careful observation of this group is urgently required to monitor responses to pathogen exposure.

Higher trophic levels are crucial for structuring ecosystem communities (Montoya et al. 2003). Parasitic bee species are strongly specialized as they depend on the host species (Amiet and Krebs 2014). They are involved in large proportions of species interactions, and are important in establishing ecological networks and ensuring stability (Lafferty et al. 2006). In comparison to their hosts, parasite population sizes are generally lower and more variable, compromising their ability to colonize their host (Kruess et al. 1994).

During habitat perturbation, interaction diversity is considered to exhibit higher sensitivity to changes than species diversity (Albrecht et al. 2007). Trophic species interactions can be substantially altered with no noticeable changes in other parameters such as species richness or abundance (Tylianakis et al. 2007). The detected negative association between infected honey bees and parasitic wild bees suggests a potential impairment on the function, structure and biodiversity of inherent host-parasite interactions within the pollinator community. Furthermore, the nesting specialization of above-ground nesting species requires certain nest structures that are not available in many sites (Amiet and Krebs 2014) and this restriction narrows their niche.

Red List species

In our analysis species of particular rarity and conservation status were negatively associated with pathogen presence in the pollinator network. The detected Red List species were predominantly solitary or parasitic.

Red List species are often restricted to particular habitat structures or food resources due to high specializations (Westrich et al. 2011), which is linked with reduced flexibility of the species to reestablish in other sites. Consequently, such species should be more vulnerable

to direct or indirect stressors linked to pathogen infection in sympatric honey bee populations. We acknowledge that our study only incorporated the pathogen *N. ceranae*, but honey bee viruses have also been detected in several species of wild bees (Genersch et al. 2006, Singh et al. 2010, Evison et al. 2012, Levitt et al. 2013, Fürst et al. 2014, McMahon et al. 2015) and other bee-associated parasites such as *Nosema bombii*, *Crithidia* spp., *Sphaerularia bombi* or gregarines were not examined. Consequently, current results could be impacted by the co-occurrence of any number of established or emerging pathogens.

Habitat and Berlin-Brandenburg analysis

Low availability of bee habitat is another potential stressor for wild bees. Red List species showed a positive association with proportion of foraging and nesting habitat. Taken together with the negative association with *N. ceranae* infection in honey bees, the preferred environment for wild bees could be characterized by low pathogen pressure and specific habitats, like calcerous grasslands or heathland. Solitary-oligolectic species were negatively associated with low proportion of bee habitats as well but did not show the association with honey bees infected with *N. ceranae*. In contrast, social-polylectic species were not associated with either of these two potential stressors.

The results confirm that endangered and more specialized species are more restricted to specific habitats in comparison to more generalistic bumble bees and honey bees (Osborne et al. 1991, Banaszak 1992, O'Toole 1993). With the additional stress imposed by honey bee colonies increasingly being infected with putative pathogens, the suitable areas for Red List and particularly specialized species could be further constrained.

Interestingly, we found higher bee abundances in smaller sampling areas within the city of Berlin. The results indicate denser pollination networks, corroborating a recent study by Theodorou et al. (2016). Further studies point out that urbanization has an impact on pathogen transmission due to increased population densities on fragmented resource patches (Patz et al. 2004; Bradley and Altizer 2007). Consequently, a higher pathogen exposure due to an enhanced contact between infected and non-infected foraging pollinators is likely. In our study, all three functional groups were positively associated with areas in Brandenburg with lower pollinator density and thus potentially lower pathogen exposure. However, the associations for the solitary species of both foraging types were much stronger than for the generalist social-polylectic species. The results corroborate the positive association between social-polylectic wild bees and presence of infected honey bees. The generalist group might again be least affected by pathogen exposure.

Other important factors impacting pollinators that was not tested in our study are pesticides. Particularly the interaction between infection with *Nosema* and pesticide exposure has been

reported to impact honey bee mortality and energetic stress (Alaux et al. 2010). This remains to be investigated for wild bees in future studies tackling synergistic effects among multiple stressors.

1.6 Conclusion

Our findings suggest that wild bee pollinator species composition is associated with *N. ceranae* infections in honey bees and therefore could be influenced by factors linked to emerging pathogens in honeybees. Whether these associations are caused by direct pathogen exposure and infection or to indirect effects remains unclear. Responses differed between ecological groups with social wild bees being positively associated with *N. ceranae* in honey bees. Bees that are rare or have a specialized lifestyle are particularly impacted by pathogen presence in honey bees and low availability of bee habitats. Such bees are an important component of functional diversity and therefore pollinator service provision. Strategies to provide adequately sized and resourced habitats harboring against anthropogenic pressures, including reduced levels of honey bee associated pathogens and minimal pesticide exposure are urgently required. Ensuring that these issues are addressed can help to sustain vulnerable populations. Further studies are required to test associations between bee habitat availability, pesticide application, honey bee densities and pathogen spillover.

1.7 Acknowledgements

We thank the German Environmental Foundation (DBU) and the BioMove project for funding the project. The Bee Institute in Hohen Neuendorf provided valuable data on *N. ceranae* in honey bees in the relevant areas. We thank Dr. Christoph Saure for taxonomic identification and the Department of Urban Development and Environment Berlin as well as the Brandenburg State Office of Environment, Health and Consumer Protection, who granted permission to catch wild bees and provided landscape data.

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Chapter 2: Susceptibility of the wild bee *Osmia bicornis* to the honey bee pathogen *Nosema ceranae*

2.1 Abstract

1. Wild bees are important pollinators for agricultural crops and solitary species such as *Osmia bicornis* are particularly suitable for pollination management. Wild bees share floral resources with managed honey bees and may be exposed to emerging infectious diseases. While current studies have explored the prevalence of pathogens in solitary wild bee species data regarding the impacts of pathogens on solitary bee health have been lacking.

2. We carried out experiments to examine whether the solitary bee species, *O. bicornis* is susceptible to infection with the emerging pathogen *Nosema ceranae*, and recorded the impact of exposure on survival.

3. Our results indicate that *N. ceranae* may be able to infect *O. bicornis*, but that its impact on host fitness is negligible: survival rates did not differ between control and infected bees, although male survival was marginally lower following infection. To explore the possible field-relevance of our findings, we collected wild bees near an infected and a non-infected hive, and showed that *N. ceranae* was shared between managed and wild bees but only the in presence of infected honey bees.

4. Our findings show that *O. bicornis* is susceptible to pathogen spillover and could act as a potential reservoir host for *N. ceranae* in pollinator networks. Additional studies on this species incorporating sublethal effects, multiple infections and other interacting stressors are warranted.

2.2 Introduction

A diverse wild bee community provides important pollination services and is vital for maintaining agricultural yields and wild plant diversity, with important economic and ecological consequences around the globe (Garibaldi et al. 2013). Aside from the widely applied social bees from the genus *Bombus* some solitary wild bee species can also be managed for crop pollination, including species from the genus *Osmia* which are used in orchards in Europe and Japan (Bosch et al. 2002, Kornmilch 2010). In contrast to honey bees they are active at lower temperatures, show a higher visitation rate of flowers per unit of time and can transport more pollen (Kornmilch 2010).

Pollinator declines have been widely documented across the northern temperate regions of the globe (Biesmeijer et al. 2006, Vanbergen et al. 2013, Goulson et al. 2015, Hallmann et al. 2017) and are thought to be caused by a combination of factors, including land use change, pesticides, climate change and pathogens (Vanbergen and Garratt 2013, Goulson et al. 2015; Brown et al. 2016). Although land use change and pesticides have risen in recent years, the role of emerging pathogens have also been considered to play an important role in the decline of some pollinators (Potts et al. 2010; Schroeder and Martin, 2012, Fürst et al. 2014).

One prevailing pathogen is the microsporidium *Nosema ceranae* which reduces honey bee lifespan (Natsopoulou et al. 2016) and has been associated with significant colony losses in Spain (Higes et al. 2008), although the wider impacts of *N. ceranae* at the honey bee colony and population level have been a topic of discussion (Cox-Foster et al. 2007, Invernizzi et al. 2009, vanEngelsdorp et al. 2009, Gisder et al. 2010, Fernández et al. 2012, Wilfert et al. 2016). Here, climatic or genetic variation may be involved in explaining differences in observed virulence effects across honey bee populations (Martín-Hernández et al. 2009, Genersch et al. 2010, Williams et al. 2013, Natsopoulou et al. 2015). The pathogen is of further relevance because it has been found to be transmitted in the field from honey bees to bees from the genera *Bombus* and *Osmia* (Graystock et al. 2013a, Graystock et al. 2013b, Fürst et al. 2014, Ravoet et al. 2014). Numerous studies have conducted laboratory inoculation experiments using *N. ceranae* in honey bees (Higes et al. 2007, Higes et al. 2008, Forsgren and Fries 2010, Eiri et al. 2015, Natsopoulou et al. 2015, Natsopoulou et al. 2016, Jack et al. 2016) and, to a lesser extent, in social bumblebees (Graystock et al. 2013a, Graystock et al. 2013b, Fürst et al. 2014, Porrini et al. 2017) but to our knowledge no study has explored the effects on solitary bees such as *Osmia*. The majority of wild bees have a solitary lifestyle (Westrich 1989) and physiological impacts observed in social species may not apply to solitary species as has recently been demonstrated with regard to pesticide vulnerability in managed social vs solitary wild bees (Rundlöf et al. 2015). In light of the need

to secure future food sources and plant diversity it is important to understand the impacts of honey bee pathogens on solitary wild bee species. Even if a pathogen does not result in fitness impairments for a particular species, its establishment and transmission in numerous vectors can lead to increased circulation in pollinator networks. For example *B. terrestris* larvae can act as reservoirs for *Crithidia bombi*, an important disease of bumblebees that can reduce queen fitness (Folly et al. 2017).

Here, we carry out experimental infections of *Osmia* with *N. ceranae* with the aim of testing the susceptibility of *O. bicornis* to *N. ceranae*, and by asking whether *N. ceranae* exposure can lead to reduced host fitness. We also analyze data from the field to investigate whether spillover of *N. ceranae* can occur between infected managed honey bee populations and neighbouring *O. bicornis*

2.3 Material & Methods

Study species *Osmia bicornis*

Osmia bicornis is a common wild bee species in Germany. In contrast to the social honey bee it is characterized by a solitary, univoltine lifestyle. Its flight period ranges from March to June. Nesting takes place in wood cavities or crevices in buildings (Kornmilch 2010, Strohm 2011). Concerning pollen collection the species is polylectic and pollen resources close to the nest site are favoured (Westrich 1989, Radmacher and Strohm 2011). Females do not cooperate in brood care and provide food only for their own offspring. The nest cells in which the eggs are laid are provided with pollen for the larvae and sealed. No contact between larvae and parents exists (Westrich 1989).

The pathogen *Nosema ceranae*

Nosema ceranae has an oral-fecal transmission route. Once spores are ingested they germinate in the midgut by polar tube extrusion. The pathogen was found to cause gut tissue degeneration and prevent epithelium renewal 7 days following infection (Dussaubat et al. 2012). Consequences of impairment are energetic stress and reduced longevity (Mayack and Naug 2009, Eiri et al. 2015, Huang et al. 2015, Natsopoulou et al. 2016). *N. ceranae* is shared between bee species and is probably transmitted from honey bees to bumble bees (Graystock et al. 2013a, Graystock et al. 2013b, Fürst et al. 2014) as well as to stingless bees and social wasps (Porrini et al. 2017). However, the directionality of transmission in the field is difficult to discern and has not been demonstrated unequivocally. More precisely, the widespread occurrence of *N. ceranae* in South American and Asian *Bombus* species suggests the pathogen may also spread in the opposite direction (Plischuk et al. 2009, Li et al. 2012). Concerning solitary wild bees, the pathogen has been found in some species,

including *O. bicornis* (Ravoet et al. 2014). However, it is unknown, if the exposure can result in an infection.

Inoculation experiment

Spore detection and molecular identification

In order to inoculate individuals of *O. bicornis*, we acquired fresh *Nosema* spores from honey bees from several hives in Berlin which were checked for the pathogen prior to use in the experiments. A gut of a bee was dissected and homogenized in 200 ml of NaCl (0.9%). One drop was investigated microscopically (400x). If spores were present, the solution was processed with the DNeasy Plant Mini Kit from Qiagen (Hilden, Germany) according to the manufacturer's instructions with the following modifications: 4 µl Proteinase K were added with RNAse and incubated at 65°C for 30min. We added 3-5 metal beads to the samples and put them in the disrupter 3 times for 30sec at full speed during the incubation period. For the final suspension we used 30 µl of AE buffer.

In order to confirm that infections were caused by *N. ceranae*, we tested the DNA extracts with *N. ceranae*-specific primers in a PCR and sequenced the products. 5 µl DNA of each sample were mixed with 5 µl RNAse free H_2O, 12.5 µl KAPA 2GFast ReadyMix with dye (Kapabiosystems) and 2.5 µl of the primers Mnceranae-F and Muniv-R based on the 16S ribosomal rRNA gene (Fries et al. 2013). The PCR programme was set according to Fries et al. (2013). The amplified products were analysed in a 1.5% agarose gel stained with SYBER GOLD Nucleic Acid Stain (Thermofisher) and run for 80min at 80V. In each run a positive control with confirmed *N. ceranae* bands was included as well as a negative control with ddH_2O. Differentiation of the samples was based on the 143 bp-fragment of the 5'-end of the complete *N. ceranae* gene sequence. A subset of the positive samples was sequenced by GATC Biotech and analyzed with BLAST to confirm the identity of the pathogen.

Honey bee maintenance for spore cultivation

Honey bees were kept according to Williams et al. (2013). 1-2 days after hatching young honey bees were maintained in sterilized cages in groups of one hundred individuals in a breeding chamber at 28°C. Bees were fed with 50% sucrose solution in syringes and were starved for 24h prior to inoculation.

For the inoculum we used samples from one of the previously tested hives. We killed fresh bee samples and immediately processed them according to Fries et al. (2013) with minor modifications as follows. We dissected the guts and homogenized them in 500 µl ddH_2O. One drop of the suspension was screened for *Nosema* spores under the microscope. If confirmed, samples were combined in one sample, centrifuged at 5000g for 2 minutes and

the supernatant discarded. The pellet was resuspended in 500µl ddH_2O. We filtered the suspension with sterilized cotton wool. Spore concentrations were determined with a Neubauer chamber as described by Cantwell (1970). DNA extraction was performed to confirm the identity of the spores as described before.

The inoculum was added to a 50% sucrose solution with a find concentration of 10^6 spores/ml. One hundred bees were collectively fed via a syringe supplying 1ml of spore suspension. Control cohorts were fed with autoclaved spore suspension (Fig. 6). After having taken up the whole amount of inoculum, infected and control bees were fed sucrose solutions *ad libitum* at 28°C in a breeding chamber. Dead individuals were removed daily.

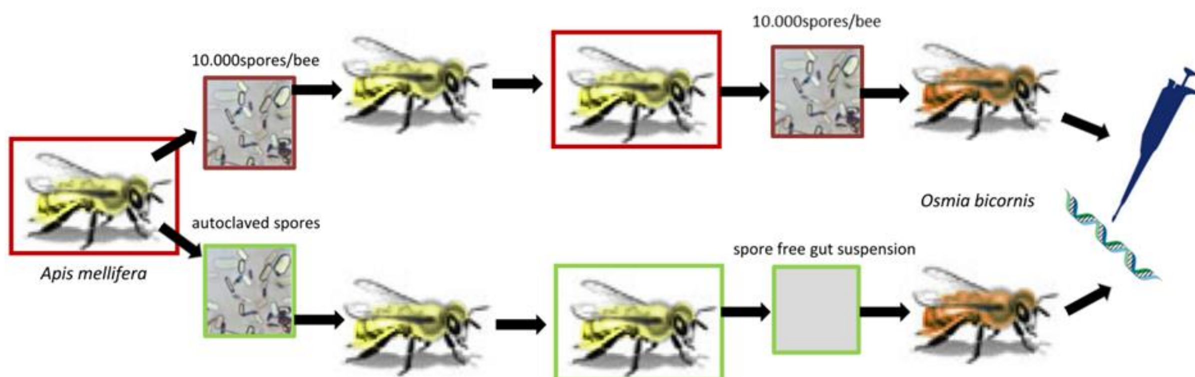

Figure 6: Inoculation and control procedure for *Nosema* infection of *Osmia bicornis*.

Osmia samples

O. bicornis cocoons and mother bees were acquired from BIENENHOTEL.de (www.bienenhotel.de) for the second and third experiment. Cocoons were kept at room temperature (20-25°C) to induce hatching and were supplied with ad libitum 50% sucrose solution *ad libitum* thereafter. We checked for *N. ceranae* in 5 individuals via phase-contrast microscopy to confirm the absence of spores. All individuals were deprived of food twelve hours prior to the administration of spore and control suspensions. Inocula were prepared from the lab-infected honey bees at day 10 post inoculation (Fig.6) and were prepared in a 50% sucrose solution as described above.

Inoculation experiment

In the first experiment sixty females of *O. bicornis* were confined individually in plastic boxes of 300ml volume and starved for 12h prior to infection. Thirty bees were individually supplied with a 10 µl droplet of inoculum containing 10^4 spores in a 50% sucrose solution. The droplet

was pipetted onto the bottom of each box containing one individual. The inoculum was only used up to one day after preparation to ensure the viability of the spores. The other 30 individuals were fed with the same amount of inoculum prepared from control honey bees. Each bee was observed individually to ensure that the whole drop was ingested. Individuals who that did not completely take up the inocula were excluded from the experiment. Bees were subsequently returned to their boxes and were provided with 50% sucrose solution *ad libitum*. The same was conducted with a set of 15 infected and 15 control male *O. bicornis*.

The survival of bees was recorded and food was resupplied daily. Dead individuals were frozen immediately at -80°C and the experiment was terminated 20 days following infection, after which all remaining ones bees were freeze-killed and stored at -80°C.

Time-course experiment

In a second experiment male *Osmia* were infected as above (30 treated, 30 control) and in parallel 30 *Apis mellifera* workers were individually fed with the same *N. ceranae* spore suspension (10^4 spores) or the equivalent control. This was done to confirm that the suspension was infective. As in the first experiment, honey bee survivals were recorded for 20 days. After 2 and 6 days post infection, 10 *Osmia* bees from each group were killed (or removed if they had died already) and frozen at -80°C until further processing. Samples were crushed in liquid nitrogen. 200 µl RNAprotect (Qiagen) were added to the samples and DNA extraction was performed with TRIzol Reagent (Thermo Fisher Scientific) according to manufacturer's protocol. TRIzol was chosen for simultaneous extraction of DNA and RNA.

Field collected wild bees

Between April and July 2017 we caught free flying wild bees sharing flowers with honey bees from neighbouring hives known to be infected with *N. ceranae*. Sampling was conducted in the garden within a radius of 15m from the hives. Another garden with hives of honey bees tested to be *Nosema*-free was chosen and sampled in the same way. The distance between the two locations was 75km and no other honey bee hives were observed in the surrounding areas of the study sites. Field work took place between 10 am and 4pm only on sunny days with a minimum temperature of 15°C, low wind and dry vegetation. The collected wild bees were observed to forage on flowers also visited by honey bees. Caught bees were killed by being frozen at -80°C as soon as possible.

Identification and categorization

Identification of defrosted and pinned bee samples followed literature by Amiet & Krebs (2014), Gokcezade et al. (2010), Scheuchl (1995, 2006), Schmid- Egger (1997) Amiet et al. (1999, 2006). Identifications were confirmed by the expert Dr. Christoph Saure.

DNA was extracted from all wild bees. PCR, agarose gels and sequencing of *N. ceranae* positive samples were performed as described above.

Statistical analysis

All analyses were carried out in R 3.3.4. The survival analysis was performed with the package Survival (Therneau 2008). Differences in survival were tested with the *survreg* command using either *exponential* or *weibull* distribution. We produced Kaplan-Meier survival curves with the *survfit* function. Pathogen prevalence between *Osmia* cohorts were checked with a Chi^2 test.

2.4 Results

Pathogen prevalence

We were able to successfully inoculate *Osmia* bees in both experiments as confirmed by significant differences in pathogen prevalence between treated and control groups (all $p<0.01$, *Osmia* female (*f*) inoculation: $\chi^2=10.512$, df=1, 21, *Osmia* male (*m*) inoculation: $\chi^2=9.7793$, df=1, both 21 days post infection; *Osmia* (*m*) time-course: $\chi^2=9.3919$, df=1, 6 days post infection). In the first inoculation experiment, *N. ceranae* was detected in 45% of *Osmia* female individuals (N=9 of 20) and 75% of the male individuals (N=9 of 12) (Fig. 7). In the second time-course experiment, *N. ceranae* prevalence in *Osmia* males was 33% (N=10 of 30) (Fig. 7). Analysis of *N. ceranae* prevalence in honey bees from the second experiment showed that infections had been successful ($p<0.01$, $\chi^2=21.012$, df=1). Among the 30 honey bees that were fed *N. ceranae* spores were found in 57% of the individuals (N=17 of 30). One sample among the control group in each experiment was contaminated and excluded from the analysis.

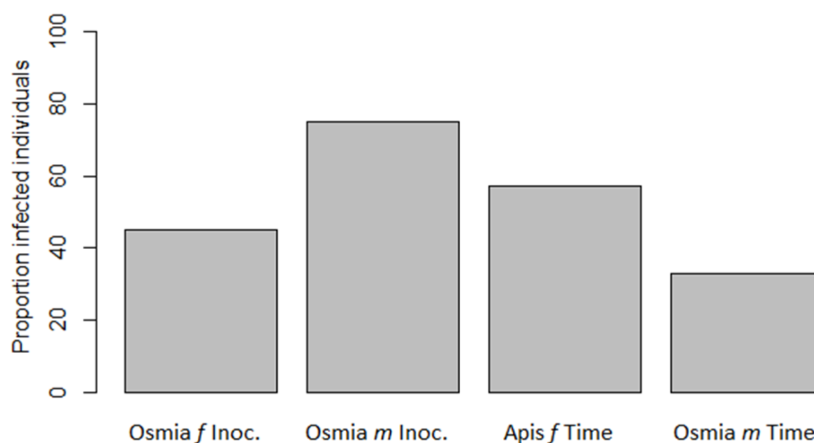

Figure 7: Prevalence of *N. ceranae* in individuals in treated groups: of female and male *Osmia* from inoculation and time-course- and female honey bees from the time-course experiment.

Differences in survival

The survival regression analysis revealed that survival of male and female infected and control *O. bicornis* individuals did not differ at 6 days post inoculation (N female=45, 20 infected, 25 control; N male= 12 infected, 10 control, χ^2=2.81, df=3, *p*=0.42, treatment: z=1.206, *p*=0.228, sex: z=0.336, *p*=0.721, interaction treatment sex: z=0.044, *p*=0.965, Table 7). However, after 20 days the same groups differed due to lower survival in males (χ^2=13.09, df=3, *p*=0.044, treatment: z=1.6, *p*=0.109, sex: z=-1.9, *p*=0.0569, interaction treatment sex: z=-0.81, *p*=0.418, Table 7, Fig.8).

When analyzing differences within the first six days of male *Osmia* between both experiments no differences in male *Osmia* survival were detected in both experiments (N Inoculation experiment: 12 infected, 10 control, N Time-course experiment: 30 infected, 29 control; χ^2=3.27, df=3, *p*=0.35, treatment: z=0.877, *p*=0.38, Exp.: z=-0.487, *p*=0.626, interaction treatment Exp.: z=-0.729, *p*=0.466, Table 7). In the second time-course experiment pathogen prevalence in male *Osmia* did not differ between day 2 and day 6 post infection (χ^2 =0, df=1, *p*=1, Table 8).

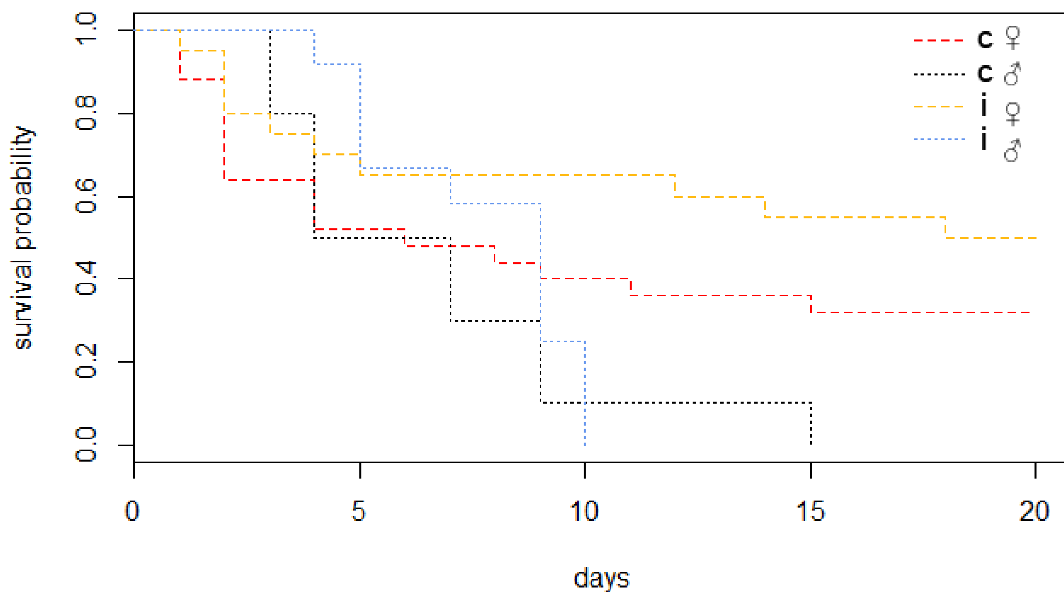

Figure 8: Kaplan-Meier survival curves for male and female *Osmia* in inoculation experiment within 20 days post inoculation, (i): infected, fed with spores of *N.ceranae*, (c): control, fed with control suspension.

Table 7: Survival regression test results between survivals of treated and control bees of *Osmia bicornis*

Exp.	days	Bees	infected	control	χ-square	df	*p*	z (t)	*p* (t)	z (Exp.)	*p* (Exp.)	z (sex)	*p* (sex)	z (t*Exp.)	*p* (t*Exp.)	z (t*sex)	*p* (t*sex)
Inoculation	6	*Osmia* female, male	20/12	25/10	2.81	3	0.42	1.206	0.228			0.336	0.731			0.044	0.965
Inoculation	20	*Osmia* female, male	20/12	25/10	13.09	3	**0.0044**	1.6	0.109			-1.90	0.0569			-0.81	0.418
Inoculation&Time	6	*Osmia* male	12/30	10/29	3.27	3	0.35	0.877	0.38	-0.487	0.626			-0.729	0.466		

Table 8: Proportion of *Nosema* detected in male *Osmia* in Exp. 2 in the 2nd and 6th day post infection cohort. Differences were tested using Chi^2 tests.

Cohorte	Indiv.num.	nat. death	% Nosema +	censored	% Nosema +	% all Nosema +	χ-square	df	*p-value*
2 p.i.	13	3	100	10	40	54			
6 p.i.	9	0	0	9	44	44			
2 vs 6							0	1	1

Pathogen spillover to wild bees in the field

Among the 51 field collected wild bees near the spore source hive, 58% were positive for *N. ceranae*. Caught species could be allocated to 22 species from 13 genera. 49% (N=25) of each were social and solitary species while 2% were parasitic (Table 9).

Table 9: Wild bee species, level of sociality and detection of *N. ceranae*, from individuals collected near the infected honey bee hive that was used as the source of *N. ceranae* spores in the reported infection experiments.

Species (Number of Individual)	Sociality	*N.cer.*	Species (Number of Individual)	Sociality	*N.cer.*
Andrena flavipes (1)	solitary	yes	*Chelostoma campanularum (5)*	solitary	no
Andrena flavipes (2)	solitary	yes	*Chelostoma campanularum (6)*	solitary	yes
Andrena nitida	solitary	yes	*Chelostoma campanularum (7)*	solitary	no
Andrena subopaca	solitary	yes	*Chelostoma campanularum (8)*	solitary	no
Anthophora plumipes (1)	solitary	no	*Chelostoma campanularum (9)*	solitary	no
Anthophora plumipes (2)	solitary	yes	*Colletes daviesanus*	solitary	yes
Bombus lapidarius (1)	social	yes	*Halictus rubicundus*	social	no
Bombus lapidarius (2)	social	yes	*Halictus subauratus*	social	yes
Bombus pascuroum (1)	social	no	*Heriades truncorum*	solitary	yes
Bombus pascuroum (2)	social	no	*Hyaleus communis*	solitary	yes
Bombus pascuroum (3)	social	no	*Lasioglossum calceatum (1)*	social	no
Bombus pascuroum (4)	social	no	*Lasioglossum calceatum (2)*	social	yes
Bombus pascuroum (5)	social	yes	*Lasioglossum calceatum (3)*	social	yes
Bombus pascuroum (6)	social	no	*Lasioglossum calceatum (4)*	social	no
Bombus pascuroum (7)	social	no	*Lasioglossum calceatum (5)*	social	yes
Bombus pascuroum (8)	social	yes	*Lasioglossum pauxillum*	social	yes
Bombus pascuroum (9)	social	yes	*Lasioglossum sexstrigatum*	social	yes
Bombus pascuroum (10)	social	yes	*Megachile ericetorum*	solitary	no
Bombus pascuroum (11)	social	no	*Melecta albifrons*	parasitic	no
Bombus pratorum	social	no	*Melitta nigricans (1)*	solitary	yes
Bombus terrestris (1)	social	yes	*Melitta nigricans (2)*	solitary	yes
Bombus terrestris (2)	social	yes	*Osmia bicornis (1)*	solitary	no
Chelostoma campanularum (1)	solitary	yes	*Osmia bicornis (2)*	solitary	no
Chelostoma campanularum (2)	solitary	yes	*Osmia caerulescens (1)*	solitary	yes
Chelostoma campanularum (3)	solitary	yes	*Osmia caerulescens (2)*	solitary	yes
Chelostoma campanularum (4)	solitary	no			

Spores were detected in 64% of solitary and 56% of social bees (Fig. 9). Infection rates did not differ significantly between social and solitary bees (χ^2: 0,083, df= 1, p= 0.7728). No spores were detected in the parasitic species. Among the caught samples, 2 individuals of *O. bicornis* were found which were not positive for *N. ceranae*. None of 48 individuals from the site near non-infected honey bees was infected resulting in a highly significant differences compared to the infected honey bee site (χ^2: 37.774, df=1, p= < 0.001). At this site, 21 species from 8 genera were caught (Table 10). Between the two sites, 16.7% of the detected species were shared. 83.3% of the shared species were positive for *N. ceranae* at the field site near the spore source hive.

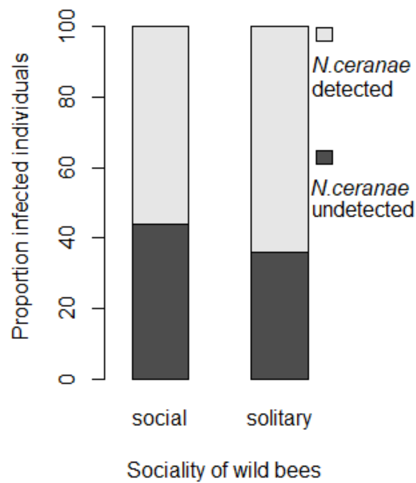

Figure 9: Proportions of individuals infected with *N. ceranae* in social and solitary wild bees caught near infected honey bees serving as spore source for the inoculation. Prevalence did not differ significantly between the social groups.

Table 10: Wild bee species, level of sociality and detection of *N. ceranae*, from individuals collected near the honey bee hive where *N. ceranae* could not be detected.

Species (Number of Individual)	Sociality	*N.cer.*	Species (Number of Individual)	Sociality	*N.cer.*
Andrena dorsata	solitary	no	*Andrena nigroaenea (1)*	solitary	no
Andrena flavipes(1)	solitary	no	*Andrena nigroaenea (2)*	solitary	no
Andrena flavipes (2)	solitary	no	*Andrena subopaca (1)*	solitary	no
Andrena fulva (1)	solitary	no	*Andrena subopaca (2)*	solitary	no
Andrena fulva (2)	solitary	no	*Bombus pascuroum (1)*	social	no
Andrena gravida (1)	solitary	no	*Bombus pascuroum (2)*	social	no
Andrena gravida (2)	solitary	no	*Bombus pascuroum (3)*	social	no
Andrena haemorrhoa (1)	solitary	no	*Bombus pascuroum (4)*	social	no
Andrena haemorrhoa (2)	solitary	no	*Bombus pascuroum (5)*	social	no
Andrena haemorrhoa (3)	solitary	no	*Colletes cunicularius*	solitary	no
Andrena haemorrhoa (4)	solitary	no	*Dasypoda hirtipes (1)*	solitary	no
Andrena haemorrhoa (5)	solitary	no	*Dasypoda hirtipes (2)*	solitary	no
Andrena haemorrhoa (6)	solitary	no	*Halictus rubicundus*	social	no
Andrena haemorrhoa (7)	solitary	no	*Halictus subauratus*	social	no
Andrena haemorrhoa (8)	solitary	no	*Halictus tumulorum*	social	no
Andrena haemorrhoa (9)	solitary	no	*Lasioglossum pauxillum*	social	no
Andrena haemorrhoa (10)	solitary	no	*Lasioglossum quadrinotatum*	social	no
Andrena haemorrhoa (11)	solitary	no	*Lasioglossum sexnotatum*	social	no
Andrena haemorrhoa (12)	solitary	no	*Nomada furcuata*	parasitic	no
Andrena haemorrhoa (13)	solitary	no	*Osmia aurulenta*	solitary	no
Andrena haemorrhoa (14)	solitary	no	*Osmia caerulescens*	solitary	no
Andrena haemorrhoa (15)	solitary	no	*Osmia leaiana (1)*	solitary	no
Andrena helvola (1)	solitary	no	*Osmia leaiana (2)*	solitary	no
Andrena helvola (2)	solitary	no	*Osmia leaiana (3)*	solitary	no

2.5 Discussion

This is the first study to test experimental infection of a solitary wild bee with the emerging managed honey bee pathogen *N. ceranae*. We detected a clear difference in pathogen prevalence between treated and control groups of both sexes suggesting susceptibility of *O. bicornis* to *N. ceranae*. We did not detect significant differences in survival between control and treatment groups of both sexes in *O. bicornis* across two experiments, except for a slightly negative effect for males over a period of 20 days. Additionally, we showed that *N. ceranae* is likely to be shared between honey bees and wild bees in the natural environment.

Pathogen prevalence

Our results show susceptibility of both sexes of *O. bicornis* to *N. ceranae.* In contrast to an infection rate of almost 100% in bumblebees after day 5 post infection (p.i.) with a dose of 6500 spores/bee (Graystock et al. 2013a) proportions of infected individuals were lower in our study. However, our infection rates of 45 and 33% in female and male *Osmia* of Exp. 1 and 2 were higher than the 0-25% in bumblebees collected in the first 4 days p.i. (Graystock et al. 2013a). The high infectionn rate of 75% in males in Exp. 1 could not be corroborated in

Exp. 2 and regarding the lower sample size (12 vs 30) it should be considered carefully. Furthermore, our results caution against generalizing findings for the highly diverse group of wild bees. For instance, the biology of social bumblebees shows more commonalities with honey bees than does those of the solitary *O. bicornis*. Species-specific physiological features can account for differing sensitivity to stress factors as has been shown for pesticides impacts on different pollinators (Rundlöf et al. 2015).

Likewise, infection rates from various studies might not be consistent due to differing susceptibilities across different species. In contrast to our results, infection rates of 100% in honey bees have been reported using the same dose as our study (Forsgren and Fries 2010). However, in another study with the same dose, only 23.5-35.8% of spore treated honey bees were found to be infected 16 days post infection (Jack et al. 2016). In our results, the infection rates of 57% in honey bees of Exp. 2 range between these values and demonstrate the likely infectivity of our inocula.

Survival effects

Survival rates of infected and control male *Osmia* within 6 days did not differ between the two experiments and between males and females of Exp. 1 and the lower survival of males within the 20 day period of Exp. 1 was only marginal.

Our results for *Osmia* contrast with findings from infection experiments with *N. ceranae* in bumblebees where infected individuals show a distinctly lower survival (Graystock et al. 2013a). Additionally, in a 15 day experiment bumblebee survival was significantly reduced when faeces or pollen from commercial bumblebee colonies was ingested (Graystock et al. 2013b). Our results are not likely to be explained by too low a dose. Our infectious suspension made of bee gut contents contained even more spores (10.000/µl vs. 6500 / respectively 6100 as used in (Graystock et al. 2013a, Graystock et al. 2013b)). More precisely, a mean of 10.053 (+-4147) spores of *N. ceranae* for 24h old honey bees, 3217 (+-1268) for 5 day old bees and 5009 for 14 day old bees were identified as a lethal dose in an experimental setting by (Huang et al. 2015). However, a lethal dose in one experimental setting may not apply in other environments using bees from a different origin (Huang et al. 2015). Our results suggest that our dose of 10^4 spores can lead to an establishment of *N. ceranae* in *Osmia* but without resulting in compromised survival. As mentioned before, differences in physiology of solitary wild bee species and social bee can account for contrasting outcomes of pathogen infection. Our survival test was carried out with a certain bee species and environment. Thus, changing parameters such as including other environmental stressors like pesticide applications or extreme temperatures might lead to different results. Furthermore, diet is an important variable to consider in infection

experiments as protein can result in higher proliferation of *N. ceranae* (Porrini et al. 2015). As the present samples were maintained on sucrose solution alone this may also impact *N. ceranae* infection rate.

Eventually, it would be of interest to scrutinize if the applied concentration corresponds to the natural situation. Currently, to our knowledge, no information about spore loads on flowers in a natural transmission setting exists. In a previous study an average of 12.000 spores of *N. ceranae* was recorded at flowers when honey bees acted as donors and bumble bees as vectors whereas an average of 7200 spores per flower was detected when the species roles were reversed (Graystock et al. 2015). These data provide clues as to the possible exposure levels presented by different hosts on flowers in a plant-pollinator network. Our spore dose therefore represented a reasonable starting point for further infection experiments in solitary wild bees.

Detection of *N. ceranae* spores at different time points

The detection of *N. ceranae* after 2 and 6 days post inoculation demonstrates successful establishment of the pathogen in *O. bicornis*. The development of a *Nosema* infection was found to take 10-14 days in bees inoculated at an age of 5 days (Forsgren and Fries 2010). However, the results could not be reconfirmed in other trials in different locations (Huang et al. 2015). Both of our 2 and ongoing 6-day cohorts are within this time-frame and no differences in infection rates suggest that 2 days are sufficient for establishment of the pathogen. Nevertheless, other studies provided contrasting outcomes: Samples dying within 6 days of exposure were pathogen free yet pathogens were found in samples of bumblebees surviving after day 7 p.i. (Graystock et al. 2013b). With regard to our experiments, initial dosage can influence pathogen development and proliferation (Fries 1988, Forsgren and Fries 2010; Cuomo et al. 2012, Fries et al. 2013). Thus, the early detection of spores in our experiments could have been affected by the higher spore dose used, as compared with Graystock et al. (2013b).

Hosts of *N. ceranae* in the field

Data from our field collected wild bees reveal how readily *N. ceranae* may be shared between managed honey bees and wild bees in a natural environment. In a wider context 21% *N. ceranae* prevalence was recorded from a large bumblebees study (N=764) in the UK (Graystock et al. 2013a). In our study, infection rates of 58% and 0% at the *N. ceranae* positive and negative sites respectively are consistent with these findings and suggest that close vicinity to infected honey bees and intense sharing of floral resources can lead to significant cross over of pathogens. Although we acknowledge that, as our sample sizes of

51 and 48 individuals were significantly smaller, our evidence of spillover is vital for assessing further scenarios in pollination management particularly in the light of movement and re-location of honey bee hives for agricultural purposes or honey production.

Moreover, individuals can carry and distribute pathogens without becoming infected themselves as demonstrated for bumblebee larvae and *Crithidia bombi* (Folly et al. 2017). Also, covert infections can still impact survival and foraging in the long term as demonstrated for Deformed Wing Virus (DWV) in honey bees (Benaets et al. 2017). Our successful infection study together with reports on *Nosema* in *O. bicornis* collected from the field (Ravoet et al. 2014) show that *Osmia* could be a reservoir for *N. ceranae* in pollinator networks. Our study also reveals that other wild bee species differing significantly in ecology have the potential to transmit this emerging pathogen. Nonetheless, we do not know anything about effects in other solitary wild bees and other non-bee pollinator species. Evidence for sub-lethal effects has been found in bumblebees (Graystock et al. 2013a, Graystock et al. 2013b) and should be investigated further in solitary bees in future. Furthermore, honey bee viruses have been found in several wild bee species (Genersch et al. 2006, Singh et al. 2010, McMahon et al. 2015, Ueira-Vieira et al. 2015, Alvarez et al. 2018) and infection experiments in solitary species are warranted.

2.6 Conclusion

O. bicornis proved to be susceptible to *N. ceranae*. Survival was not shown to be negatively affected as demonstrated for honey bees and bumblebees. Reasons could be different pathogen virulence and/or host susceptibility. Pathogen spillover was detected in social as well as solitary wild bee species sharing flowers with spore-source honey bees suggesting numerous vectors for *N. ceranae* in the pollinator network. Future studies should incorporate multiple infections and stressors as well as investigate sub lethal effects.

2.7 Acknowledgements

We would like to thank Erhard Strohm for sharing his expertise on working with *O. bicornis* and Lena Wilfert for her advice on infection experiments. Further we thank the *Senate Department for Urban Development and the Environment* for granting their special permission for capturing and killing wild bees as required by §45 (7) Nr.3 Federal Nature Conservation Act Germany. We additionally thank Lorin Schellenberg for her practical support in the experiments.

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Chapter 3: Life history effects in the solitary bee *Osmia bicornis* after larval exposure to the honey bee pathogen *Nosema ceranae*

3.1 Abstract

1. Wild bees are important pollinators of wild plants and agricultural crops and threatened by a number of environmental stressors including emerging pathogens. Honey bees have been suggested as a source of pathogens. One prevalent pathogen that has recently emerged as a disease of honey bees is the microsporidian *Nosema ceranae*. While the impacts of *N. ceranae* in honey bees have been well documented, virtually nothing is known about its effects in solitary wild bees.

2. The solitary mason bee *Osmia bicornis* is a common pollinator in orchards and can be managed. Here we experimentally exposed larvae of *O. bicornis* to food contaminated with *N. ceranae* and document spore presence during larval development. We measured mortality, growth parameters and timing of pupation in a semi-field experiment. Hatched individuals were assessed for fitness correlates including fatbody mass, wing muscle mass and body size.

3. We recorded higher mortality and delayed pupation in the spore-exposed group, but could only detect a low number of spores among exposed individuals. Spore-exposed males had a reduced body size compared to the control group. Both infected males and females showed increased head capsule width, possibly attributable to longer development time. No fitness effects in hatched individuals were detected.

4. Although we did not find overt evidence of *O. bicornis* infection, our findings indicate that exposure of larvae to *N. ceranae* spores affects bee development.

3.2 Introduction

Pollination provided by wild and domesticated bees is essential for wild flowers and agricultural crops. Biodiversity and food security rely on healthy pollinator communities (Klein et al. 2007, Potts et al. 2010, IPBES 2016). Bee population declines have been reported globally (Biesmeijer et al. 2006, Fitzpatrick et al. 2007, Oldroyd 2007, Stokstad 2007, Brown and Paxton 2009, Neumann and Carreck 2010, Potts et al. 2010, Cameron et al. 2011, Bartomeus et al. 2013, Vanbergen and Garratt 2013, Nieto et al. 2015). Causes for decline include land use changes, habitat loss and fragmentation (Winfree et al. 2009), pesticide use (Rundlöf et al. 2015, Woodcock et al. 2016, Woodcock et al. 2017) pathogens, climate change and invasive species (Brown and Paxton 2009, Goulson et al. 2015, Brown et al. 2016, Potts et al. 2016). Multiple stressors have also been considered to interact additively or synergistically as causes of bee decline (Bryden et al. 2013, Di Pasquale et al. 2013, Di Prisco et al. 2013, Brunner et al. 2014, Doublet et al. 2015, Potts et al. 2016, Renzi et al. 2016, Sánchez-Bayo et al. 2016, Manley et al. 2017).

The importance and effectiveness of wild bee pollinators has increasingly been acknowledged in recent years (Garibaldi et al. 2013) These studies have emphasized the importance of a diverse community for ecosystem service pollination provision and stability. Social bees such as honey bees and bumble bees are regularly used in pollination management in European agriculture, but only one genus of the solitary bees is currently used in pollination management in European agriculture, the mason bees in the genus *Osmia* (Bosch et al. 2002). In addition they represent an important group of pollinator species which makes *Osmia* a highly suitable study species to explore the effects of honey bee pathogens on solitary wild bees.

For honey bees, a variety of pathogens are known. Those comprise viruses, fungi and bacteria. At least 23 viruses are known from honey bees (McMenamin and Genersch 2015) with several new viruses being discovered every year (Mordecai et al. 2016, Remnant et al. 2017, Galbraith et al. 2018). The ectoparasitic mite *Varroa destructor* and the viruses it transmits, particularly DWV, are considered the major cause of elevated honey bee mortality (Highfield et al. 2009, Genersch 2010, Nguyen et al. 2011, Dainat et al. 2012, Nazzi et al. 2012, Francis et al. 2013, Budge et al. 2015, Di Prisco et al. 2016). Over the last two decades, the emerging microsporidium *Nosema ceranae* also became prevalent globally and has been known to cause mortality of honey bees (Paxton et al. 2007, Higes et al. 2008, Natsopoulou et al. 2016) although the extent to which it is responsible for colony loss has been debated (Gisder et al. 2010, Fernández et al. 2012). More recently, the wider risk posed by *N. ceranae* to sympatric wild bee populations has been explored, with studies revealing transmission of the parasite to bumble bees (Graystock et al. 2013a, Graystock et al. 2013b, Fürst et al. 2014) and solitary bees such as *Osmia bicornis* (Ravoet et al. 2014).

This indicates frequent transmission potential to a broad range of bee hosts (Shafer et al. 2009).

Pathogen detection alone does not provide information about consequences for host fitness. Defense against pathogens is usually energetically costly because of the upregulation of the immune system of the host resulting in physiological trade-offs (Sheldon and Verhulst 1996, McKean and Nunney 2001, Zuk and Stoehr 2002, McKean et al. 2008, Kirschman et al. 2018). Studying fitness correlates can reveal physiological costs due to an activated immune system. However, the costs can also depend on the host life stage.

Here we exposed *O. bicornis* larvae experimentally to food containing *N. ceranae* spores to investigate if this exposure results in fitness costs. To our knowledge, no such experiments in solitary wild bees with *N. ceranae* have been conducted and nothing is known about life-history effects of this pathogen on solitary bees.

We adressed the following questions: (I) Does *N. ceranae* cause detectable infection in the larval stage of *O. bicornis*? (II) Does infection with *N. ceranae* affect the development? and (III) does infection with *N. ceranae* result in fitness costs in the imago?

3.3 Materials and Methods

Study species

O. bicornis is a common solitary wild bee species in Germany with an univoltine lifestyle and a flight period from March to June. The species uses wood cavities or crevices in buildings for nesting (Kornmilch 2010, Strohm 2011). *O. bicornis* is polylectic and favours pollen resources close to the nest site (Westrich 1989, Radmacher and Strohm 2011). For our experiment we ordered cocoons of *O. bicornis* from a breeder (BIENENHOTEL at www.bienenhotel.de).

Ethical statement

Permissions for the study were provided by the Senate Department for Urban Development and the Environment of Berlin and included the allowance to release *Osmia bicornis* bees bought from the breeder according to §40 subsection 4 BNatSchG. Moreover, a permission according to § 45 subsection sentence 1 No. 3, sentence 2 BNatSchG for scientific reasons and a permission to catch and kill the bees according to § 44 subsection 1 No. 1 BNatSchG (ibid.) was granted.

Preparation of spore suspension for inoculation

For our inoculation suspension fresh *Nosema* spores from honey bees were required. Wcollected samples from several hives around Berlin and investigated pathogen presence. A gut of a bee was dissected and homogenized in 200 ml of NaCl (0.9%). One drop was investigated microscopically (400x). If spores were present, the solution was processed with the DNeasy Plant Mini Kit from Qiagen (Hilden, Germany) according to the manufacturer's instructions with following modifications based on previous tests for effective extraction: 4 µl Proteinase K were added with RNAse and incubation on the heater was extended to 30min. We added 3-5 metal beads to the samples and put them in the disrupter 3 times for 30sec at full speed during the heat incubation. For the final suspension we used 30 µl of AE buffer.

For PCR 5 µl DNA of each sample were mixed with 5 µl RNAse free H_2O, 12.5 µl KAPA 2G Fast ReadyMix with dye (Kapabiosystems) and 2.5 µl of the primers designed by Gisder & Genersch (Gisder and Genersch 2013) applying the described PCR programme. The amplified products were analysed in a 1.5% agarose gel stained with SYBER GOLD Nucleic Acid Stain (Thermofisher) and run for 80min at 80V. In each run a positive control with confirmed *N. ceranae* bands was included as well as a negative control with ddH_2O. Differentiation of the samples was based on the 662 bp (Gisder and Genersch 2013). A subset of the positive samples was sequenced by GATC Biotech and analyzed with BLAST to confirm the identity of the pathogen. If *N. ceranae* was detected, we chose the honey bees as spore source for the infection experiment.

Fresh *N. ceranae* spore suspension for use in infection experiments were maintained in laboratory kept groups of *A. mellifera* workers. Honey bees were kept according to Williams et al. (Williams et al. 2013). After hatching young honey bees were maintained in sterilized cages in groups of 50-200 individuals in a breeding chamber at 28°C. For the inoculum we used samples from one of the previously tested hives. We killed fresh bee samples and immediately processed them according to Fries et al. (2013) as described before. Ten days post infection up to three bees were killed and processed further by the modified protocol of Fries et al. (2013). The bee gut was homogenized in 300 µl 1 5mM 9.0 ph buffered ammonium chloride (NH_4Cl), and centrifuged (Eppendorf centrifuge 5810 R) at 5,000 G (Relative Centrifugal Force) for five minutes. The supernatant was discarded and the pellet was resuspended with another 500 µl NH_4Cl by vortexing and filtering again. Spores were checked under a microscope with 400x magnification, counted with a hemocytometer (improved Neubauer chamber) (Human et al. 2013) according to Cantwell (1970) and molecularly confirmed for species identity as described before. The positive tested suspensions were diluted with a 50 % sucrose solution (w/w). The inocula was applied to

each larvae of *O. bicornis* aged 2 or 3 days with a concentration of 10.000/spores per bee and autoclaved for the controls. The spore dose referred to previous infection experiments by Fries et al. (2010), Forsgren and Fries (2010), Jack et al. (2016) and Eiri et al. (2015).

Study site and experimental set-up

The study site was a 14 ha orchard meadow in Brandenburg 32 km south of Berlin within the FFH-area Nuthe-Nieplitz-Niederung (N52°21`59.6", E13°07'53.4", GeoBasis-DE/BKG, 2017). Eighteen honey bee colonies from a beekeeper were located within a 250m distance to the nesting box. The hives have been there since several years. A nest box measuring 1,90 x 1,00 x 0,80m was constructed. As nests, 60 nest boards accommodating 10 nests each were used with an acrylic, high temperature resistant cover to allow access for experimental manipulation. The diameter of the entrance was 8 mm, suitable for *Osmia bicornis* (www.bienenhotel.de, 01.04.2016). Bee cocoons and nest boards were ordered on www.bienenhotel.de and kept in 5°C until placement in the nest boxes in the field on the 29th of April.

One characteristic for sex determination in *O.bicornis* is body size with females being generally bigger and heavier than males (Raw 1972). We estimated sex via cocoon size. In order to avoid a low return rate of released bees, the recommended 1:1 sex ratio by the breeder was increased to 1.14 f:m resulting in 800 female and 700 male cocoons that were placed in the nest boxes.

Bees were observed building brood cells and laying eggs. The hatching date was recorded in order to determine the age of the sampled larvae. Parasites were cleared and brood cells with parasites were discarded from the experiment. Temperature and weather conditions were also recorded.

Treatment with *N. ceranae*

One µl of *N. ceranae* spore-containing solution was placed either on the food source (treatment SpF) (N=229) or directly on the larvae (treatment SpL). Corresponding controls were treated with solutions with autoclaved spores termed treatments CoF (N=321) and CoL (N=206), respectively. Food consisted of pollen in the larval brood chamber provided by the mother bee. For SpF and CoF treatments a droplet of spore suspension was applied on the food directly in front of the larvae. For SpL and CoL treatments the suspension was directly applied on the larval body. The randomized treatments of larvae were conducted between 13th and 29th of May 2016.

Sampling of *Osmia* bee larvae

Finished brood cells were defined as closed cells with pollen provided and an attached egg and recorded daily. The hatching date was observed in order to determine larval age for inoculation.

Between the 5th and the 16th of June, 150 *O. bicornis* larvae were sampled on the 19th or 20th day post infection corresponding to the 4th larval instar (22-24 days old). From a total of 150 larvae at least 12 larvae were discarded for different reasons. The sampled larvae were transferred individually into a 1.5 ml Eppendorf tube and immediately frozen at -20°C for later measurements and dissection.

Fitness parameters of *Osmia* bee larvae

The physical measurements head capsule width and fresh body weights were taken from larvae. The head capsule width was measured on the widest point with a binocular microscope (Olympus SZX16, ColorView) with a caliper (1/50 mm nonius) according to Vogelweith et al. (2013). Referring to Bosch & Vicens (2002) the head width constitutes the best estimators of adult weight and provision weight in both sexes besides wing lengths.

Sampling and fitness parameters of Imago I (imago enclosed in cocoon)

526 imagines were sampled as fully developed bees within the cocoon (155 to 157 days old). The cocoon was gently cleaned by hand with a dry brush to remove feces and debris and stored immediately in a 1.5ml microcentrifuge tube.

After recording the weight of the cleaned cocoon with the imago still inside, the cocoon was gently opened with a scalpel. The cocoon weight and fresh body weight were measured. The sex was determined based on the color of the clypeus (Raw 1972, Amiet and Krebs 2014). Afterwards the imago was frozen at -80°C for 12 min, the fresh body weight was recorded and the gut was removed from the body. Both groups, the sampled larvae and the imago, were further dissected for the microscopic examination of *N. ceranae* infection.

Hatching record and fitness parameters of Imago II (hatched imago)

In total, 72 control and treated individual cocoons were stored at 4°C over winter and placed outside in two separated cages on April 13th 2017. Temperature of night and day as well as hatching was recorded daily. Hatching took place between 3rd and 20th of May. Remaining cocoons were observed until May 29th when the experiment was ended. The unhatched

cocoons were counted. The heads of the hatched individuals were frozen at -80°C.The remaining bee body was stored at -20°C.

The distance referred to as inter-tegular span is used as a measure of insect size as it is well correlated with flight range for bees (Cane 1987). The fat content of a bee body is essential to determine immunity and longevity. It stores energy and synthetizes immunoproteins (Amdam and Omholt 2002).

The fat content was measured following methods described by Mikolajewski et al. (2008) and De Block et al. (2008). The bodies of all hatched individuals were weighed and subsequently dried in a drying cabinet at 60°C for 48h. Afterwards, 1.5ml of dichlormethan were added. Samples were put on a shaker for 24h after which the dichlormethane was removed and the bees weighed again. Fat content was determined by subtraction

Body size and flight muscle ratio were measured as described by Plaistow et al. (1996). The dry fatless thorax was placed in 0.2M potassium hydroxide at room temperature for 48h which results in digestion of the flight muscle. Afterwards, the remaining cuticle was washed in distilled water, dried and re-weighed. Flight muscle ratio was determined as the quotient of dry flight muscle mass and total dry abdomen and thorax mass.

Detection of *N. ceranae* in *O. bicornis* in larvae & Imago I (pupae)

Larvae (N =138) were processed either as whole body or separated gut only (Supplement, Table A2). The gut of the Imago I (N=507) was dissected as described for honey bees (Fries et al. 2013). The guts of the two developmental stages were processed according to Fries et al. (2013) as described before with a few modifications. The gut was mixed with 300 µl sterile ddH_2O for homogenization and centrifugation. The supernatant was discarded, and the pellet was resuspended with 300 µl sterile ddH_2O and filtrated once through cotton wool. Gut suspensions and remaining imago bodies were frozen.

After spore preparation, determination of *N. ceranae* spores and possible infection were inspected with a phase contrast microscope (400x). If spores were present, the solution was processed with the DNeasy Plant Mini Kit from Qiagen (Hilden, Germany) as described before. PCR and gel electrophoresis were performed as described with primers by Gisder & Genersch (2013).

Statistical Analysis

All statistical analyses were conducted in R 3.3.4. Data were tested for normal distribution of residuals (larva, Imago I) and homogeneity of variance or Shapiro-test (Imago II) and log-

transformed if necessary. In case of normal distribution the data was tested with linear models. The family gaussian was applied for all models.

Statistical analysis in Larvae and Imago I

The conditions for linear models were met and we tested the differences in pupation start and head capsule width using the explanatory variable ‚treatment' (spore exposed/control). Finally a linear multi factorial model was fitted to the data with an one-way ANOVA. The pupation start (in days) as response was tested against the treatment as fixed factors and the head capsule width as co-variate. Another ANOVA was applied with treatment as fixed factor, head capsule width as response variable and bee age as co-variate in the larvae sampling group. In two ANCOVAs head capsule width as response was tested with weight difference of cocoon and fresh weight as fixed factor and sex as co-variate for the larvae as well as for the imagines separately. For the imagines, weight difference as a response was tested with treatment as fixed factor and sex as co-variate.

A linear regression was conducted with measures of head capsule width to fresh weight or cocoon weight within the 'imago' and the 'larva' dataset. Another regression analysis was performed to see relationship of the head capsule width to fresh body weight of the both sexes.

Statistical analysis Fitness parameters in Imago II

As no normal distribution could be reached, differences between groups were tested with general linear models. Assumptions for general linear models were confirmed.

For the measure of head capsule width, cocoon weight and fresh weight an ANCOVA with a linear model was fitted to the data (Table 12).

3.4 Results

From a total of 1,592 recorded brood cells 1,085 *O. bicornis* were treated with either viable or autoclaved spores. A total of 662 *Osmia* bees were sampled (150 larvae and 526 Imago I). 31 bees had to be discarded as they were parasitized which resulted in 138 larvae and 507 Imago I for the analysis (Table 11). For Imago II (N=72) bee cocoons were separated for both treatments.

Table 11: Overview of study sampling with exclusion of brood cells.

Samples	No. of brood cells with eggs	Sample size (from 1,085)	Discarded bees	Head capsule width / Weight	Spore check
All bees	1,592	676	31	645	545
Larvae		150	12	138	138
Imago (I+II)		526	19	507	407

Mortality rate of infected *O. bicornis* Larvae and Imago I (Pupae)

In total, 664 dead bees were recorded at either egg, larval and imaginal stage.

From 676 sampled bees 69 bees died, CoF (control on food): 12, CoL (control on larvae): 9, SpF (spores on food): 18, SpL (spores on larvae): 30. A Pearson's Chi-squared test with Yates' continuity correction revealed a significant difference between 'Control' and 'Spore' treatments (χ^2= 9.4153, df = 1, p = 0.002).

Detection of *N. ceranae* spores

The spore control under the microscope was conducted with 545 samples from which 334 were treated with spores and yielded a total of five *N: ceranae* positive homogenates with only a few spores each. With this low amount of spores it was not possible to do a further determination of the spore density with a haemocytometer and the amount of material was too little for a further investigation.

Sex ratio Imago I

The sex ratio from sampled imagines was calculated from N=506 (140 f, 366 m). The overall sex ratio for all sampled imagines is 1: 2.6 females to males, including those bees which were parasitized but a sex could still be determined from the head capsule rest. The proportion of females in the 'Spore' group was 32.2% and 24% in the 'Control' group. The proportions differed significantly (χ^2: 4.808, df= 1, p=0.0283). In order to take the differences into account, sex was incorporated as a co-variate in the statistical analysis.

Fitness correlates

Head capsule width was lowest in control treatment in the larvae as well as the imago dataset. An ANCOVA revealed a significant higher mean head capsule width in the 'Spores' groups within the larvae ($F_{1,133}$= 6.632, p= 0.011, Table 12, Fig. 10). For all 502 imagines a mean head capsule width of 3.19 ± 0.34 mm, a cocoon weight of 74.82 ± 22.68 mg and a fresh weight of 64.01 ± 19.33 mg were measured.

The body size of the imagines showed a strong correlation between head capsule width and fresh weight of the bees (r=0.782). The strongest positive correlation was detected between cocoon weight and fresh weight (r=0.995). The body size of imago females was 1:1.5 time

that of males for head capsule width, cocoon weight and fresh body weight (Table A2, A3). The analysis of covariance of the head capsule width to fresh body weight ($F_{2,499}$= 950.1, *p*<0.001, Table 12) as well as head capsule width to cocoon weight ($F_{2,499}$= 956.4, *p*<0.001, Table 12) with sex as co-variate is increased by a sex significant effect.

Table 12: Results of a linear multi factorial model for influence of treatment (Control and Spores) on the pupation start, head capsule width and weight differences during development and results of regressions with three datasets for two development stages. As co-variates head capsule width, bee age or sex was used. lm=linear model

Samples	Dependent variable	Independent variable	co-variate (C)/ Interaction (I)	Num. D.f.	F-value	*p-value*	Analysis
larvae	pupation start	treatment		1, 74	5.23	0.025	lm: anova
larvae	pupation start	treatment, inoculation type	C: head capsule width	1, 74	16.42	< 0.001	lm: anova
larvae	pupation start	treatment	I: head capsule width	1, 74	4.61	0.0349	lm: anova
larvae	head capsule width	treatment, inoculation type	C: bee age	1, 133	6.632	0.011	lm: anova
imago	head capsule width	fresh weight	C: sex	2, 499	950.1	< 0.001	regression
imago	head capsule width	cocoon weight	C: sex	2, 499	956.4	< 0.001	regression
imago	fresh weight	cocoon weight	C: sex	2, 499	5-871e+04	< 0.001	regression
imago	weight difference	treatment	C: sex	1, 500	3.934	0.0479	lm: anova

Regressions of head capsule width to fresh body weight for both sexes are shown in Fig. A1, A2.

The ANCOVA of the cocoon weight to fresh body weight with co-variate sex shows a significant effect of cocoon weight ($F_{2,499}$= 5.871E04, *p*<0.001, Table 12) as females are bigger than males in this study and show a reduction in all measurements concerning the 'Spores' treatment. In contrast the males have an increase of cocoon weight and fresh body weight in the 'Spores' treatment.

Weight differences between cocoon and fresh body weight

There was a significant lower difference of cocoon and fresh weight in the 'Spores' group ($F_{1, 500}$= 3.934, *p*=0.04786, Fig. 11). Weight loss in females in the pupal stage in the 'Control'

group was higher than in males ($F_{1,499}$=4.58, p=0.033, Fig. 12). Within the 'Spores' group the males (9.95 ± 2.95 mg) revealed a significant lower mean weight difference than females (13.06 ± 4.31 mg) as shown in Fig. 12 ($F_{2,499}$=43,77, p<0.001).

Development time of the larvae

The development time was investigated for N = 87 *Osmia* bees (larvae). The ANOVA of the transformed linear model showed that the treatment has a significant effect ($F_{1,74}$ = 5.23, p= 0.025, Table 12) as well as the head capsule width on the pupae start ($F_{1,74}$ = 16.42, p< 0.001, Table 12, Fig. 13). This results in a later onset of pupation of the 'Spores' group (22.3 ± 1.3 days) than in the 'Control' group (21.8 ± 1.4 days) (Fig. 13, Fig. 14, Table A3).

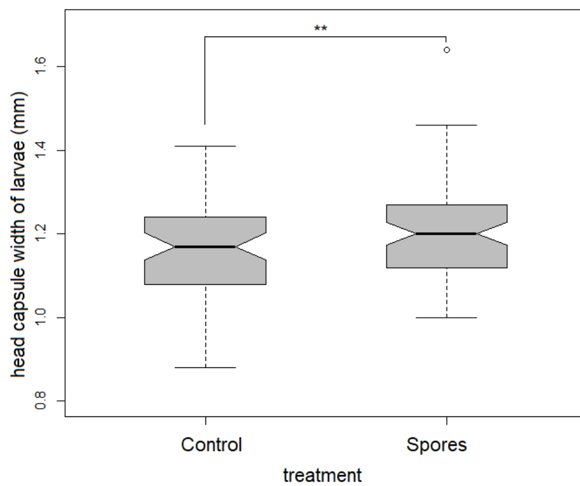

Figure 10: Significant differences in head capsule width in treatment groups within the larvae dataset.

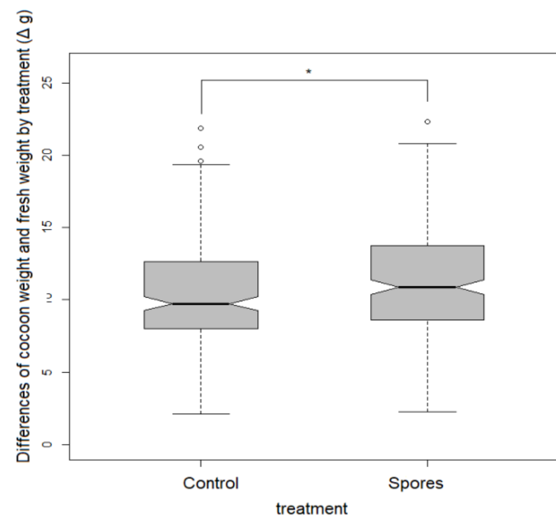

Figure 11: Significant weight differences of cocoon and fresh body weight between the treatments.

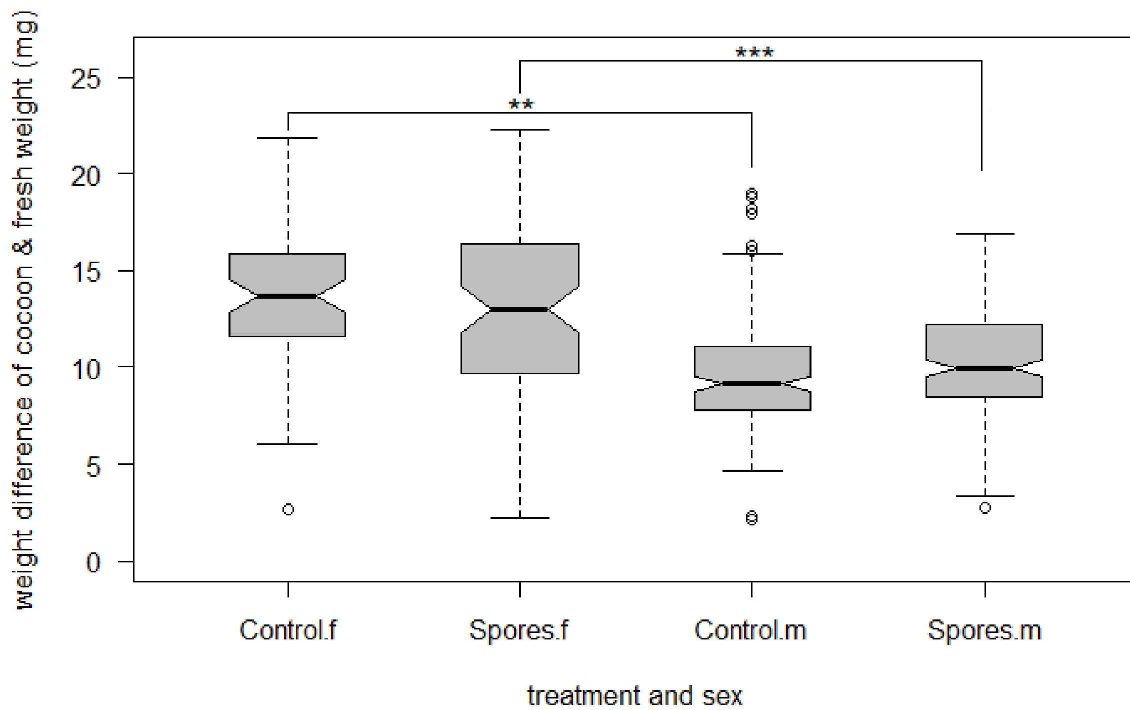

Figure 12: Significant weight differences between cocoon weight and fresh weight of *O. bicornis* by treatment and sex, Control. f: 60 females, Spores. f: 80 females, Control. m: 193 males, Spores. m: 173 males.

The group ‘Spores on larva’ had the smallest N noticeable with a minimum pupation start of 21 days and the latest pupae start within the group on the 22nd day. The interaction between treatment and the head capsule width significantly affected the start of the pupal stage ($F_{1,74}$ = 4.61, p=0.035, Table 2, Fig, 13).

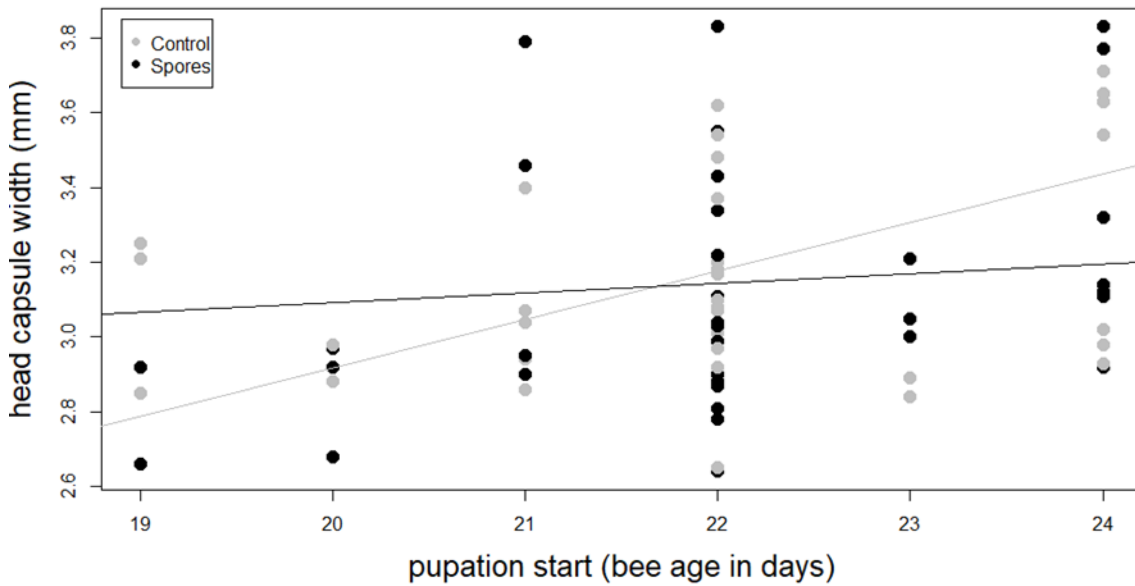

Figure 13: Linear regression between head capsule width and pupation start of main treatment groups 'Control' and 'Spores'.

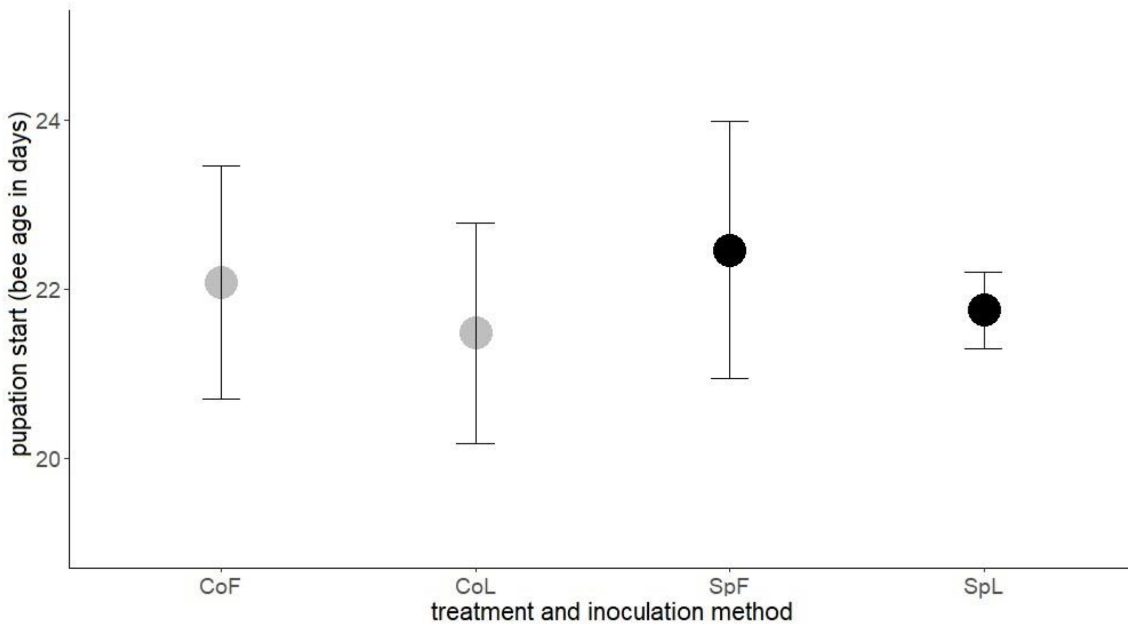

Figure 14: Distribution of mean pupation start time shown as 'bee age in days' as mean and standard deviation by each treatment. Letters represent treatment and inoculation type (Co= Control, Sp= Spores, L= applied on larva, F = applied on food).

Fitness correlates Imago II

The hatching rate was determined for the individuals that reached pupation. Among the controls hatching rate was 93%, among the treated 91.6%. The control group contained 26 females and 41 males, the treated group 26 females and 40 males.

Fat content weight was compared between treated and control males. As the data was not normally distributed (Shapiro-Test p=0.03255) a general linear model was performed which showed no significant difference between the treatments ($F_{79,81}$= 0.257, p=0.798). In the females, the data was not normally distributed either (Shapiro-Test p=0.03404). The glm revealed no significance difference in fat content weight ($F_{36,52}$=1.72, p=0.0941). For the tegulae size the data was normally distributed for the males (Shapiro-Test p=0.3696). A linear model indicated no significant difference between the groups ($F_{79,81}$=0.4379, p=0.5101). Equivalent results were found for the females (Shapiro-Test p=0.5313, lm: $F_{36,52}$=-1558, p=0.1281). The wing muscle weight analysis excluded 16 individuals due to measurement errors and showed no significant difference within the males (Shapiro-Test p=0.01899, glm: $F_{76,78}$=1360, p=0.178) as well as within the females (Shapiro-Test p=1.033e-04, glm $F_{37,39}$=0.994, p=0.32667, Table 13).

Table 13: Fitness parameter tests of hatched imagines (males/females) from spore-treated and control groups

Dependent variable	**Independent variable**	**Shapiro-Test (*p-value*)**	**Model**	**Residual deviance**	**Num. D.f.**	**F-value/ t-value**	***p-value***
fat content	males s/c	0.03255	glm	2753.3	79	0.257	0.798
fat content	females s/c	0.03404	glm	0.0025140	36	(-)1.72	0.0941
tegulae distance	males s/c	0.3696	lm		79	0.4379	0.5101
tegulae distance	females s/c	0.5313	lm		36	(-)1558	0.1281
wing muscle weight	males s/c	0.01899	glm	0.00036717	76	(-)1360	0.178
wing muscle weight	females s/c	1,03E-04	glm	0.00055552	37	0.994	0.32667

3.5 Discussion

In this study on a solitary bee exposed to a honey bee pathogen during development we found (1) significantly higher mortality in spore-exposed individuals as well as (2) delayed onset of pupation and a reduced body size in males. During development, (3) increased head capsule width in treated individuals of both sexes was observed. However, (4) no fitness effects were found in hatched individuals.

(1) Higher mortality in spore-treated individuals (larvae & prepupae)

The higher mortality in the spore-treated larvae could be explained by the high effort of synthesis of storage proteins during larval development. As demonstrated in honey bees, triggering a stronger larval immune response can result in lower spore load in adults but impose the cost of the reduced life-span (Eiri et al. 2015). This could elucidate the higher

mortality in the Imago I of the infected individuals. Moreover, in *A. mellifera N. ceranae* impairs its host by damaging intestinal tissue and preventing renewal by inhibiting responsible genes (Dussaubat et al. 2012), uses host-ATP for its own growth and reproduction (Hacker et al. 2014) and was shown also shown to inhibit immune responses (Antúnez et al. 2009, Huang et al. 2012, Aufauvre et al. 2014, Badaoui et al. 2017). The deprived hosts consequently lack functional tissue, energy necessary for its own metabolic maintenance and immune capacity to combat the infection which would be the next necessary step and could be a reason for increased mortality in the spore-treated cohort.

(2) Delayed pupation start in spore-treated bees

Immune activation and maintenance in infected hosts require high nutritional and energetic resources which can be expected to be lacking for growth and development processes (Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000). The observed delayed pupation start in the spore-cohort could be attributed to a trade-off between combat against the infection and investment in development.

(3) Weight effects during development

Individuals treated with spores showed a higher weight loss during the pupal stage which is likely to be due to costs of an immune response. Higher weight losses in females than in males are attributed to sexual dimorphism in body size. However, the higher weight losses in control compared to spore-treated female bees are due to the higher body size of controls. The increased cocoon and body weight in spore-treated males might be explained by lower male immune defense investment in arthropods (Kurtz et al. 2000, Kurtz and Sauer 2001, Rolff 2001, Siva-Jothy et al. 2001). Respective results were obtained for head capsule width. For males with lower contribution in parenting resources are allocated more towards immunity whereas females invest in maternal care (Vincent and Gwynne 2014). As *O. bicornis* displays a sexually biased investment in parental care our results are in concordance with the findings of Vincent & Gwynne (2014) who found higher investment in immune response in a positive relation to parental investment. Moreover, an infection with *N. ceranae* increases hunger level, thus food intake, and reduces survival in honey bees (Mayack and Naug 2009). This could also explain increase in weight and mortality in spore-treated individuals in our study. Immune system capacity was also shown to increase with age in honey bees. Higher pathogen susceptibility was observed in younger than in older honey bee queens (Chaimanee et al. 2014). The different immune response could be attributed to the upregulation of certain immune related genes with increasing age (Chaimanee et al. 2014)

Sex ratio

The shift towards higher male ratio in the imago dataset could be due nest competition of female Osmias. In this process they adopt other nests resulting in another order of sexes within one nest (Seidelmann et al. 2010). Furthermore, females could have tried to avoid risk of open-cell parasitism by reducing offspring body size favoring towards the smaller sex, males (Seidelmann 2006). Females are generally larger than males needing higher maternal provision thus longer foraging times for their brood cells.

The sex ratio with over 72 % of males could be one explanation for the result of the bigger head capsule in the 'Spores' group, which is shifted towards males with a bigger sample size and smaller variance. We found only very few individuals with spores of *N. ceranae* in the treated cohorts. The results contrast with infection experiments with *N. ceranae* on honey bee larvae (Eiri et al. 2015) and *Nosema bombi* in larvae of *Bombus* spp. (Schmid-Hempel and Loosli 1998). Differences to other studies might be attributed to initial spore doses, bee species, strains of *Nosema* the bee immune responses at different developmental stages or combinations of these factors (Fontbonne et al. 2013, Higes et al. 2013). We used the same dose of the same species as in one cohort in Eiri et al. (2015) where it resulted in spore elevations in larvae of *A. mellifera*. Our low spore detection would suggest a clearance of the infection. It was demonstrated in honey bees that some individuals exhibited resistance to the pathogen by countering its manipulation of apoptosis and defecating the infected cells (Kurze et al. 2016).

(4) No fitness effect in hatched imagines (Imago II)

We found no effect on fat content between spore-exposed and control individuals. Apart from energy storage, the fat body has an essential role in immunity in insects. Consequently, variations in fat body mass might impact effectiveness of immune response. The present study suggests that the infection with *Nosema* spores does not result in immunity compromises in hatched individuals. Body size, measured through tegulae distance, was not differing either. Body size is determined by conditions during larval development (Heinrich 1974). All bees were provided with adequate amount of pollen. However, due to exposure with the pathogen the bee might have encountered a stress which to combat requires more resources.

3.6 Conclusions

Spores of *N. ceranae* were only detected in low numbers in a few of the exposed individuals indicating no establishment of the pathogen. However, in the larval stage, higher mortality and slower development time in the exposed group as well as reduced body size in exposed males argue for fitness costs early in life history. Among the hatched imagines no fitness consequences could be found. Even in absence of evidences for successful infection, the pathogen can still compromise the development of *O. bicornis*.

3.7 Acknowledgments

We thank the Deutsche Bundesstiftung Umwelt (DBU) for funding the research. We would thank Erhard Strohm for his sharing his expertise on working with *O. bicornis*, Benedikt Polazec for providing honey bees for our experiments and the *Landschaftspflegeverein Nuthe-Nieplitz* for their support at the orchard. We thank several beekeepers for their cooperation in taking samples. We appreciate the help of Julia Lorenz and Nicolas Bramke at the field site.

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General discussion

Wild bees share pathogens with honey bees but the actual impacts of at the individual- and community-level are hardly known. The present thesis investigates indirect effects of the presence of the pathogen *N. ceranae* in honey bees on wild bee communities and the direct impacts of this pathogen on *O. bicornis* as a model species for solitary bees.

Chapter 1 revealed that environmental factors associated with pathogen load in honey bees particularly affect rare and more specialized wild bee species. However, the results could not be ascribed to causal responsibility of the pathogen as susceptibility and consequences of infection are unknown in solitary bees. In order to approach this knowledge gap, infection experiments were carried out to test fitness impacts of *N. ceranae* in a solitary bee model. Chapter 2 showed that *N. ceranae* can successfully infect adult *O. bicornis* with a marginal impact on survival in male individuals. Accordingly, *O. bicornis* could play a role as a pathogen reservoir and a potential vector in the pollinator network. Widespread pathogen presence in wild bees sharing a habitat with infected honey bees suggests an even wider host range, in which impacts still remain to be investigated. Chapter 3 demonstrated that even without proliferation pathogen exposure can still result in life history effects during development.

1. Species-specific effects

Previous inoculation experiments proved that honey bee derived pathogens directly affect social bumble bees (Graystock et al. 2013a, Graystock et al. 2013b, Fürst et al. 2014). However, it would be inappropriate to use these data to extrapolate effects on solitary species. This is because taxonomic relatedness does not necessarily allow generalization of stress responses as sensitivity to a pathogen is not the same for all host taxa as demonstrated for bumblebees (Arbetman et al. 2017). Likewise, immune defenses were shown to vary among species of Diptera (Bronkhorst and Van Rij 2014). As outlined for pesticides, impacts of infection therefore depend on species and developmental stage (Rundlöf et al. 2015, Nicholls et al. 2017). Wild bees are a highly diverse group with a variety of life histories (Westrich 1989). Concerning the outcomes of Chapter 2, it has to be kept in mind that the polylectic, cavity nesting and early emerging *O. bicornis* might differ in reactions to pathogens compared to oligolectic, ground-nesting or parasitic species. Differences in reactions and risks to pesticides have been explored for only a few wild bee species (Thompson 2001, Tasei 2002, Devillers et al. 2003, Hardstone and Scott 2010, Arena and Sgolastra 2014, Kunz et al. 2014, Rundlöf et al. 2015). Traits like fecundity (Mommaerts et al. 2010, Laycock et al. 2012, Whitehorn et al. 2012) and feeding rate (Cresswell et al. 2012) were afflicted in *Bombus terrestris*. We lack similar studies for

pathogen infection. With regard to the results from Chapter 1 knowledge about direct impacts in particularly sensitive rare and specialized species would be of high importance. Some solitary wild bee species can be reared in the lab (Becker and Keller 2016) which enables inoculation experiments that would allow us to build this understanding.

For pesticide impacts differences in responses between species can occur because each species is characterized by an intrinsic sensitivity and difference in biological features such as life history, physiology, nesting and foraging behavior (Arena and Sgolastra 2014). The same factors might underpin varied responses to a pathogen. For example, in social bees immunity related behavioural mechanisms, like removal of infected individuals, can exceed the ability of individual resistance by only physiological reactions and prevent the spread of diseases (Fefferman et al. 2007, Arena and Sgolastra 2014). On the other hand, genetic homogeneity, high densities, physical contact and trophyllaxis argue for numerous opportunities for pathogen transmission and establishment (Fries et al. 2001). In contrast, in solitary bees, the only contact between adult individuals occurs during mating and no physical contact between the female and the hatching larvae exists (Westrich 1989). Hence, horizontal transmission between adult solitary bees is therefore less likely to occur than in social bees, which suggests a minor transmission and a conceivably lower infection rate. Nevertheless, pathogen transmission via pollen and nectar was established in previous studies (Singh et al. 2010) and pollen can act as a reservoir for *N. ceranae* spores (Higes, Martín-Hernández, Botías, et al. 2008). As brood cells of solitary species are provided with nectar and pollen to nourish the larvae, (Westrich 1989) pathogen exposure during development is highly likely. Moreover, food-borne infections in adults via shared flowers (Durrer and Schmid-Hempel 1994) as well as sexual transmission during mating are possible.

Life history stage specific reactions

As demonstrated for pesticides, impacts of pathogens do not only depend on host species but also on their host’s developmental stage (Nicholls et al. 2017). The costly fitness impacts of infection during larval development in Chapter 3, correspond to a consensus of several studies indicating higher disease susceptibility in juvenile hosts (Duca 1948, Zuckerman and Yoeli 1954, Francis 1961, Sait et al. 1994, Baird 1998, Kurtis et al. 2001, Panter and Jones 2002, Kubi et al. 2006, Armitage and Boomsma 2010, Garbutt et al. 2014, Bruns et al. 2017). The observed effect might be due to increased energetic demands during growth and development traded off against mechanisms of resistance (Ashby and Bruns 2018). Consequently, juveniles might be even more vulnerable to synergistic effects of stressors for example when food provisions are insufficient, contain pesticide compounds or climate

extremes that occur during development. In honey bees and social bumblebees only a few infection experiments on early developmental stages have been conducted (Schmid-Hempel and Loosli 1998, Eiri et al. 2015, Folly et al. 2017). The results from Chapter 3 argue for inclusion of different life stages of wild bees in investigating pathogen impacts.

2. Spore doses

In contrast to field studies, experiments in a laboratory allow for exclusion of natural variation and ease manipulation of specific parameters (Becker and Keller 2016). Thus, they are a suitable method to precisely focus on the impact of a selected factor, in this case, pathogens. Attention has to be paid to ensure that data collected in a laboratory experiment are informative about the natural environment as has been discussed for pesticide studies already (Carreck and Ratnieks 2014). One important aspect is the applied spore dose. *N. ceranae* spore doses in previously conducted infection experiments have a wide range (Table A1, Appendix). The infective dose to result in 100% infected bee samples was found to be 10^4 spores per bee (Forsgren and Fries 2010) and was applied in the present study. However, the possible spore densities that pollinators encounter while visiting flowers in their natural environment is unknown. Interspecific vectoring with a cohort of infected donors and uninfected receiver bumble- and honey bees sharing flowers was proven in an experiment by Graystock et al. (2015). However, the amount of spores on flowers dispersed by pollinators was not tested. Furthermore, the limited dispersal space of pollinators in cages (4 x 6 x 1.5m) has to be considered as a potential bias. For scrutinizing pathogen transmission, information about the range of spore doses on flowers in the natural environment is crucial to mimic a realistic infection scenario in individuals in the lab.

3. Multiple stressors in the environment

The detected effects from the inoculation experiments of Chapter 2 and 3 have to be considered in the context of the hosts in their natural environment in which several sublethal effects can interact synergistically. This means that to really understand the effects of pathogens such as *N. ceranae* on honey bees the effects need to be considered in the context of the biotic and abiotic factors honey bees experience in their environment (Paris et al. 2018). Host-parasite interactions often involve multiple infective agents (Alizon et al. 2013). Consequently, not only relationships between single parasites and the host but also the interactions between parasites in mixed infections determine the impact of an infection (Brown 2013). Pathogen screens in solitary wild bees revealed the occurrence of *N. ceranae* alongside Neogregarinorida, Acosphaera, Spiroplasma and viruses (Ravoet et al. 2014). Multiple viruses were found in a variety of wild bees (McMahon et al. 2015) and both *N. ceranae* and the DWV complex were detected in bumblebees (Fürst et al. 2014). In order to

account for a variety of pathogens, individuals of the study from Chapter 3 were screened for the common honey bee viruses CBPV, DWV, SBV and SBPV. The low prevalence in both exposed and control groups indicated that observed effects can indeed be attributed to *N. ceranae*. Furthermore, the host's gut microbiota can affect the general immune response and the specific host-pathogen interaction (Koch and Schmid-Hempel 2012, Raymann and Moran 2018) and respective parameters should be incorporated in future fitness analysis.

Pathogens interact with abiotic stressors bees are exposed to in their environment (Vanbergen and Garratt 2013, Brown et al. 2016). Increased virulence has been observed under environmental stress in a range of host-pathogen systems (Koella and Offenberg 1999, Moret and Schmid-Hempel 2000, Jokela et al. 2005, Restif and Kaltz 2006) and this is also likely to be the case in wild bees and their infections. One important abiotic stressor faced by bees is habitat loss and homogenization leading to flower scarcity (Carvell et al. 2006, Naug 2009, Kennedy et al. 2013, Goulson et al. 2015). A lack of floral resources can cause starvation stress which in turn enhances pathogen impact (Brown et al. 2000, Manley et al. 2017). Low flower diversity also negatively impacts immunocompetence (Alaux et al. 2010) as rich floral resources deliver a range of antimicrobial compounds in pollen and nectar which can lower disease intensity (Richardson et al. 2015, Palmer-Young et al. 2016). Furthermore, energetic stress due to an infection can result in lower foraging activity and pollen carriage (Lach et al. 2015). This is particularly relevant for solitary wild bees as they provide their brood chambers with pollen as the food resource on which larvae depend after hatching (Westrich 1989). As a result adults might experience nutritional stress and larval food provision could be jeopardized. Moreover, desynchronization of bees and their foraging plants as a result of a changing climate causes nutritional stress and particularly affects early emerging species such as *O. cornuta* and *O. bicornis* (Schenk et al. 2018). Consequently, the benign mortality increase in pathogen exposed individuals detected in Chapter 2 as well as the developmental impacts in Chapter 3 could unpredictably exacerbate in a perturbed environment with starvation stress.

Furthermore, pesticides can increase sensitivity to pathogens (Di Prisco et al. 2013, Sánchez-Bayo et al. 2016). Synergistic effects between *Nosema* sp./ *N. ceranae* and imidacloprid (Alaux et al. 2010, Pettis et al. 2012), between *N. ceranae* and thiacloprid (Doublet et al. 2015) and *N. ceranae* and fiprinol (Vidau et al. 2011, Aufavre et al. 2012, Kairo et al. 2017) have been detected in *A. mellifera*. However, effects on honey bees cannot be generalized for wild bees as was demonstrated for effects of neonicotinoids (Rundlöf et al. 2015). Differences in risk profiles to pesticides for three bee species have been detected and suggested to be due to biological features such as foraging behaviour

(Kunz et al. 2014). Accordingly, results from polylectic honey bees might not apply to oligolectic wild bees as might hold true for pathogens as well.

Beyond that, global environmental change can affect emerging infectious diseases (Daszak et al. 2000, Woolhouse et al. 2005). Examples concerning pollinators are modified movement patterns due to shifts in resource distribution and newly enabled contacts between previously isolated populations (Bartel and Altizer 2012). The widespread habitat fragmentation and increasing urbanization result in increased pollinator densities in smaller patches and potentially enhances pathogen transmission (Patz et al. 2004, Bartel and Altizer 2012). Moreover, smaller and isolated habitats compel pollinators to conduct longer, costly flights which could lead to trade-offs between investment in mobility and immune functions (Ardia et al. 2012). Pollinator dependency in agriculture has increased (Aizen et al. 2008) and is mostly covered by the intense use of the managed honey bee resulting in a rapidly growing number of colonies (Geldmann and González-Varo 2018). However, anthropogenic habitat change and the intense use of managed pollinators can exacerbate stress on wild bees. Outcompeting effects of honey bees on bumblebees in terms of floral resource use were shown to occur only in homogeneous landscapes typical for intensive agriculture but not in heterogeneous habitats (Herbertsson et al. 2016). Lower reproduction and foraging rates were detected for *O. bicornis* in the presence of honey bees when food resources were limited and shared (Hudewenz and Klein 2015). Moreover, managed pollinator trade contributes to global spread of diseases into new geographical areas and host species (Potts et al. 2016, Wilfert et al. 2016). As a few wild bee species were shown to share pathogens with honey bees already (Table 1 Introduction, results Chapter 2) a wider distribution for many diseases can be expected to have occurred already or might very likely in the future.

Biodiversity effects: Importance of functional complementarity

A functional diverse bee community is crucial for complimentary and stable pollination services (Hoehn et al. 2008, Albrecht et al. 2012, Mori et al. 2013). Several previous studies corroborate the significance of functional diversity as the most relevant factor of overall biodiversity (Tilman et al. 1997, Hulot et al. 2000, Lavorel and Garnier 2002). A higher response diversity (Moretti et al. 2009, Fründ et al. 2013, Neame et al. 2013, Sheffield et al. 2013) consolidates the insurance hypotheses about ecosystem protection against functional declines through biodiversity (Yachi and Loreau 1999). Chances are increased that a species with a particularly effective service delivery under the given environmental circumstances is present (Tilman et al. 1997). Additionally, long-term stable habitat functioning (Tilman et al. 2006, Ruijven and Berendse 2007, Winfree and Kremen 2009) and

higher buffer capacity towards climate change (Isbell et al. 2015) and land use (Garibaldi et al. 2018) are positively associated with higher diversity. Higher buffering capacity promotes faster recovery after disturbances (van Ruijven and Berendse 2010). Therefore, the negative correlation between functional diversity of wild bees and pathogen presence in honey bees detected in Chapter 1 is alerting and indicates infections in honey bees as potential hazard for other wild bee species.

Certain functional groups such as solitary oligolectic bees with shorter flight periods that rely on a smaller range of flowers are particularly vulnerable to floral shortages (Ogilvie and Forrest 2017). Furthermore, they are more vulnerable to consequences of temporal mismatch with floral resources due to climate change (Ogilvie and Forrest 2017) than long-season and/or polylectic species. Additionally, they are particularly affected by foraging competition from honey bees, due to their being less able to shift their feeding plants (Wasser and Ollerton 2006). Moreover, foraging distances of most small solitary species are relatively short and result in a dependency on foraging and nesting habitats within a close neighborhood (Zurbuchen et al. 2010). The wild bees positively detected with *N. ceranae* in Chapter 2 are comprised of oligolectic species such as *Chelostoma campanularum* or *Melitta nigricans*, as well as very small species like *Andrena subopaca* or *Hyaleus communis* that are characterized by particularly short foraging distances which make them highly dependent on resources within a small radius. Polylectic species like bumble bees on the other hand have a greater capacity to use a wider range of floral resources and lower competitive pressure (Walther-Hellwig et al. 2006). In concordance, results from Chapter 1 suggested higher sensitivity of rarer and more specialized bees to environmental factors associated with a higher disease burden in honey bees. Taken together, evidence is mounting that some functional groups are under particular pressure.

Furthermore, intact functionally diverse pollinator communities are of high importance as pollination effectiveness for certain crops differs between species (Rader et al. 2013, Rader et al. 2016). Specific pollinator groups might thus determine crop yield more than the whole pollinator community (Fijen et al. 2018). For example *O. bicornis* showed better performances in oilseed rape pollination than hoverflies (Jauker et al. 2012). Bumblebees are known for their highly effective strategy of buzz-pollination (Goulson 2003, de Luca et al. 2013) making them an ideal pollinator for crops like tomatoes and potatoes (Goulson 2003). *Bombus* queens and *Andrena* spp. proved to be have a higher pollination effectiveness than *A. mellifera* (Javorek et al. 2002).

Community level impacts: Pathogen reservoirs

Host-pathogen interactions have to be viewed not only from the individual level but also from the pollinator community perspective. As adult *O. bicornis* were proven to be susceptible, the species can act as a reservoir and thus as a potential vector for *N. ceranae* within the pollinator network. The detection of *N. ceranae* in multiple species of wild bees encountering infected honey bees presented in Chapter 2 corroborates other studies that evidence the widespread occurrence of assumed honey bee-associated pathogens within the pollinator community (Table 1 Introduction). Niche overlap and shared floral resources (Durrer and Schmid-Hempel 1994, Mazzei et al. 2014, Bailes et al. 2018), as well as an association with *A. mellifera* hives (Levitt et al. 2013) or nectar robbing (Porrini et al. 2017) can facilitate transmission to new hosts. Pathogen sharing was not only detected within the *Apis*-family but also between other insect taxa like hoverflies (Bailes et al. 2018), wasps (Singh et al. 2010, Lester et al. 2015), ants (Sébastien et al. 2015) the class *Aranaea* and seven orders of *Insecta* (Levitt et al. 2013). Together with the present results, this suggests that the previously defined honey bee-specifity of a range of pathogens is unrealistic and the distribution of previously considered honey bee-pathogens in other taxa is suggested to be heavily underestimated so far (Gisder and Genersch 2017).

Additionally, apart from honey bee-associated agents the pathogen landscape of non-pest insects is mostly unstudied (Bartel and Altizer 2012), which was clearly shown for wild bees as well (Table 1 Introduction). However, the direction of transmission as well as pathogen impacts in its different hosts still remain elusive (Tehel et al. 2016, Gisder and Genersch 2017). Impacts of insect pathogens can vary between highly detrimental (Roy et al. 2006) and asymptomatic symptoms (Lange and Lord 2012). Furthermore, even if replication of a virus as a proxy for an active infection is tested it does not elucidate if a single infection with no further transmission or effective host-to-host circulation within the population takes place (Levitt et al. 2013). Greater numbers of pathogen reservoirs clearly increase chances for pathogen circulation with increased contact on shared resources or between individuals in social species. Given the high diversity among potential hosts, transmission potential might be clearly affected by special life history traits of different insect pollinator species such as long-distance migration of hoverflies (Francuski and Milankov 2015, Bailes et al. 2018) cleptoparasitism among wild bees (Sheffield et al. 2013) or disease seasonality in overwintering honey bees (Runckel et al. 2011) with unknown consequences.

An enhanced pathogen presence and circulation threatens both managed and wild pollinators. For all managed species potential mass rearing in production might provide new centers of infection and outbreaks of epidemics not occurring in the natural environment as

pathogen prevalence is greater in managed than in feral individuals (Colla et al. 2006, Eilenberg et al. 2015). As *O. bicornis* presents one candidate species already reared for commercialization every precaution has to be taken not to facilitate establishment and spread of pathogens to which the species is susceptible, such as honey bee associated viruses and *N. ceranae*.

Management implications for sustainable agriculture and conservation

The results from Chapter 1 argue for a focus on associations between stressors, functional diversity of the pollinator community and pollination success. In applied conservation, such analysis can provide valuable information for species-specific support measures. Moreover, meticulous control of the health of managed bees sharing wild bee habitats has to be implemented (Fürst et al. 2014, Goulson et al. 2015) for example with surveillance programs that quantify diseases and causative risk factors (Lee et al. 2015). However as pathogens are not the exclusive causative agent for bee declines (Brown et al. 2016) more starting points for beneficial measures exist which ideally should be pursued simultaneously. Ensuring satisfactory nutrition through sufficient flower resources can improve bee resilience towards other stressors (Dolezal and Toth 2018). Promoting floral resources and nesting sites is suitable on the farm and field scale (Carvell et al. 2011). Enhancement and conservation of semi-natural habitats supports pollinators at the landscape scale (Garibaldi et al. 2011). Suitable nesting resources, the prevention of pesticide exposure and careful monitoring of invasive bee and bee pathogen species and climate change consequences are further steps that have to be incorporated in effective management schemes.

In the agricultural context, optimization of pollination is the crucial aim. Higher individual pollination effectiveness by a diverse wild bee community in contrast to relying on honey bees alone was demonstrated by (Garibaldi et al. 2013). However, synergistic effects between honey bees and wild insects were detected in other studies (Greenleaf and Kremen 2006, Carvalheiro et al. 2011, Brittain et al. 2013). Even though beekeeping is increasing on a global scale, the expanding agricultural crops demanding pollination are not supplied sufficiently (Aizen and Harder 2009). Consequently, the co-occurrence of both managed and wild pollinators is required to ensure sufficient crop yields in the light of a growing world population (Dicks et al. 2016, IPBES 2016, Garibaldi et al. 2018). However, it is necessary to carefully decide where, when and to what extent honey bees are indispensable and how management can be performed without threatening wild bees (Geldmann and González-Varo 2018). For example, placement of honey bee hives in protected areas should be prevented (Geldmann and González-Varo 2018). Protected areas are important refuges for

specialized and often threatened species which are potentially more sensitive to stress associated with disease prevalence in honey bees as revealed in Chapter I.

Conclusion

My results show that pathogen exposure in the natural environment leads to distinctive associations with functional groups and argues for their differential consideration when analyzing stress impacts for the highly diverse wild bees. Furthermore, exposure can result in pathogen establishment and/or sublethal effects depending on the life history stage of a solitary model species. Therefore we suggest that wild bees can be involved in disease ecological networks within the pollinator community as both pathogen reservoirs and vectors. Moreover, fitness effects caused by pathogens could probably exacerbate impacts of other stressors affecting bees and have to be incorporated in risk assessments.

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Declaration of authorship

I hereby declare that the thesis submitted is my own unaided work. All direct or indirect sources used are given as references. All contributions of co-authors are acknowledged.

Berlin, January 9 th 2019 Uta Müller

Appendix

Supplementary Material General Introduction

Table A1: Overview of spore doses administered in infection experiments

Infection experiments *A.mellifera* with *N. ceranae*		
Publication	**Year**	**Spores administered per bee**
Fries et al.	2010	1.000
		10.000
Higes et al.	2009	6.250
Graystock et al.	2013	6.500
Jack et al.	2016	10.000
Forsgen & Fries	2010	10.000
Eiri et al.	2015	10.000
		40.000
Antunez et al.	2009	32.000
Natsopoulou et al.	2016	100.000
Martin-Hernandez et al.	2009	100.000
Paxton et al.	2007	100.000
Higes et al.	2007	125.000
Vidau et al.	2014	125.000
Nanetti et al.	2015	150.000
Bombus spp. with *N. ceranae*		
Graystock et al.	2014	6.500
Fürst et al.	2014	100.000

Supplementary Material Chapter 3

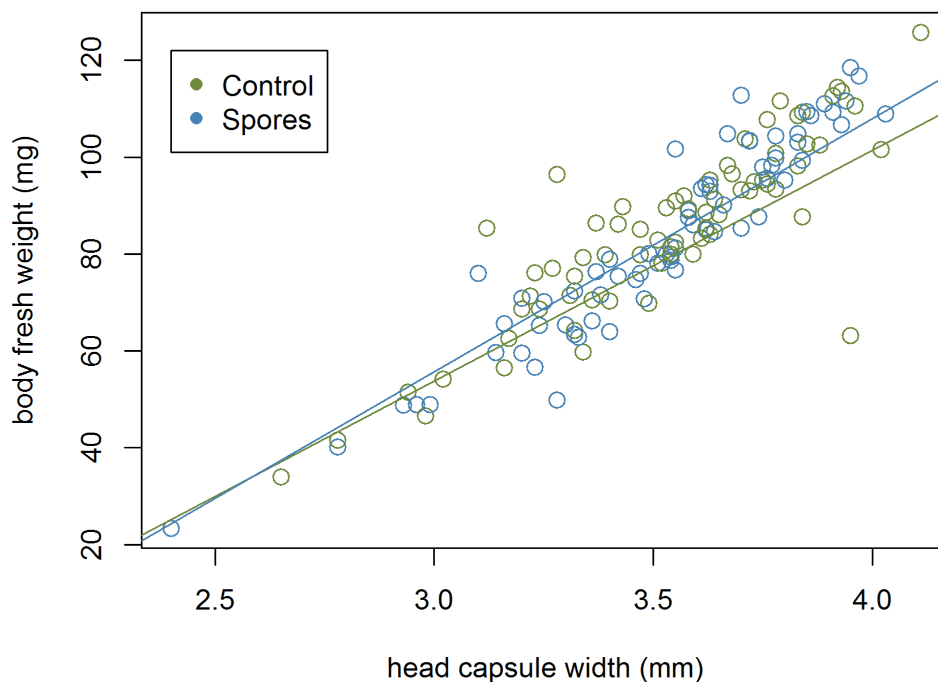

Figure A1: Female head capsule width and fresh body weight (imago dataset), N = 141

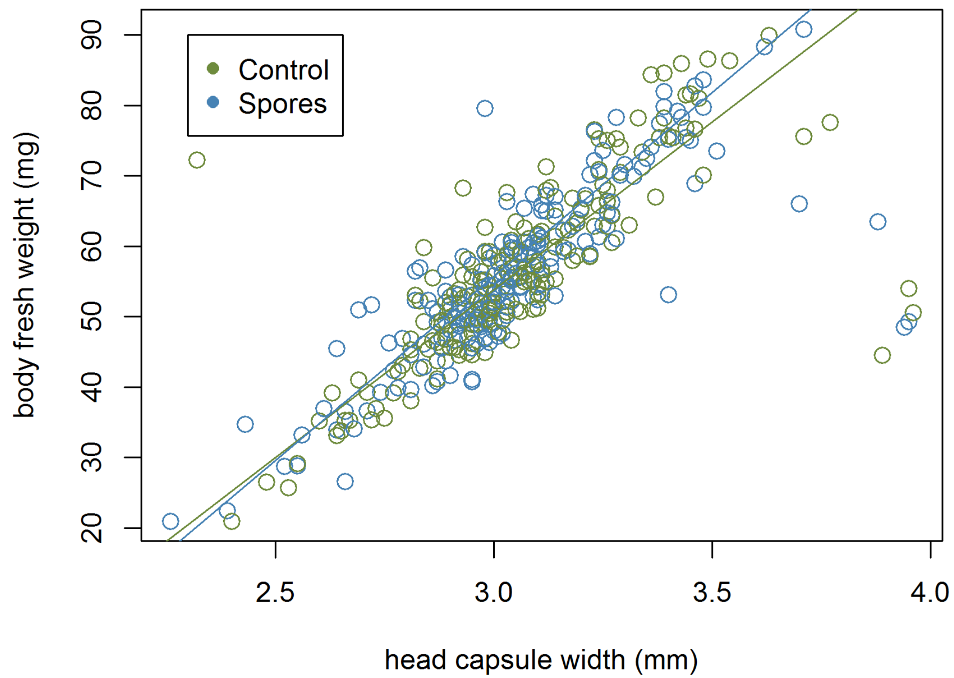

Figure A2: Male head capsule width and fresh body weight (imago dataset), N = 365.

Table A2: Overview for processed larvae and imago during sampling.

		Count of processed part of the larva for spore check	
Sum of larvae	**Treatment**	**whole larvae**	**separated larvae (body and gut)**
61	Control on food	15	18
	Control on larva	20	8
77	Spores on food	35	2
	Spores on larva	21	19
Sum of imagines	**Treatment**	**separated Imago (gut separated for spore check, spore check with n=407)**	
255	Control on food	127	
	Control on larva	128	
257	Spores on food	129	
	Spores on larva	128	

Table A3: Overview of measurements dependent on sex within the imago dataset.

sex	kind of measurement	N	mean	sd	min	max
females	head capsule width 'Co'	59	3.54	0.28	2.40	4.03
	head capsule width 'Sp'	80	3.51	0.32	2.65	4.11
	cocoon weight 'Co'	59	97.59	21.91	25.98	131.12
	cocoon weight 'Sp'	80	97.13	24.18	37.83	148.10
	fresh body weight 'Co'	59	84.23	18.38	23.29	112.81
	fresh body weight 'Sp'	80	84.22	20.08	34.01	125.80
males	head capsule width 'Co'	193	3.06	0.27	2.26	3.96
	head capsule width 'Sp'	170	3.05	0.24	2.32	3.89
	cocoon weight 'Co'	193	64.78	15.10	23.03	108.75
	cocoon weight 'Sp'	170	67.83	15.70	25.89	102.14
	fresh body weight 'Co'	193	55.18	12.32	20.89	90.83
	fresh body weight 'Sp'	170	57.53	12.78	22.43	85.92

Table A4: dispersion of dataset history for the set of pupation start.

	treatment	N	mean	sd	min	max
	Complete dataset	87	22.0	1.4	19	24
Control	Control on food	24	22.1	1.4	19	24
	Control on larva	23	21.5	1.3	19	24
	Control sum	47	21.8	1.4	19	24
Spores	Spores on food	28	22.5	1.5	19	24
	Spores on larva	12	21.8	0.5	21	22
	Spores sum	40	22.3	1.3	19	24