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Nosema ceranae in European honey bees (*Apis mellifera*)

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ABSTRACT

Nosema ceranae is a microsporidian parasite described from the Asian honey bee, *Apis cerana*. The parasite is cross-infective with the European honey bee, *Apis mellifera*. It is not known when or where *N. ceranae* first infected European bees, but *N. ceranae* has probably been infecting European bees for at least two decades. *N. ceranae* appears to be replacing *Nosema apis*, at least in some populations of European honey bees. This replacement is an enigma because the spores of the new parasite are less durable than those of *N. apis*. Virulence data at both the individual bee and at the colony level are conflicting possibly because the impact of this parasite differs in different environments. The recent advancements in *N. ceranae* genetics, with a draft assembly of the *N. ceranae* genome available, are discussed and the need for increased research on the impacts of this parasite on European honey bees is emphasized.

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1. Background

Nosema ceranae is a microsporidian parasite presently known to infect the Asian honey bee, *Apis cerana*, and the European honey bee, *Apis mellifera* (Fries et al., 1996; Higes et al., 2006). All microsporidians are intracellular parasites, disperse between hosts as spores and have unique organs for cell invasion. The infection mechanism is based on mechanical injection of a polar filament protruding from the germinating spore. With physical force, the filament penetrates a host cell membrane into the host cell. Through the filament, the infective sporoplasm is injected into the host cell cytoplasm where parasite replication, and later spore production is initiated (Larsson, 1986). Based on molecular evidence, microsporidia are now included into the cluster Fungi (Sina et al., 2005). Thus, taxonomically, Microsporidia are highly specialized parasitic fungi.

N. ceranae was first described from the Asian honey bee (*A. cerana*) in samples from the Bee Institute of the Chinese Academy of Agricultural Sciences outside Beijing, China (Fries et al., 1996). Cross-infection experiments using both *N. ceranae* and *Nosema apis* in both *A. cerana* and *A. mellifera* demonstrated that both parasites were cross-infective across hosts, but that *N. ceranae* developed better in *A. mellifera* compared to *N. apis* in *A. cerana* (Fries and Feng, 1995; Fries, 1997). The infectivity of *N. ceranae* to *A. mellifera* is not surprising since many microsporidia exploit multiple hosts. As an example, the species *Nosema necatrix* (described by Kramer (1965)) and later redescribed as *Vairimorpha necatrix* (Pilley, 1976), which is phylogenetically closer to *N. ceranae* than to *N. apis* (Fries et al., 1996; Chen et al., 2009), successfully completes development in a variety of lepidopteran hosts (Darwish et al., 1989; Kramer, 1965; Nordin and Maddox, 1974; Pilley, 1976). Microsporidia infections in *A. cerana* had been described prior to the description of *N. ceranae* and were assumed to be infections by *N. apis* (Lian, 1980; Singh, 1975; Yakobson et al., 1992). However, it is possible that some earlier observations of microsporidian infections in *A. cerana*, and possibly also in *A. mellifera*, may have been observations of *N. ceranae* (Fries et al., 2006a).

Although it was known that *N. ceranae* was infective for *A. mellifera* (Fries, 1997), there is no record of natural infections of *N. ceranae* in *A. mellifera* until Higes et al. (2006) found 10 out of 11 samples from 2005 from Spanish apiaries positive for microsporidia to contain *N. ceranae*, based on homology to the original 16S ssrRNA GenBank entry for *N. ceranae* (accession number U26533). Also, in samples from 2005, Huang et al. (2007) reported on natural infections of *N. ceranae* in *A. mellifera* in an apiary containing colonies of both *A. mellifera* and *A. cerana*.

2. Phylogeny and genetics

The first genetic analysis of *N. ceranae* based on the 16S small sub-unit rRNA gene suggested that *N. apis* was not as phylogenetically close to *N. ceranae* as one may have suspected (Fries et al., 1996). Later analysis, based on the same gene and from GenBank entries have given some conflicting results. Three analyses found *N. ceranae* to be closer to *N. bombi* than to *N. apis* (Fries et al., 2001; Wang et al., 2006; Chen et al., 2009), whereas the analysis of Slamovits et al. (2004) placed *N. apis* closer to *N. ceranae*. In contrast, the analysis of Vossbrinck and Debrunner-Vossbrinck (2005)

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put *N. apis* closer to *N. bombi* than to *N. ceranae*. In the most recent attempt to compile a phylogeny of microsporidians infecting bees, Shafer et al. (2009) used multiple sequence data sets, rather than sequences for a single gene, and concluded that *N. ceranae* is a sister species to *N. bombi* and that *N. apis* is the basal member of the clade. Based on their analysis, they (Shafer et al., 2009) suggest that either an ancestral *N. bombi* switched host from a *Bombus* lineage to *A. cerana*, or an ancestral *N. ceranae* switched host to *Bombus*.

Chen et al. (2009) sequenced the DNA of the rRNA gene from *N. ceranae* and found the size to be 4475 bp, slightly larger than reported by Huang et al. (2007). The GC content of the 16S SSU-rRNA cistron is approximately 36% (Huang et al., 2007; Chen et al., 2009). The internal transcribed spacer (ITS) region consists of a 39-bp sequence and is located between nucleotides 1260 and 1298 (Huang et al., 2007; Chen et al., 2009).

The use of sequence similarities in the conserved rRNA gene is common for building phylogenies among eukaryotes. In the case of microsporidian parasites this strategy may not be optimal. The presence of multiple copies of rRNA is common in Microsporidia (Gatehouse and Malone, 1998; Tay et al., 2005) possibly representing a case of concerted evolution, the duplication of entire loci within a genome. However, analyzing the rRNA gene from a single spore of *N. bombi*, O'Mahony et al., 2007 demonstrated multiple copies of rRNA which were not all homologous. Multiple non-homologous copies of rRNA may be a common feature of Microsporidia. Thus, homologs cannot be compared between isolates, which reduces the utility of the rRNA genes of microsporidians for phylogenetic analysis (O'Mahony et al., 2007). For future attempts to study *N. ceranae* phylogeny, there is a need to develop single-locus polymorphic markers (O'Mahony et al., 2007).

Based on pyrosequencing data, a draft assembly of the *N. ceranae* genome (7.86 MB) has recently been presented (Cornman et al., 2009). The genome of *N. ceranae* is extremely reduced and strongly AT-biased (74% A + T) (Cornman et al., 2009). Polymorphism among rRNA loci, as reported for *N. bombi* (O'Mahony et al., 2007) is likely to occur also in *N. ceranae*, which complicates the genome assembly of this operon (Cornman et al., 2009). The genome analysis predicts 2614 protein-coding sequences, arguably an underestimate since a fraction of the genome likely did not assemble in this draft project (ca. 5–10%). About 50% of the predicted protein-coding sequences in the *N. ceranae* genome share significant similarity with the microsporidian *Encephalitozoon cuniculi*, so far the most closely related published genome sequence Cornman et al., 2009). Interestingly, both parasites appear to differ from yeast and other fungi by using a larger fraction of the genome for growth related gene categories and a reduced fraction to transport and to chemical stimuli (Cornman et al., 2009). This is likely to reflect the extreme parasitic life form represented by microsporidians. Many aspects of the *Nosema*-honey bee interactions remain enigmatic. Identification of genes with specific functions is a first step in resolving such host–parasite interactions at the gene level. Cornman et al. (2009) stress the 89 gene models encoding signal peptides as being of particular interest because these proteins are candidate secretory proteins that may interact with host tissue. Antúnez et al. (2009) attempted to measure gene responses following microsporidia infections. Their results suggest differences in upregulation of genes encoding the antibacterial peptides abaecin, defensin and hymenoptaecin between infections with *N. ceranae* and *N. apis*. However, previous work based on antibacterial properties of hemolymph from *N. apis* infected bees did not show any antibacterial effects from such hemolymph (Craig et al., 1989). The results of Antúnez et al. (2009) are interesting, because they also suggest that immuno suppression results from *N. ceranae* infections. Given that their study (Antúnez et al., 2009) includes time limited data only, it is premature to conclude that the gene expression data available are indicative of variations in virulence between *N. ceranae* and *N. apis*.

3. Distribution

Although infective for *A. mellifera*, *N. ceranae* was previously believed to be geographically limited to the natural distribution area of *A. cerana* (Fries, 1997). However, Huang et al. (2008) sequenced rRNA spacer regions in *N. ceranae* samples from both honey bee host species and found little or no differences between samples, suggesting that no transmission barrier exists for *N. ceranae* between *A. mellifera* and *A. cerana*. Based on historical data (Klee et al., 2007), it appears likely that *N. ceranae* has entered into a new host (from *A. cerana* into *A. mellifera*), and is presently spreading within that species, although this scenario still needs to be confirmed. Thus, we do not know when or where this proposed host shift may have occurred. In the US there are confirmed infections of *N. ceranae* dating back to the mid-1990s (Chen et al., 2008) and in Uruguay one sample pre-dating 1990 has been confirmed to contain *N. ceranae* (Invernizzia et al., 2009), which is the oldest record of *N. ceranae* in *A. mellifera*.

Analysis of samples from all continents where apiculture is practiced demonstrate that *N. ceranae* infections of *A. mellifera* occur worldwide (Klee et al., 2007; Giersch et al., 2009) but is so far only detected in North Africa (Higes et al., 2009). However, it appears from historical records that *N. ceranae* infections progressively have become more common over time, at least in some regions (Klee et al., 2007; Paxton et al., 2007). In Finland analysis of ten year old bee samples demonstrated only infections of *N. apis,* whereas more recent samples contained mixed samples of *N. apis* and *N. ceranae* or pure infections of *N. ceranae* (Paxton et al., 2007). There data were interpreted as the process of one parasite possibly replacing the other (Paxton et al., 2007). If their is a general tendency for *N. ceranae* replacing *N. apis* this will be evident as more data on microsporidia infections in honey bees become available. Data from the German bee monitoring project, for which both *Nosema* species were distinguished, does not yet suggest that one parasite is replacing the other (Monitoring-Projekt "Völkerverluste", 2008). A national survey for microsporidia infections in Sweden, using species specific molecular detection techniques to distinguish between *N. apis* and *N. ceranae,* demonstrated no pure infections of *N. ceranae,* but only mixed infections (17%) and pure *N. apis* infections (83%) in 319 samples positive for microsporidia in light microscopy (Fries and Forsgren, 2008). Later surveys will document if the proportion of *N. ceranae* will increase over time.

Both on the North American continent (Williams et al., 2008) and in Europe (Martín-Hernández et al., 2007; Fries and Forsgren, 2008) the proportion of *N. ceranae* infections appears to dominate in warmer climates compared to more temperate regions, whereas *N. apis* presently may be more prevalent in colder climates. It is unclear whether this difference in prevalence from north to south reflects the direction of spread. In Finland the prevalence of *N. ceranae* is much higher compared to Sweden and Norway (Paxton et al., 2007; Fries and Forsgren, 2008) although the climates are similar. One difference between these countries is that Finland imports bees from southern Europe, where the prevalence of *N. ceranae* is high, whereas Sweden and Norway have not imported bees from areas with *N. ceranae* in recent years. Nevertheless, climate may be an important factor explaining differences in species distribution and impact. Using both *N. ceranae* and *N. apis* spores, Martín-Hernández et al., 2009 compared the increase in spore numbers in individual bee abdomens at different times post infection and found *N. ceranae* to increase more in numbers over a wider temperature range compared to *N. apis*. It is not yet clear how this difference may influence the distribution of *N. ceranae*. The viability of *N. ceranae* spores is significantly reduced following one week in a deep freezer, which is not the case for *N. apis* (Fries and Forsgren, 2009). This difference in temperature sensitivity between parasite species probably has epidemiological

implications and may decrease transmission opportunities for *N. ceranae*, at least on wax exposed to freezing temperatures during storage.

4. Pathology and epidemiology

To understand pathology and evolutionary epidemiology of honey bee diseases, it is imperative to distinguish between colony level and individual bee effects from certain disease agents (Fries and Camazine, 2001). Commonly, larval diseases may be highly virulent at the individual level killing infected individuals, whereas they rarely kill entire colonies (Fries and Camazine, 2001). The fact that colonies are killed by American foulbrood may largely be an apicultural phenomenon (Fries et al., 2006b). Adult bee diseases are most often benign, both at the individual and colony level (Fries and Camazine, 2001). However, if *N. ceranae* is a comparatively recent introduction into populations of European honey bees, the host–parasite relation may not yet have been molded by natural selection to a predictable level of virulence, either at the individual or colony level. The introduction of an exotic parasite, such as *N. ceranae*, into a novel host system (*A. mellifera*) could potentially lead to local eradication of this honey bee species. The invasion of an exotic species into an ecosystem is currently viewed as one of the most important sources of biodiversity loss and may even lead to host eradication (Deredec and Courchamp, 2003).

4.1. Prevalence

The typical pattern for *N. apis* infections in temperate climates is low prevalence or hardly detectable levels during the summer with a small peak in the fall. During the winter there is a slight increased prevalence with a large peak in the spring before the winter bees are replaced by young bees (Borchert, 1928; Bailey, 1955). The pattern is similar both in the southern and northern hemisphere (Doull and Cellier, 1961). Unfortunately, very few data exist for *N. apis* on the seasonal prevalence from tropical or subtropical conditions. The only published year round sampling under conditions where bees could fly all year round, revealed detectable levels of *N. apis* with no seasonal pattern of prevalence (Fries and Raina, 2003). Thus, a seasonal pattern of prevalence may be dependent on climatic conditions. However, from older Spanish records, *Nosema* spp. infections did have a seasonal pattern of prevalence, similar to descriptions from temperate climates. From 2003 onward a change in seasonality occurred with an increase of *Nosema* spp. positive samples throughout the year until 2005, when there was a total absence of seasonality in infection prevalence (Martín-Hernández et al., 2007). This strongly suggests that fundamental epidemiological parameters, such as transmission rates and/or routes may be different between the two parasites.

4.2. Colony virulence

Several studies from Spain suggest that *N. ceranae* is a colony level virulent parasite and that infections eventually lead to colony collapse unless the infections are controlled (Higes et al., 2008a; Higes et al., 2009; Martín-Hernández et al., 2007). However, most published data on colony losses linked to *N. ceranae* infections are correlations and fail to provide evidence of cause and effect. Based on correlative data, Higes et al. (2008a) describe the different phases that infected colonies go through, until they eventually succumb to the infections. One small-scale experiment did suggest that *N. ceranae* was causal in colony losses. Higes et al. (2008a) established ten small (nucleus) colonies with mated queens with endemic *N. ceranae* infections. Five of these colonies received fumagillin treatment and five colonies received only sugar solution. Fumagillin is effective in controlling *N. ceranae* infections (Williams et al., 2008). Within 15 months all untreated colonies were dead whereas all fumagillin treated colonies were alive (Higes et al., 2008a). Nevertheless, *N. ceranae* infections are present in many areas where not all colonies are treated with fumagillin, without significant colony losses linked to such infections. In the US, infections of *N. ceranae* have been present for more than a decade (Chen et al., 2008) and a meta-genomic study of the so called Colony Collapse Disorder (CCD) failed to link colony failure in the US to *N. ceranae* infections, although the data revealed an overrepresentation of *N. ceranae* in collapsing colonies (Cox-Foster et al., 2007). Furthermore, data from Uruguay where the incidence of microsporidia infections of honey bees have been monitored for decades did not find a correlation between the arrival of *N. ceranae* and either increasing microsporidia loads or increased colony losses (Invernizzia et al., 2009). In Germany, where microsporidia infections are monitored to species, there appears to be no clear causal link between winter losses and infections with *N. ceranae* (Siede et al., 2008). Although *N. ceranae* appears to be the most prevalent microsporidian parasite in German honey bees, the disease prevalence actually decreased between 2004–2007, to increase somewhat again in 2008 (Monitoring-Projekt "Völkerverluste", 2008). This situation certainly does not suggest that the occurrence of *N. ceranae* in colonies from central Europe will lead to the dramatic effects described from Spain.

The few data yet available on colony level virulence of *N. ceranae* infections are obviously contradictory. The discrepancies may be due to climatic or other as yet unresolved factors. There may even be differences in virulence in different isolates of the parasite and different variants of *N. ceranae* have been described (Huang et al., 2008; Williams et al., 2008). Given that regional or climatic differences may be important for the impact of this disease, data from long term monitoring of *Nosema* spp. disease prevalence in different parts of the world is badly needed.

4.3. Individual virulence

The first report using cage experiments with individually infected honey bees, suggested that bees infected by *N. ceranae* die within eight days of infection and the authors suggested that this reflected the high virulence of the parasite (Higes et al., 2007). However, neither later cage experiments by the same authors (Martín-Hernández et al., 2009) nor other experiments (Mayack and Naug, 2009; Paxton et al., 2007) have confirmed this rapid cage mortality. Mayack and Naug (2009) found that *N. ceranae* have a higher hunger level that leads to a lower survival, but when fed *ad libitum*, the mortality of infected bees was not different from uninfected bees. The cage mortality results of Paxton et al. (2007) did suggest a higher virulence in *N. ceranae* compared to *N. apis*, but the authors urged caution in interpreting these results as final. Longevity is reportedly reduced in bees infected by *N. apis* (Hassanein, 1953; Wang and Moeller, 1970) although this effect is not always evident in all bees (Malone and Giacon, 1996). Survival data comparisons from cage experiments suggest that dosing newly-emerged bees with *N. apis* may result in a relatively fast death for some bees and a slower death for most of the bees. Also, spore-loads may vary greatly, with no clear relationship to survival time (Malone and Giacon, 1996). Although useful for certain purposes (i.e. infectious dose, parasite growth rate), cage experiments are probably not suitable for studying the effects of infection on longevity, because of the artificial conditions and variations in response to cage conditions *per se*. Although there is regional evidence for higher individual virulence of *N. ceranae* compared to *N. apis* (Higes et al., 2007; Paxton et al., 2007) this aspect needs further study and to be ascertained in field colonies before it can be accepted as a general phenomenon.

5. Diagnosis and biology

There is no specific outward sign of disease in bees infected with *N. apis*, although the ventriculus of heavily infected bees may appear whitish and swollen (Fries, 1997). Similarly, there are no outward symptoms reported for *N. ceranae*. Thus, diagnosis requires light microscopy, or more sophisticated molecular methods. The spores of *N. ceranae* are slightly smaller than in *N. apis*, but the two species are nevertheless difficult to tell apart with certainty under a light microscope (Fig. 1; Fries et al., 2006a). Using transmission electron microscopy, the species can be separated based on the number of polar filament coils on that basis that *N. ceranae* always have fewer coils compared to *N. apis* (Fries et al., 1996; Chen et al., 2009) (Fig. 2). Several PCR based molecular techniques for the diagnosis and identification of *N. apis* and *N. ceranae* have been described. Protocols for the PCR–RFLP method (Higes et al., 2006; Klee et al., 2007), a uniplex PCR using species specific primers (Chen et al., 2008) and a multiplex PCR for amplification of DNA from the two species simultaneously (Martín-Hernández et al., 2007) have been published. The latter methodology is accepted and recommended by The World Organisation for Animal Health (OIE Terrestrial Manual, 2008). Because mixed infections of the two parasites are common in some areas (Paxton et al., 2007) the development of quantitative molecular tools is necessary to describe relative parasite prevalence.

Colony level symptoms of dysentery may be aggravated by infections of *N. apis* (Bailey, 1981) but this agent is not the primary cause of this condition (Bailey, 1967). Nevertheless, dysentery certainly aids the fecal–oral route of parasite transmission. For *N. ceranae* no specific colony level symptoms of infection have been described. In Spain, infected colonies have been associated with gradual depopulation, higher autumn and winter colony death and a decrease in honey production (Higes et al., 2008a). Strikingly, no dysentery is reported to be associated with infections of *N. ceranae* (Faucon, 2005; Higes et al., 2008a). Whether this indicates that the main transmission routes of *N. ceranae* are different from *N. apis*, where soiled comb is believed to be the primary

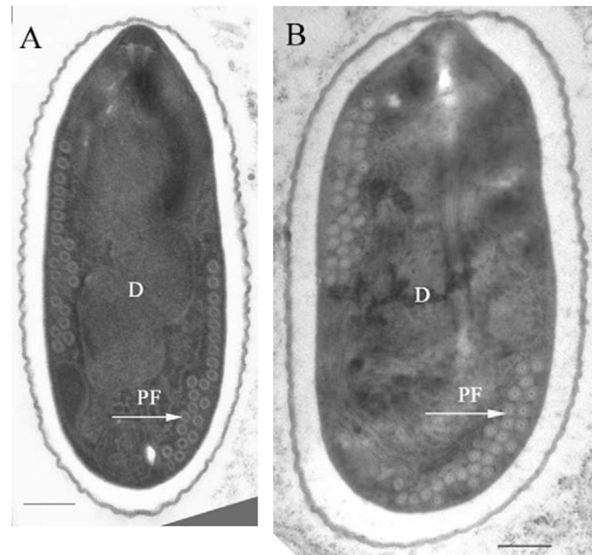

Fig. 2. The internal structures of the diplokaryotic (D) *N. ceranae* (A) and *N. apis* (B) are similar. Notably, *N. ceranae* spores contain fewer polar filament (PF) coils compared to *N. apis*. Bars = 0.5 μm. (from Fries et al. 2006a).

source of infection (Bailey, 1955) remains to be investigated. Disease transmission through soiled comb is possible because *N. apis* spores may remain viable in fecal deposits for more than a year (Bailey, 1962) whereas the effect of time on the viability of *N. ceranae* spores in the hive environment is unknown. Nevertheless, even if freezing significantly reduces *N. ceranae* viability (Fig. 3, Fries and Forsgren, 2009), the spores of this parasite do remain viable for extended periods outside of bees, given that viable spores have been found in regurgitated pellets of the bee eating bird *Merops apiaster* (Higes et al., 2008b).

The intracellular development of *N. ceranae* in the ventricular cells appears to be similar to that of *N. apis* (Fries et al., 1996; Higes et al., 2007; Chen et al., 2009). The spores enter the bee through the food canal and germinate in the midgut where the epithelial cells become infected. There is as yet no evidence for *N. ceranae* spores being produced in any other cell type than in the ventricular epithelial cells (Fries et al., 1996; Higes et al., 2009). However, using PCR, Chen et al. (2009) report on finding *N. ceranae*-specific nucleic acid, not only in the ventricular epithelium, but also in the Malpighian tubules, hypopharyngeal glands, salivary glands and fat body cells. These data suggest that *N. ceranae* may not be cell specific, but it remains to be demonstrated whether the parasite can complete its life cycle outside of the ventricular cells. Using light microscopy, no spores of *N. ceranae* were found in any other tissue type in *A. cerana* when the parasite was first described (Fries et al., 1996). Nevertheless many microsporidian species infect multiple tissues. As an example, *Nosema bombi*, a parasite of different bumble bee species, completes its life cycle not only in the ventricular cells, but also in the Malpighian tubules, fat body cells and even in the brain and nerve tissue cells (Fries et al., 2001).

Following a dose of 10,000 spores to bees kept at +30 °C, the growth rate of *N. ceranae* is similar to that of *N. apis*, but mature spores are produced a day or so later (Paxton et al., 2007). Both parasites reach approximately 30 million spores in the midgut after 10–12 days although bees may need to be kept longer to reach the maximum spore number in *N. ceranae* (Paxton et al., 2007). Martín-Hernández et al., 2009 reported different growth curves for both parasites at +33 °C with spores produced much faster in *N. ceranae*. However, they (Martín-Hernández et al., 2009) used a dose of 100,000 spores per bee and investigated the whole abdomen and not just the ventriculus. The discrepancy in growth

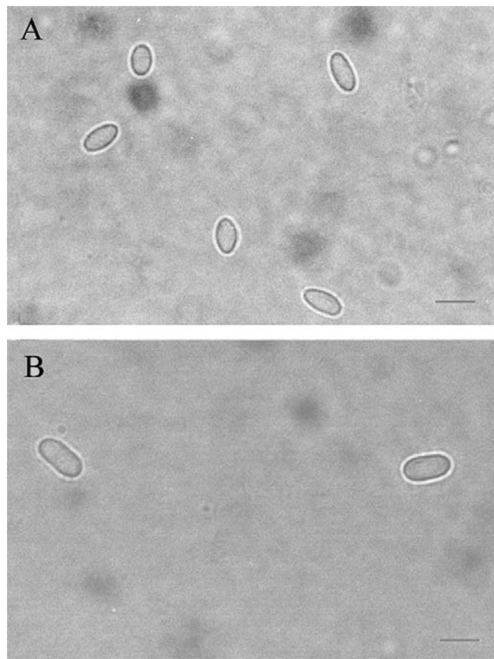

Fig. 1. Spores of *N. ceranae* (A) are distinctly smaller than spores of *N. apis* (B). Nevertheless, they can be hard to distinguish by light microscopy, in particular where mixed infections occur. Bars = 5 μm. (From Fries et al. 2006a).

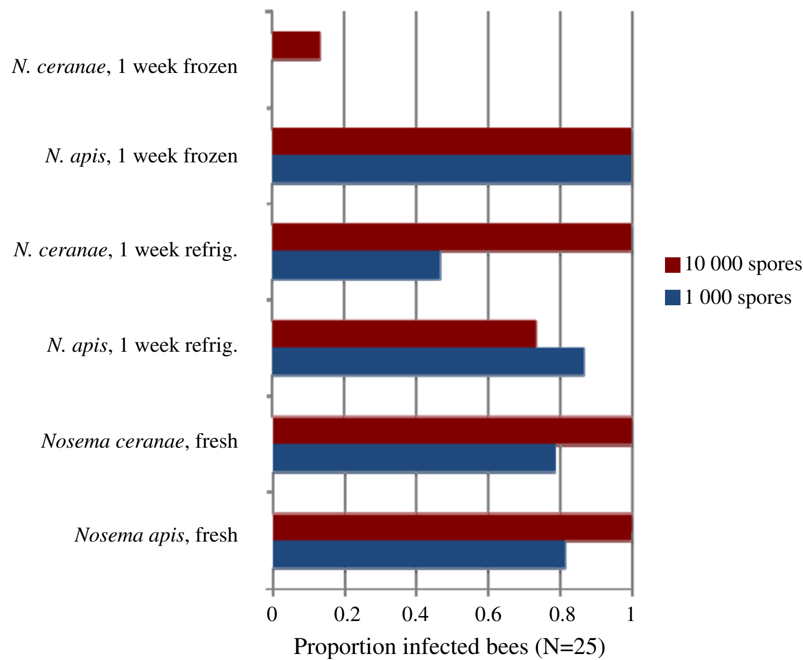

Fig. 3. The effect of freezing on *N. ceranae* spore viability is dramatic. Each bar represents 25 bees individually fed with 10 µl sugar suspension containing 1000 or 10,000 spores of either *N. ceranae* or *N. apis*. Spores were fed from fresh infections (fresh) or (using the same suspension) after one week in a refrigerator at +8 °C (refrig.) or in a deep freezer at −18 °C (frozen). (From Fries and Forsgren, 2009).

pattern may be due to different methods used, but also to different temperatures at which infected bees were exposed. Further infection experiments are warranted to study the progress of infection in individual bees.

The infectious dose of *N. apis* has previously been reported to be close to 100 spores per bee (Fries, 1988). Recent experiments suggested that the infectious dose of *N. ceranae* may be of this magnitude, although the infectious dose was slightly higher for *N. apis* in these experiments. It needs to be verified if there is a difference between the two species in infectious dose. When both parasites occur in the same bee, it does not appear as if one parasite outcompetes the other, when bees infected with similar doses of both parasites are subsequently analyzed for parasite DNA using qPCR (Fries and Forsgren, 2009, unpublished).

It was specifically noted in the species description of *N. ceranae* that emptied spores of the parasite were not present in the host cell cytoplasm of *A. cerana* (Fries et al., 1996). Emptied spores in the host cytoplasm are always present when mature spores of *N. apis* can be seen in *A. mellifera*, and are interpreted as the means by which the parasites can spread between cells in the ventricular epithelium (Fries et al., 1992). Interestingly, Higes et al. (2006) found emptied spores of *N. ceranae* in infected ventricular cells of *A. mellifera* but such structures were not evident in the micrographs presented by Chen et al. (2009). This aspect may require further attention. If intracellular germination of spores occurs in one region and not in the other, or in some isolates and not in others, disease progression may also be different. The presence of emptied spores probably means that within host transmission is more effective for that microsporidian in that tissue. Without intracellular germination the parasite spores probably need to re-enter the ventricular lumen and re-infect the epithelium for intra-host transmission whereas intracellular germination also offers intercellular transmission.

A major effect of *N. apis* infections on individual bees that can lead to strong colony level impacts is the atrophy of the hypopharyngeal glands of infected bees (Lotmar, 1936, 1939). This atrophy leads to poor spring build up and low honey production (Lotmar, 1936). Because *N. ceranae* infects this same tissue, it is likely that both infections have the same effect, but this needs to be verified. It is clear that the parasites do not necessarily have the same effects either on individual bees (infection with *N. ceranae* is not associated with dysentery), or on infected bee colonies (colonies are reported to collapse in Spain). Thus, all work done on *N. apis* biology and epidemiology now needs to be repeated for *N. ceranae*.

6. Control

Until more research is available on the biology and transmission of *N. ceranae* it is difficult to say if general recommendations for *N. apis* (i.e. wax renewal, acetic acid fumigation of stored comb) are also relevant for *N. ceranae* control. The major commercial medication available, based on the antibiotic fumagillin, is effective on both parasites (Williams et al., 2008). However, in contrast to some other parts of the world where *N. ceranae* infections may be controlled using fumagillin, antibiotic treatments of honey bee colonies are not legal in most parts of Europe.

7. Conclusions

Several puzzles remain with respect to the importance of *N. ceranae* for honey bee health. Infections of *N. ceranae* appear to give different colony level effects in different geographical regions. Furthermore, the seasonal variations and gross colony level symptoms described for *N. apis* seem not to be present in *N. ceranae*. At the individual level, there are differences between the two parasites, but virulence differences remain to be conclusively verified. The spores of *N. ceranae* appear to be much more vulnerable than spores of *N. apis*, in particular to freezing, and the apparent replacement of *N. apis* for *N. ceranae* remains enigmatic. These and other remaining questions warrant increased research on the impacts of *N. ceranae* on individual bees, colonies, and populations of European honey bees.

The impact of *N. ceranae* infections on the development of *A. cerana* colonies also needs to be investigated. There is presently a growing interest in *A. cerana* beekeeping both for conservation purposes and because this species of honey bee has some advantages compared to *A. mellifera*, such as tolerance to the mites *Varroa destructor* and *Tropilaelaps clareae*. An increased understanding of how *N. ceranae* invades its presumed original host, and how *A. cerana* resists or tolerates such invasions, will therefore be of interest for these species and, by inference, *A. mellifera.*

Conflicts of interest

There are no conflicts of interest to be declared.

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