

Chapter 7

Nosema apis and *N. ceranae* Infection in Honey bees: A Model for Host-Pathogen Interactions in Insects



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Abstract There has been increased focus on the role of microbial attack as a potential cause of recent declines in the health of the western honey bee, *Apis mellifera*. The *Nosema* species, *N. apis* and *N. ceranae*, are microsporidian parasites that are pathogenic to honey bees, and infection by these species has been implicated as a key factor in honey bee losses. Honey bees infected with both *Nosema* spp. display significant changes in their biology at the cellular, tissue, and organismal levels impacting host metabolism, immune function, physiology, and behavior. Infected individuals lead to colony dysfunction and can contribute to colony disease in some circumstances. The means through which parasite growth and tissue pathology in the midgut lead to the dramatic physiological and behavioral changes at the organismal level are only partially understood. In addition, we possess only a limited appreciation of the elements of the host environment that impact pathogen growth and development. Critical for answering these questions is a mechanistic understanding of the host and pathogen machinery responsible for host-pathogen interactions. A number of approaches are already being used to elucidate these mechanisms, and promising new tools may allow for gain- and loss-of-function experiments to accelerate future progress.

Keywords *Nosema* · Microsporidia · Honey bee · Infection · Host-pathogen interactions

7.1 Introduction

The western honey bee, *Apis mellifera*, is crucial to key agricultural and ecological systems, and the pollination of many important crops is dependent on this species (Potts et al. 2016). If this pollination crisis remains unsolved, it will contribute to serious nutritional deficiencies for humans (Smith et al. 2015) and significant

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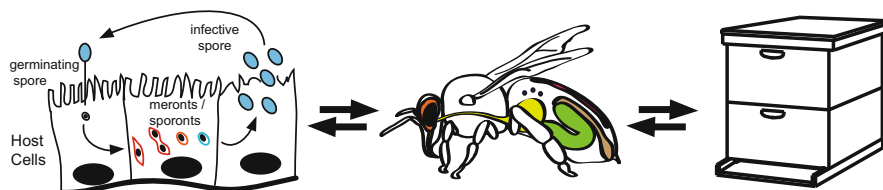


Fig. 7.1 Honey bees infected with both *Nosema* spp. display significant changes in their biology at the cellular, tissue, organismal, and colony levels

economic losses (Gallai et al. 2009) worldwide. Infection by pathogenic microbes represents one critical factor influencing honey bee declines in recent years (Goulson et al. 2015; Steinhauer et al. 2018). The microsporidian species *Nosema apis* and *N. ceranae* can cause individual pathology and mortality in honey bees that has been well documented (Fries 2014; Goblirsch 2017; Martín-Hernández et al. 2018). Such infections can also contribute to colony-level disease and collapse in conjunction with other elements, which appear to differ by region. Such factors include genetic variation on the part of both parasite and host and the presence of other stressors (e.g., climate variation or pesticide exposure) (Fries 2014; Goblirsch 2017; Martín-Hernández et al. 2018).

Honey bees infected with either *Nosema* spp. display significant changes in their biology at the cellular, tissue, and organismal levels with impacts on host metabolism, immune function, physiology, and behavior. Infected individuals lead to colony dysfunction and can contribute to colony disease in some circumstances (Fig. 7.1). Starting with the digestive tract, this review will describe some of the cellular, tissue, and organismal changes associated with infection by *Nosema* spp., with special focus on *N. ceranae*, which originated in the eastern honey bee, *Apis cerana*, and seems to have displaced *N. apis* in the western honey bee in many regions (Klee et al. 2007; Emsen et al. 2016; Gisder et al. 2017). The impacts of individual bee disease and mortality on the colony and on honey bee health more broadly have been comprehensively reviewed recently and will not be covered here (Fries 2014; Goblirsch 2017; Martín-Hernández et al. 2018).

Remarkable progress has been made in our understanding of *Nosema* disease in the years since the first report of *N. ceranae* in western honey bees. Yet many key questions remain unanswered. While there is still some debate, both *Nosema* spp. appear to display very narrow tissue tropism, being restricted to the digestive tract (Huang and Solter 2013; Higes et al. 2020). Thus, proximal changes to midgut function and the presence of a rapidly developing infectious agent must necessarily be responsible for dysfunction at the organismal level. However, the relative contribution of the host and parasite cells to these larger order changes and the molecular mechanisms through which they are achieved are incompletely understood. Conversely, the factors that influence the growth and development of cells of both *Nosema* spp. within the host environment remain unclear. Finally, our understanding of the positive and negative consequences of the observed organismal changes on both host and parasite is limited. Understanding the host and pathogen machinery

responsible for these negative changes is critical for answering these questions. A number of approaches are already being used to better understand these mechanisms, and promising new tools may allow for the gain- and loss-of-function and biochemical experiments necessary to accelerate future progress.

7.2 Changes in Digestive Tract Structure and Function After *Nosema* Infection

In worker honey bees, both *N. apis* and *N. ceranae* are restricted to infecting cells of the midgut. Typical of microsporidia, environmental spores first inject sporoplasms into host cells where they develop into a meronts that begin to rapidly proliferate. Meronts then mature into sporoblasts, which produce large numbers of primary spores and ultimately new infective environmental spores. These spores are released from the infected cell to begin the cycle anew (Solter et al. 2012). Digestive tract cells infected with *Nosema* spp. show evidence of important restructuring of sub-cellular structures, including aggregated ribosomes (Liu 1984) and association of microsporidia with the mitochondria in both *N. ceranae* (Higes et al. 2007) and *N. apis* (Graaf et al. 1994) infection. These cells also display extensive lysis, tissue disorganization, and cell sloughing into the lumen of the midgut (Liu 1984; Higes et al. 2007; García-Palencia et al. 2010; Dussaubat et al. 2012). In adults of insect species studied to date, epithelial cells are sloughed off into the lumen at some rate before being replaced by stem and progenitor cell proliferation and differentiation (Apidianakis and Rahme 2011). In the fruit fly, homeostatic self-renewal of the digestive tract has been shown to be critical for maintaining organ function after insult from damage or infection (Jiang et al. 2016; Guo et al. 2016b).

As an intracellular parasite with a long and complex lifecycle, *Nosema*-mediated regulation of cell turnover is likely paramount for maximum production of mature spores prior to enterocyte shedding and removal. Such regulation could occur through control of a variety of cellular processes including apoptosis and proliferation. In 2013, it was reported that midgut cells of *N. ceranae*-infected honey bees have reduced rates of apoptosis. The authors hypothesized that this reduced cell death was due to active manipulation of host cell apoptosis to allow for maximal pathogen reproduction (Higes et al. 2013). Further studies have confirmed this finding and also revealed that apoptosis suppression was most pronounced in the posterior regions of the midgut (Kurze et al. 2018). Studies have indicated that *N. apis* infection is initially restricted to the posterior section of the midgut (Fries 1988; Graaf et al. 1994), although this has not been observed in *N. ceranae* infection (Snow 2016). The mechanisms through which *N. ceranae* suppresses apoptosis are incompletely understood. One study implicated augmented expression of the *inhibitor of apoptosis protein-2* (*Iap2*) gene in cell survival (Kurze et al. 2015). Interestingly, both the suppressed apoptosis and the increased *Iap2* gene expression were lost in a strain of bees selected for *Nosema* tolerance. Another study found that a

number of other survival factors are aberrantly upregulated in infected midguts, including the Bcl2-like *Buffy* gene and the Iap family member *BIRC5* gene (Martín-Hernández et al. 2017). Alterations in the expression of apoptotic machinery have also been observed in infected midgut tissue using transcriptomic (Dussaubat et al. 2012; Holt et al. 2013; Doublet et al. 2017) and proteomic (Kurze et al. 2016a) approaches. Other microsporidia species infecting vertebrates and invertebrates are known to inhibit host cell apoptosis (Scanlon et al. 1999; Aguila et al. 2006). For example, *Nosema bombycis* reduces apoptosis in silkworm cells exposed to actinomycin D, an RNA polymerase inhibitor commonly used to induce apoptosis. Here, upregulation of *Buffy* was also implicated (He et al. 2015).

N. ceranae infection also impacts midgut proliferation. Panek et al. found that proliferation, as assessed by BrdU+ “crypts,” was reduced in *N. ceranae*-infected bees (Panek et al. 2018). This decrease is correlated with altered transcription of components of a number of pathways known to regulate midgut regeneration in the fruit fly (Jiang et al. 2016; Guo et al. 2016b), such as *Hippo* (Panek et al. 2018), and *Wnt* (Dussaubat et al. 2012), as well as cell cycle genes themselves (Martín-Hernández et al. 2017). Proliferation of midgut cells in the honey bee has been shown to be influenced by age, social function, and diet (Ward et al. 2008; Willard et al. 2011). However, a more complete understanding of the pathways responsible for controlling proliferation in the honey bee midgut is vital to understand how these pathways are altered by *Nosema* infection. For example, due to their sequence divergence, the honey bee JAK/STAT pathway ligands that regulate tissue regeneration in the fruit fly midgut have been elusive and were only recently discovered as being induced by thermal stress in the honey bee midgut (Bach et al. 2021). As new players in honey bee midgut biology are characterized, their role in *Nosema* infection can be explored.

A number of other interesting alterations in midgut biology have been observed after *N. ceranae* infection. Relating to cell proliferation, Panek et al. observed that *N. ceranae* appeared to be excluded from the proliferative stem cell population (Panek et al. 2018) although a previous study did not observe similar findings (Higes et al. 2007). Such exclusion likely has a significant impact on the ability of the digestive tract to retain some functionality during the long course of a typical microsporidia infection by preventing exhaustion of regeneration potential. The peritrophic membrane (PM), a key protective feature of insect digestive tracts (Hegedus et al. 2009), also appears disorganized after *N. ceranae* infection (Dussaubat et al. 2012), though full characterization of this phenomenon is lacking. Disruptions in PM function may be important for spore production and shedding but may also modify the nature of the interactions between the midgut cells and the cells of the microbiome or even other pathogens. Several studies have also suggested that *N. ceranae* infection impairs the integrity of the epithelial layer in the midgut (Higes et al. 2007; Dussaubat et al. 2012). While formal demonstration of increased intestinal barrier permeability has not yet been provided, it would be expected to enable diverse entities from the digestive tract to enter the hemocoel and future studies should clarify its role in *Nosema* disease.

Transcriptomic and proteomic data also suggests dramatic alterations in the expression of genes involved in all major functions of the digestive tract in insects, including nutrient acquisition and processing, host defense, microbiome maintenance, chemical detoxification, and barrier function (Antúnez et al. 2009; Dussaubat et al. 2012; Chaimanee et al. 2012; Holt et al. 2013; Aufauvre et al. 2014; Doublet et al. 2017). Additionally, expressions of a number of host miRNA are altered by infection (Huang et al. 2015), although the targeted mRNA are largely unknown. Our current understanding of how digestive tract function is altered will be discussed in more details in the sections below.

Infection by Nosema spp. Impacts Nutrient Acquisition and Processing Infection by *Nosema* spp. causes proximal changes to midgut nutrient acquisition and processing that impact organismal metabolism. The digestive function of the midgut epithelium is compromised at early time points after infection by *N. apis*, as both trypsin and chymotrypsin levels are decreased (Malone and Gatehouse 1998). Changes to metabolism in the gut have been inferred from gene expression studies that show changes in the genes involved in transport and processing of carbohydrate molecules. In particular, genes encoding trehalose transporters and alpha-glucosidase have been found to be upregulated during infection (Dussaubat et al. 2012), while trehalase has been shown to be downregulated (Aufauvre et al. 2014). Alpha-glucosidase protein levels have also been shown to be impacted (Vidau et al. 2014). It is not currently clear which of the above changes are specific to microsporidia infection versus generic consequences that would be found with diverse stressors. For example, trehalose transporter gene expression (Bach et al. 2021) and alpha-glucosidase protein levels (Huang et al. 2013) have both been shown to be altered by stressors not related to infection state, suggesting that these changes might not be a specific to infection. Regardless, these changes are particularly interesting because trehalose is currently thought to be the most plausible source of glucose for microsporidia (Timofeev et al. 2020). As one of the so-called “Terresporidia,” neither *N. apis* nor *N. ceranae* appear to possess the alternative oxidase of more basal microsporidia (Timofeev et al. 2020). Thus, it is unclear how regeneration of reduction equivalents such as NAD⁺ is achieved. It is possible that novel transport proteins able to move NAD⁺ (Dean et al. 2018) could provide one solution and the species of the *Nosema* genus indeed possess a rich transportome (Chetia et al. 2017). A recent study also found that the levels of eight proteins of the oxidative phosphorylation pathway were altered by *N. ceranae* infection (Houdelet et al. 2020), which is particularly striking in the context of the association between microsporidia and mitochondria previously discussed. RNAi studies indicate that *N. ceranae* is in fact dependent on its ATP/ADP transporters (Paldi et al. 2010), likely for commandeering host ATP as has been shown for other microsporidia (Jarkass and Reinke 2020). *N. bombycis* also possesses a putative ATP-binding cassette transporter, which is critical for parasite cell growth (He et al. 2019). Multiple metabolic enzymes of microsporidia are secreted into host cells (Senderskiy et al. 2014), presumably to modify host metabolism (Timofeev et al. 2020). Hexokinase, which catalyzes the first step in glycolysis, was first found

to be secreted by *Nematocida* spp. (Cuomo et al. 2012) and then by diverse microsporidia species (Timofeev et al. 2020). The enzymatic activity of hexokinase has been confirmed for both *N. ceranae* and *N. bombycis* (Dolgikh et al. 2019). RNAi studies demonstrated a critical role for this enzyme in *N. bombycis* growth (Huang et al. 2018b).

Infection leads to dramatic changes in metabolism in a number of tissues that are distal from the site of infection. Reduced trehalose levels are observed in the hemolymph of honey bee foragers, although glucose levels stay the same (Mayack and Naug 2010). Other metabolites including amino acids, lipid biosynthesis components, and the polyamine compound spermidine are also altered in the hemolymph of *N. ceranae*-infected bees (Aliferis et al. 2012; Jousse et al. 2020). Whole bee lipid loss is observed (Li et al. 2018), which likely represents changes in fat body lipid storage and provides one indicator of reduced energy reserves. Bees infected with *N. ceranae* are energetically stressed and have higher hunger levels (Alaux et al. 2009; Naug and Gibbs 2009; Mayack and Naug 2009; Martín-Hernández et al. 2011), which have important impacts on their physiology and behavior and overall colony health as discussed below. *N. apis* infection also impacts bee energetics, but the effect is less pronounced compared to *N. ceranae* infection (Martín-Hernández et al. 2011). Interestingly, decreased survival of bees infected with *N. ceranae* can be ameliorated by ad libitum feeding, suggesting that energetic stress may be a critical driver of mortality in infected bees (Mayack and Naug 2009). Energetic stress, as measured by hemolymph trehalose levels, is not observed in the *Nosema*-tolerant strain after *N. ceranae* infection (Kurze et al. 2016b), perhaps suggesting that mechanisms increasing tolerance to infection are as critical as those for resistance to infection (Kurze et al. 2016c)

Immune Responses Are Altered by Nosema Disease The immune response to microsporidiosis in invertebrates is currently thought to be composed of multiple arms. First, microsporidia infection leads to transcriptional induction of immune recognition and effector proteins, including antimicrobial peptides (AMPs). This arm has been most extensively studied in honey bees. At early time points, experimental (Schwarz and Evans 2013; Huang et al. 2016b) and natural (Li et al. 2017b) infection by *N. ceranae* induces expression of genes encoding AMPs and pattern recognition receptors (PRRs) and alters expression of components of the Imd and Toll pathways, both known to respond to pathogen-associated molecular pattern (PAMP) and damage-associated molecular pattern (DAMP) stimuli. After this initial phase, the expression of immune effector (e.g., AMPs) genes and other immune-related transcripts was reduced below the levels of infected controls. There appears to be significant study-related variability in immune gene expression (Antúnez et al. 2009; Dussaubat et al. 2012; Chaimanee et al. 2012; Holt et al. 2013; Aufauvre et al. 2014; Doublet et al. 2017), which may be due to differences in experimental procedures (dose, tissue sampled, or time of sampling) or strain variation on the part of either the host or the parasite. *N. bombycis* infection induces robust transcriptional activation of immune genes in *Bombyx mori*, including genes encoding AMPs and serine proteases and serpins (Ma et al. 2013), suggesting that

microsporidia infection induces some similar responses in different insect species (Pan et al. 2018). Thus far, there are only a few pieces of evidence that these responses have a protective effect on the host. *Naked cuticle (nkd)* is a Wnt pathway antagonist that is induced by *N. ceranae* infection and is thought to regulate immune responses. Knockdown of this gene results in reduced parasite load and induction of immune genes, such as the AMPs *abecin*, *apidecin*, and *defensin-1* and the Pattern Recognition Receptors (PRR) *PGRP-S2* (Li et al. 2016). Exposure to Pathogen-associated molecular patterns (PAMPs) increased survivorship in *N. ceranae*-infected bees, potentially by inducing a more robust immune response, although no measurement of such responses was made (Valizadeh et al. 2020). Drones from the *Nosema*-tolerant strain above also appear to have some evidence of more active immune pathways, which may influence their survival (Huang et al. 2012).

Systemic immune responses, including cellular and humoral, have not been emphasized in honey bee studies of microsporidia infection to date, presumably because of the highly restricted tissue tropism of the microsporidia infection in bees. Cellular immunity, as measured by hemocyte numbers (Alaux et al. 2010), does appear impacted by microsporidia infection, although hemocytes have been shown to phagocytose *N. apis* spores in vitro (Gilliam and Shimanuki 1967). The phenoloxidase cascade, involved in immune-mediated melanization, is similarly unaffected by *N. ceranae* infection (Alaux et al. 2010; Pasquale et al. 2013; Roberts and Hughes 2014), although serine proteases involved in melanization are upregulated by infection (Aufauvre et al. 2012; Badaoui et al. 2017). However, in silkworms, there is evidence that systemic melanization is an important mechanism of immune defense against microsporidia infection (Ma et al. 2013; Ni et al. 2020) and *N. bombycis* parasite actively suppresses melanization (Bao et al. 2019).

An intracellular immune mechanism that targets microsporidia was recently discovered in *C. elegans*. This mechanism, called the “intracellular pathogen response” (IPR), uses ubiquitin to target and clear parasite cells via autophagy (Bakowski et al. 2014; Balla et al. 2019; Reddy et al. 2019). While this specific pathway has not been found in insects, autophagy has been shown to be a critical component of immunity in the fruit fly (reviewed in (Buchon et al. 2014)), and that was recently shown to confer some protection against *N. bombycis* infection in silkworms (Hua et al. 2021). Characterization of intracellular immune mechanisms in bees that might target microsporidia represents a crucial direction for future research.

Complex Interactions Between Nosema spp. and the Honey bee Microbiome The microbiomes of insects play key roles in their biology (Engel and Moran 2013), and it is likely that some of the impacts of honey bee disease are caused by interactions between the honey bee microbiome and *Nosema* spp. Recent studies have shown that the gut microbiota of honey bees is more complex than that found in solitary insects (Kwong and Moran 2016) and that its composition can have a significant impact on honey bee health (Raymann and Moran 2018). The microbiome community provides benefits to the honey bee host that include metabolic contributions (Zheng et al. 2017) and immune modulation (Kwong et al. 2017). In addition, the

microbiome can regulate hormonal signaling found to be involved in some of the organismal-level processes perturbed by *Nosema* infection (described below) (Zheng et al. 2017). Thus, teasing out the relative contributions of host cell pathology and host dysbiosis in inciting *Nosema* disease will be challenging. Perturbation of the honey bee microbiota by diverse mechanisms, such as antibiotic exposure or dietary alterations, can negatively impact honey bee health. Changes in the microbiome can impact the severity and outcomes of infections by pathogenic microbes, including *Nosema* spp. Microbiome composition is correlated with infection intensity of *N. ceranae* infection in *A. mellifera* (Maes et al. 2016; Rubanov et al. 2019; Paris et al. 2020). The interaction appears to be bidirectional. Penicillin-streptomycin exposure can make bees more susceptible to *N. ceranae* infection (Li et al. 2017a). Conversely, reducing *N. ceranae* infection through RNAi increased the abundance of certain bacterial species, suggesting that infection directly or indirectly impacts the digestive tract microbiome (Huang and Evans 2020). Interestingly, recent data from *Apis cerana* also shows that the microbiota can suppress *N. ceranae* growth (Wu et al. 2020), suggesting an antagonistic relationship between *N. ceranae* and the microbiome is a more widespread phenomenon in different bee species.

7.3 *Nosema* Infection Induces Changes in Organismal Physiology and Behavior

Microsporidia infection in bees is associated with alterations in diverse tissues and organs that impact both physiology and behavior of infected individuals. Hypopharyngeal glands (HPG) are involved in producing secretions for feeding larvae in nurse bees and in foraging bees (Ahmad et al. 2021). HPG are decreased in size (Wang and Moeller 1969) and functionally deficient (Liu 1990) in adult bees infected with *N. apis* and show alterations in gene expression and enzyme activity in bees infected with *N. ceranae* (Li et al. 2019). The insect fat body is an important tissue for managing energy homeostasis (Arrese and Soulages 2010). Despite its importance as a metabolic hub for the organism, relatively little is known about the impacts of infection by *Nosema* spp. on this organ. One study found that fat body genes involved in metabolism and hormonal signaling were impacted by infection (Holt et al. 2013). *N. ceranae* infection also alters gene expression in the brain (Holt et al. 2013; McDonnell et al. 2013; Mayack et al. 2015; Doublet et al. 2016) and impacts learning and memory (Gage et al. 2017). Collectively, these tissue-/organ-level shifts act in concert to alter organismal physiology and behavior.

Taken together, organismal changes mimic an acceleration of those observed during normal honey bee development. Individuals of the nonreproductive worker caste of honey bees exhibit a phenomenon known as age polyethism, defined as the age-related division of labor for nonreproductive tasks (Seeley 1982). The most pronounced transition with this process is from nurse bees, which perform tasks inside the colony, to forager bees, involved in gathering resources from the

environment (Johnson 2010). This shift is associated with significant behavioral and physiological changes and is highly regulated to optimize colony function. Infection by either *N. apis* (Hassanein 1953; Wang and Moeller 1970; Woyciechowski and Moroń 2009) or *N. ceranae* (Goblirsch et al. 2013; Dussaubat et al. 2013; Natsopoulou et al. 2015; Lecocq et al. 2016) causes worker bees to accelerate this aging process as manifested by both physiological and behavioral changes. One 2013 study found that infected bees are twice as likely to engage in precocious foraging compared to controls (Goblirsch et al. 2013). Foragers infected by *N. ceranae* (Naug 2014; Alaux et al. 2014) and *N. apis* (Lach et al. 2015; Dosselli et al. 2016) perform less efficiently, and *N. ceranae*-infected foragers are less likely to return to the colony due to energetic stress (Kralj and Fuchs 2010) and reduced homing ability (Wolf et al. 2014). Infected workers also spend more time outside the nest engaged in risky behaviors such as robbing (Kuszevska and Woyciechowski 2014).

Multiple hormonal systems known to control worker division of labor are impacted in bees infected by *Nosema* spp. The vitellogenin (Vg)/juvenile hormone (JH) axis is critical for controlling age polyethism (Johnson 2010). JH levels are increased in bees infected with either *Nosema* spp. (Ares et al. 2012), while Vg expression is reduced (Antúnez et al. 2013; Goblirsch et al. 2013; Zheng et al. 2014; Garrido et al. 2016). Bees infected as larva have increased Vg levels as young adults, suggesting different impacts based on the age of infection (BenVau and Nieh 2017). Infected bees also show alterations in the octopamine pathway (Mayack et al. 2015), which is also known to participate in the transition to foraging (Johnson 2010), and insulin signaling (Holt et al. 2013), involved in regulating caste (Ament et al. 2008, 2010).

Interestingly, a number of other stressors are known to induce a similar precociousness in the physiological and behavioral shifts associated with the development from nurse to forager as well as the hormonal systems controlling these transitions. For example, infection by diverse pathogens and parasitization by an arthropod pest induce comparable changes (McDonnell et al. 2013; Doublet et al. 2016). One theory to explain the related outcomes from these distinct stressors is that they all impinge on nutritional status which is known to heavily influence this transition (Ament et al. 2010). Here again, additional foundational knowledge of honey bee biology will likely facilitate a better understanding of *Nosema* disease. Based on the complexity of the interorgan communication systems used in fruit flies to regulate organismal physiology and behavior (Droujinine and Perrimon 2016; Liu and Jin 2017), it is likely that other molecules yet to be characterized in honey bees integrate signals of nutrition status (and injury) at the physiological levels and couple these inputs with control of life stage transitions in bees. The *unpaired* family of JAK/STAT pathway ligands described above is also known to regulate both physiology and behavior in flies (Droujinine and Perrimon 2016; Liu and Jin 2017). Characterizing the role of the recently described honey bee homologs of these ligands (Bach et al. 2021) in interorgan communication may represent one potential link between the infected digestive tract and organismal pathology.

The behavioral changes observed in parasitized bees may be seen to have adaptive benefits to both the parasite and host. For example, by engaging in risky behavior, infected bees spend less time in the colony potentially limiting transmission to nestmates and benefitting the colony (Rueppell et al. 2010). This potentially altruistic self-removal by infected bees may represent one of the social immunity strategies found in eusocial insect species (Cremer et al. 2018). However, such behavior could result in more efficient spread to individuals of uninfected colonies, thereby benefiting the pathogen. Similarly, *N. ceranae*-infected bees appear to seek out the warmer areas in the colony, which could help energetically stressed bees manage thermoregulation or provide the higher temperatures preferred by this microsporidia species and a better opportunity to spread the pathogen (Campbell et al. 2010). Thus, the adaptive benefit, if any, and the beneficiary of such behavioral changes in infected honey bees are unclear (Wagoner et al. 2013).

Many groups have further reported shortened worker lifespans after infection by *Nosema* spp. (Higes et al. 2006; Alaux et al. 2009; Vidau et al. 2011; Goblirsch et al. 2013; Williams et al. 2014; Doublet et al. 2015). For example, Goblirsch et al. observed a 9-day reduction in worker lifespan (Goblirsch et al. 2013). However, it is important to note that other studies have not observed differences in mortality in infected bees and these inconsistencies may be due to similar factors involved in modulating the colony impacts of *Nosema* disease, such as genetic variation on the part of both parasite and host and the presence of other stressors (Goblirsch 2017; Martín-Hernández et al. 2018).

7.4 Organismal Pathogenesis Caused by *Nosema* Disease Disrupts Colony Organization and Function

Honey bee colonies are eusocial (Holldobler and Wilson 2008), and colony physiology is regulated by the collective activities of individual colony members (Friedman et al. 2020). Individual infection by *Nosema* spp. appears to cause significant disruptions in colony organization and function which likely contribute to colony-level disease. For example, *N. ceranae* infection alters pheromone production in workers (Dussaubat et al. 2010, 2013) and queens (Alaux et al. 2011). *Nosema* spp. infection has been observed to alter production of ethyl oleate, which is involved in the regulation of division of labor among workers (Dussaubat et al. 2010). Another recent study found increased levels of alarm pheromone in colonies infected with *N. ceranae* (Mayack et al. 2021). Other studies have demonstrated differences in cuticular hydrocarbon profiles (McDonnell et al. 2013; Murray et al. 2016), although it is not clear how these changes impact interactions between workers (Murray et al. 2016; Biganski et al. 2018).

In the honey bee colony, the health and function of the reproductive individuals in the colony are critical for colony success. While less studied, both drones and queens can be infected by *Nosema* spp. Thus far, individuals from the reproductive castes

display similar perturbations to worker bees in addition to important changes in biology unique to their colony function (reviewed in (Goblirsch 2017; Martín-Hernández et al. 2018)). The many impacts of microsporidia infection on the physiology and behavior of workers in addition to these effects on reproductive individuals likely contribute to colony death, especially in the presence of other stressors. The negative consequences of *Nosema* infection on bees are thought to be influenced by the presence of other stressors, including nutritional stress due to loss of appropriate forage, chemical poisoning from pesticides, changes to natural living conditions brought about through large-scale beekeeping practices, myriad environmental changes due to climate change, and infection by arthropod parasites and other pathogenic microbes. For example, *N. ceranae* infection can interact with chemical stressors, such as pesticides, on honey bees leading to synergistic effects on health and mortality (Alaux et al. 2010; Vidau et al. 2011; Wu et al. 2012; Pettis et al. 2012, 2013; Aufauvre et al. 2012, 2014; Retschnig et al. 2014; Doublet et al. 2015). In addition, chemical stressors are known to increase the prevalence or intensity of *N. ceranae* infection likely through impacts on host well-being (Wu et al. 2012; Pettis et al. 2013). Bees are also often coinfecting by multiple pathogens (Runckel et al. 2011; Cornman et al. 2012), and the impacts on both pathogens and the host are likely to be complex. This topic has been recently covered in great detail (Goblirsch 2017; Martín-Hernández et al. 2018) and will not be explored further here.

7.5 Impact of Host Factors on Microsporidia Growth and Development

The parameters that influence the growth and development of *Nosema* cells within the host environment are still largely unknown. Microsporidia as a group have significantly reduced genomes relative to free-living fungi, with much of the lost coding content being found in metabolic pathways (Nakjang et al. 2013). To make up for reduced biosynthetic complexity, microsporidia are known to acquire diverse array of metabolites from host cells, often through the use of expanded families of transporters (Chetia et al. 2017), which are sometimes acquired through horizontal gene transfer (Dean et al. 2018). Recent studies of *Tubulinospora ratisbonensis* which infects fruit flies (Niehus et al. 2012) have shown that specific metabolites, namely, phosphatidic acid and related lipids, are limiting for the proliferation of the microsporidium in host cells (Franchet et al. 2019). Most studies of *N. ceranae* suggest that increased protein consumption leads to increased infection intensities as measured by spore counts (Porrini et al. 2011; Basualdo et al. 2014; Zheng et al. 2014; Fleming et al. 2015; Jack et al. 2016; Tritschler et al. 2017), suggesting that for this species, amino acids may be limiting. There is also evidence that many other host-derived nutrients are important for *N. ceranae* growth. For example, iron, which is often a limiting nutrient for diverse pathogenic microbes (Cassat and Skaar 2013), was recently observed to be reduced in forager honey bees (but not nurses) after

N. ceranae infection (Rodríguez-García et al. 2021). RNAi-mediated knockdown of *transferrin* resulted in decreased available iron and led to reduced *N. ceranae* infection intensity. Limiting iron availability may be useful as a therapeutic strategy (Rodríguez-García et al. 2021).

The life cycle of *Nosema* spp. is complex and is likely influenced by the host environment and involves multiple developmental transitions characterized by distinct morphological forms and evolving patterns of expression of both mRNA (Huang et al. 2016b) and miRNA (Huang and Evans 2016; Shao et al. 2021) transcripts. For example, many microsporidia species appear to use the “early sporulation” strategy to produce primary spores for spread within host tissues before switching to the production of the secondary or environmental spore. The presence of intracellular empty spore coats indicates that intracellular germination of spores is occurring during *N. ceranae* infection (Higes et al. 2007; García-Palencia et al. 2010) as has been shown for *N. apis* (Fries et al. 1992). Another strategy for rapid spread within the tissues without the need to progress to the environmental spore is found in microsporidia infection in nematodes, which involves the removal of lateral membranes to form syncytia (Balla et al. 2016). Here, developmental timing, in particular the switch to sporulation, is triggered by parasite density (Balla et al. 2016). This may be true for *N. ceranae* as well, as inoculation dose has been shown to play a role in infection intensity and spore production (McGowan et al. 2016). Another possible signal could be related to available host resources, which may not only impact growth directly (see above) but may influence developmental decisions. Other possible cues include cell stress, which alters the developmental program of other fungi (Boyce and Andrianopoulos 2015). One stress that is known to impact *N. ceranae* infection is temperature (Martín-Hernández et al. 2009; Higes et al. 2010; McNamara-Bordewick et al. 2019), and some evidence suggests this effect is due to changes in the development of the pathogen (Higes et al. 2010). However, detailed analysis of the stage and impact has not heretofore been possible due to the difficulty in isolating the distinct developmental forms from host cells. Similarly, the current frontline drug for treating *N. ceranae* infection, fumagillin, may also impact development. At lower doses, this drug causes hyperproduction of spores compared to spore production in untreated bees (Huang et al. 2013).

While the typical focus is on the impacts on the honey bee host, the effect of various stressors on the growth and development of *Nosema* spp. in bees is also important to understand. Many environmental factors are carefully controlled by the healthy host cell, thereby reducing variability. Adaptation to this host-controlled environment would be predicted to have a significant impact on the cell stress machinery in microsporidia. In fact, comparative genomics indicates that microsporidia have lost many of the quality control and cell stress pathways found in free-living eukaryotes (McNamara-Bordewick et al. 2019; Snow 2020), perhaps leading to a loss of the redundancy and flexibility that often allow organisms to withstand cellular stresses. However, data suggests high levels of stress resistance in *Nosema* spp. For example, although the majority of fungal species prefer the 12°–30 °C range and relatively few species tolerate temperatures higher than 35 °C (Robert and Casadevall 2009), *N. ceranae* exhibits a striking ability to grow at the

high temperatures (34–35 °C) maintained in honey bee colonies (Martín-Hernández et al. 2009; Higes et al. 2010), despite the loss of the Heat Shock Factor (Hsf) gene, encoding the canonical regulator of the heat shock response (McNamara-Bordewick et al. 2019). *N. apis*, while still missing the Hsf gene, is similar to typical fungi in its sensitivity to high temperatures (Burnside and Revell 1948; Woyciechowski and Czekońska 1999), and honey bees can recover from infection when kept at the slightly elevated temperature of 37 °C (Lotmar 1943). While the canonical cell stress response systems appear reduced, there may be adaptation of the cellular machinery to allow for novel responses to cell stress. This may be achieved through mechanisms that increase tolerance to stress. Microsporidia encode aminoacyl-tRNA synthetases without editing domains, and the wrong amino acid is added in up to 6% of cases for some codons. This diversity of protein variants may allow these parasites to adapt under different stress conditions (Melnikov et al. 2018). Another mechanism may involve manipulation of the host cell. For example, *N. ceranae* infection induces decreases in both the amount of ROS and oxidative damage (Paris et al. 2017), possibly mediated through pathogen manipulation of host cell machinery. Microsporidia genomes possess a large number of genes encoding proteins with predicted signal peptides. Many of these are likely secreted into the host cell and are thus candidates for interceding in the function of host cell processes (Cuomo et al. 2012; Campbell et al. 2013). In fact, Reinke et al. were able to use exogenous machinery expressed in host cells to label a number of host-exposed proteins from *Nematocida* species. Based on analysis of other microsporidia genomes, the authors predicted that other microsporidia species might use 6–32% of their proteome to interface with the host (Reinke et al. 2017). While such an experiment is not currently possible in honey bees, there is a need for methods to identify *Nosema* proteins responsible for host manipulation.

7.6 Conclusion and Future Perspectives

Since the discovery of *N. ceranae* infecting western honey bees, there have been impressive gains in our understanding of the effects these microsporidia have on honey bee health (Goblirsch 2017; Martín-Hernández et al. 2018). A number of questions and areas for further research are evident. As parasite growth is restricted to the digestive tract, the routes through which parasite growth and tissue dysfunction in the midgut leads to the dramatic physiological and behavioral changes at the organismal level are incompletely understood. If caused by pathogen-mediated manipulation, how are these changes effected at the molecular level? If due to disruptions in normal midgut function and host responses to this pathology, are changes specific to microsporidia infection or are they observed with diverse stressors? The critical host parameters that influence the growth and development of *Nosema* cells within the host environment also remain obscure. Relatedly, elucidating the positive and negative consequences of observed changes on both host and parasite is also critical.

A number of approaches are already being used to perform gain- and loss-of-function experiments to answer these questions. RNAi machinery exists in *N. ceranae* (Paldi et al. 2010), and RNAi has been shown to be a powerful tool in elucidating the machinery of both the parasite (Paldi et al. 2010; Huang et al. 2016a, 2018a; Rodríguez-García et al. 2018; Huang and Evans 2020) and the host (Li et al. 2016; Rodríguez-García et al. 2021). New genetic tools are being developed that could greatly facilitate investigations into the mechanisms involved in host-pathogen interactions. Recently, CRISPR/Cas9 protocols have been developed for the honey bee (Kohno et al. 2016; Kohno and Kubo 2018; Roth et al. 2019; Hu et al. 2019; Nie et al. 2021). Such tools could allow confirmation of host pathways and processes suspected to be involved in *Nosema*-induced pathogenesis. For example, loss-of-function experiments could be used to probe host factors involved in the reduced apoptosis, such as the candidate *Iap* (Kurze et al. 2015; Martín-Hernández et al. 2017).

Methods to generate genetically modified microsporidia would similarly represent an indispensable resource for making future advances (Reinke and Troemel 2015). Some critical progress has been made in culturing sporoplasm in *N. bombycis* for this purpose (He et al. 2019 2020) and in delivering genetic material (Guo et al. 2016a). A heterologous cell culture system has been established (Gisder et al. 2011; Gisder and Genersch 2015) that could facilitate biochemical studies although additional more robust cell culture models are necessary.

Such future studies could also provide much needed potential target molecules for treatment strategies for *Nosema* disease in honey bees. *N. apis* infection has long been controlled by treatment with the drug fumagillin, a methionine aminopeptidase 2 inhibitor (Heever et al. 2014). Yet, the effectiveness of fumagillin treatment for treating *N. ceranae* at the colony level appears transient. High doses of this drug impair host cell function, and evidence suggests that *N. ceranae* can escape suppression in some circumstances, although the mechanisms remain unknown (Huang et al. 2013). Most critically, the long-term availability of fumagillin is uncertain, making efforts to find alternative treatment strategies critical to protect honey bees from this parasite (Heever et al. 2014; Holt and Grozinger 2016). New treatment strategies to reduce the impact of *Nosema* disease on this pollinator species are currently being explored (see (Huntsman et al. 2021) and references therein), which could have significant positive impacts on the health of both agricultural and ecological systems.

Taxonomic Note

Nosema apis and *N. ceranae* have recently been redefined as *Vairimorpha apis* and *V. ceranae* based on a recent molecular phylogenetics analysis of the *Nosema* and *Vairimorpha* clades (Tokarev et al. 2020). For the purposes of this review the *Nosema* Genus will still be used.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no conflict of interest.

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