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REVIEW ARTICLE

Does infection by *Nosema ceranae* cause "Colony Collapse Disorder" in honey bees (*Apis mellifera*)?

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Summary

Nosema ceranae is an emergent and potentially virulent pathogen of the honey bee (*Apis mellifera*) that has spread across the world in the last 10 or so years. Its precise origin and timing of spread are currently unclear because of a lack of appropriate genetic markers and inadequate sampling in putative Asian source populations. Though it has been dismissed as a cause of CCD in the USA based on correlational analyses of snapshot sampling of diseased hives, observations of naturally infected colonies suggest that it leads to colony collapse in Spain. Experiments are sorely needed to investigate its impact on individuals and colonies, and to pin down a causal relationship between *N. ceranae* and colony collapse. Whether *N. ceranae* is displacing *N. apis* is uncertain. For temperate zone apiculturalists, global climate change may mean that *N. ceranae* presents more of a challenge than has hitherto been considered the case.

¿ Causa el "Síndrome del Colapso de las Colmenas" en la abeja de la miel (*Apis mellifera*) la infección por *Nosema ceranae*?

Resumen

Nosema ceranae es un patógeno emergente y potencialmente virulento de la abeja de la miel (*Apis mellifera*), que se ha propagado en todo el mundo en los últimos 10 años. En la actualidad su origen exacto y el tiempo de propagación no están claros debido a la falta de marcadores genéticos adecuados y a la toma inadecuada de muestras en poblaciones de supuesto origen asiático. Aunque ha sido descartado como causa del Síndrome del Colapso de las Colmenas (SCC) en los EE.UU. a partir de análisis de correlación de la toma instantánea de muestras de colmenas enfermas, las observaciones de las colonias infectadas de forma natural sugieren que *Nosema ceranae* conduce al colapso de las colmenas en España. Los experimentos son muy necesarios para investigar su impacto en los individuos y las colmenas, y para definir una relación causal entre *N. ceranae* y el colapso de las colonias. El desplazamiento de *N. apis* por *N. ceranae* es incierto. Para los apicultores de las zonas templadas, el cambio climático global puede significar que *N. ceranae* representa un desafío mayor del considerado actualmente.

Keywords: CCD, Microsporidia, nosemosis, emergent, invasive, disease, *Nosema apis*

Introduction

Microsporidia are a highly derived group of fungi (Lee *et al.,* 2008) that are obligate intracellular parasites of many animal species, particularly insects (Larsson, 1986). Nosema disease (nosemosis), the original causative organism of which was identified as the unicellular microsporidium *Nosema apis* exactly 100 years ago (Zander, 1909), is considered to be one of the most prevalent and economically damaging of diseases of the western honey bee, *Apis mellifera*, and much information has accumulated on its biology, site of infection (ventricular cells of adult bees) and its impact on individual bees and colonies (Fries *et al.,* 1984; Fries, 1993). Yet it often goes unnoticed because *N. apis*, like all microsporidia, is microscopic in size and invisible to the naked eye and because *N. apis* rarely leads to the death of a diseased colony. Matheson (1993) reported the widespread distribution of *N. apis* across the natural and introduced world range of *A. mellifera*, and DNA sequence data support the view that this microsporidium was indeed the sole causative agent of Nosema disease in the western honey bee up to the 1990s (Klee *et al.,* 2007).

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History of discovery of *Nosema ceranae*

In 1994, Fries *et al.* (1996) discovered and described a new microsporidium, *Nosema ceranae*, infecting adults of the eastern honey bee, *Apis cerana*, around Beijing, China. Differences between the two microsporidia, *N. apis* and *N. ceranae*, lie in their ultrastructure and small subunit (16S) rRNA gene sequence (see Fig. 1; Fries *et al.* 2006), allowing ready identification by transmission electron microscopy and DNA sequencing respectively. Indeed, the rRNA gene sequence seems to be an excellent DNA barcode (sensu Valentini *et al.,* 2009) to differentiate among these and other microsporidian species (Klee *et al.,* 2006), but not for intraspecific characterisation of variants (O'Mahony *et al.,* 2007).

Though cross-infection experiments demonstrated that *N. ceranae* was infective for the western honey bee (Fries and Feng, 1995), little more was made of the observation until *N. ceranae* was detected in *A. mellifera* in the spring of 2005 in Taiwan (Huang *et al.,* 2007), within the natural range of *A. cerana*. In summer 2005 the first confirmed record of *N. ceranae* in *A. mellifera* was made within the western honey bee's natural range, in Spain (Higes *et al.,* 2006), and outside the range of *A. cerana*. Its spread has undoubtedly come about through increased international trade, as has that of many other infectious microorganisms whose human mediated dispersal is occurring at unprecedented levels through the global transport network (Wilson *et al.,* 2009).

Origins of *Nosema ceranae*

Because *N. ceranae* was first isolated from *A. cerana* near Beijing, it may be assumed that its natural host is the eastern honey bee and its natural distribution is Asia. There is little evidence of its occurrence outside Asia pre-1994 (Klee *et al.*, 2007; Paxton *et al.,* 2007), supporting an Asian origin, though Invernizzi *et al.* (2009) report a single pre-1990 isolate from Uruguayan *A. mellifera* to contain *N. ceranae*. Whether *A. cerana* was its only host, or its principal host, or a source of cross-species infection for *A. mellifera*, is less clear. This is because sampling of other Asiatic honey bee species or other potential Asian host (bee or other insect) species has not been undertaken for *N. ceranae*. In addition, its recent discovery in Argentinean bumble bees (*Bombus* spp.; see Plischuk *et al.,* 2009) suggests that it may have a wider host range in Asia than *A. cerana*.

An understanding of the original source, with respect to both host and geographic location, of *N. ceranae* infections in western honey bees will require use of polymorphic genetic markers to differentiate intraspecific variants or strains. So-called haplotypes of *N. ceranae* have been defined on the basis of rRNA sequence variants (Huang *et al.,* 2008; Williams *et al.,* 2008a), but rRNA may be inappropriate for such studies. Micropsoridia contain multiple copies of rRNA, not all of which are identical in sequence (Tay *et al.,* 2005). In the closely related *Nosema bombi*, each spore contains a range of rRNA sequence variants (O'Mahony *et al.,* 2007), making invalid the use of rRNA as a haplotype marker because homologues cannot be reliably compared between host isolates. As is the case for other microsporidia, there is a need to develop single-locus polymorphic markers for *N. ceranae* to allow study of its phylogeography and the source of this emergent invasive disease (EID) in *A. mellifera*.

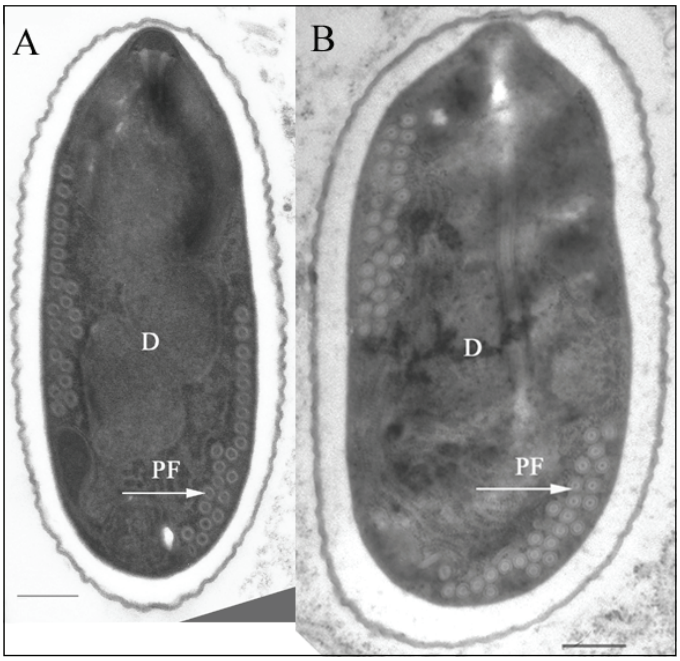

Fig. 1. Transmission electron micrographs of spores of (A) *Nosema ceranae* and (B) *Nosema apis*. D: diplokarya; PF with arrows: coils of the polar filament. The scale bar is 0.5 µm. (photos: Ingemar Fries).

Date of spread of *Nosema ceranae*

When originally discovered in Europe in 2005, it was assumed that *N. ceranae* was a recent arrival (Higes *et al.,* 2006). Sampling of historical material has demonstrated that it was present in European *A. mellifera* from 1998 (Klee *et al.,* 2007) and perhaps from the mid-1990s in the USA (Chen *et al.,* 2008) and possibly elsewhere (e.g. Invernizzi *et al.*, 2009). Older records of *Nosema* from *A. mellifera* are, however, with one exception (Invernizzi *et al.*, 2009), all of *N. apis*. We can therefore be fairly confident in assuming that *A. mellifera* was not an original host of *N. ceranae*, and that *N. ceranae*, or a particularly virulent strain of *N. ceranae*, has recently jumped the species barrier into *A. mellifera* to become an EID.

Current distribution of *Nosema ceranae*

Klee *et al.,* (2007) analysis of *Nosema* isolates from *A. mellifera* from across the world, interrogation of DNA databank entries and published records (based on rRNA sequence data) indicated that, post-2003, *N. ceranae* was widespread, and already found in North and South America, across Europe and Asia. It has been subsequently detected

across Canada and USA (Chen *et al.,* 2008; Williams *et al.,* 2008a) and has been confirmed in Central America (Calderón *et al.,* 2008), Australia (Giersch *et al.,* 2009) and North Africa (Higes *et al.*, 2009b).

Impact of *Nosema ceranae* and "Colony Collapse Disorder" (CCD)

Since its emergence as a novel pathogen of *A. mellifera*, *N. ceranae* has been generally associated with heavily diseased and moribund colonies (Vejsnæs *et al.*, 2010). For example, the first report of *N. ceranae* in European *A. mellifera* attributed heavy winter 2004-2005 colony losses in Spain to this EID (Higes *et al.,* 2006). Our studies of *Nosema* in Vietnamese *A. mellifera* were prompted by heavy nosemosis and poor colony health in that country's Bee Research and Development Centre, Hanoi, in spring 2005 (Dr Dinh Quyet Tam, personal communication). Of course, the association between *N. ceranae* and poor health of *A. mellifera* colonies may simply represent reporting bias; a novel disease organism in moribund colonies will be understandably publicised whereas it may go unsampled and unnoticed if it does not have a marked pathological effect on its novel host.

The detailed metagenomic survey of CCD affected colonies of *A. mellifera* in the USA (Cox-Foster *et al.,* 2007) recognised *N. ceranae* as a potential causative agent of CCD but statistically ruled it out as the primary agent responsible for CCD. It is worth considering the results of this study in more detail. Of 30 CCD-affected colonies, all were positive for *N. ceranae*. Ten of 21 (47%) non CCD affected colonies were also, however, positive for *N. ceranae* (see Table 2 of Cox-Foster *et al.,* 2007). Statistically, then, the presence of *N. ceranae* in a colony was not a good predictor of whether the colony had collapsed. As the authors themselves are, however, careful to point out, their metagenomic survey may be inappropriate for determining the cause of CCD (Cox-Foster *et al.,* 2007). Firstly, it was a correlational study and, secondly, it only took a "snapshot in time" of the prevalence of disease organisms in colonies. Disease organisms build up over time (i.e. increase in larval / adult incidence of infection) before causing colony mortality, and generally do not act instantaneously. As *N. ceranae* has been reported to build up in prevalence within a colony over an 18 month period before causing colony demise (Higes *et al.,* 2008; 2009b), the dynamic nature of this and other infectious agents cannot be captured by a snapshot analysis of disease organisms in colonies at one point in time. The study of Cox-Foster *et al.* (2007) therefore still leaves open the possibility that *N. ceranae*, alone or in combination with other factors, causes CCD.

Evidence from Europe of naturally infected hives is compelling. Three recent publications on Spanish honey bee colonies suggest that *N. ceranae* causes colony collapse within 18 months of a colony becoming infected (Martín-Hernandéz *et al.,* 2007; Higes *et al.,* 2008, 2009b). If *N. ceranae* is so virulent as to kill colonies in 18 months then it is surprising that greater colony losses have not been reported from across Europe, given that *N. ceranae* has been in the continent since at least 1998. Spanish *A. mellifera* may be more susceptible to *N. ceranae* than other honey bee races, or the variant of *N. ceranae* within Spain may be more virulent than that found elsewhere, suggestions which deserve attention. Experimental infection of colonies is also necessary to show causation between *N. ceranae* and colony collapse; such experiments are currently lacking.

Experiments on caged worker bees have nevertheless revealed *N. ceranae* to be a potentially highly virulent pathogen (Higes *et al.,* 2007), one that seems to be more pathogenic than *Nosema apis* (Paxton *et al.,* 2007). Worryingly, Martín-Hernandéz *et al.* (2009) have recently suggested that *N. ceranae* may even have superior growth within its host than *N. apis* at a realistic range of environmental temperatures. In addition, *N. ceranae* places additional nutritional stress on individual bees (Mayack and Naug, 2009; Naug and Gibbs, 2009), which may lead to riskier foraging and greater mortality of forager bees away from the hive. These effects of *N. ceranae* on the nutritional stress of individual worker bees may be exacerbated by a shortage of resources (pollen and nectar) (Naug, 2009), providing a mechanistic model for CCD that links an EID with habitat loss. How these laboratory differences translate into field effects on individual bees and colonies needs to be investigated so as to evaluate whether *N. ceranae* leads to colony demise and causes CCD.

Outlook

Nosema ceranae is clearly a novel, emergent pathogen of *A. mellifera* with potentially very serious effects on the individual and colony. Whether it causes CCD, alone or in combination with other synergistic factors such as habitat loss and lack of floral resources, is still an open question. Under a scenario of global climate change, *N. ceranae* may exert an increasing impact on world beekeeping with *A. mellifera*, analogous to the impact of emergent fungal pathogens tied to global warming on amphibian populations (Pounds *et al.,* 2006). Research is sorely needed (i) to develop genetic markers for *N. ceranae* to differentiate intraspecific strains and (ii) to experimentally demonstrate a causal link between *N. ceranae* and CCD. That *N. ceranae* can be controlled by the fungicide fumagillin is of some reassurance for apiculture with *A. mellifera* (Martín-Hernandéz *et al.,* 2007; Higes *et al.,* 2008; 2009b; Williams *et al.,* 2008b).

There is little knowledge of microsporidian pathogens of other bee species (Murray *et al.,* 2009). *Nomema bombi* is a widespread pathogen of many bumble bee species (Tay *et al.,* 2005) that reduces colony productivity (Otti and Schmid-Hempel, 2008). *Antonospora scoticae* infects the communal bee *Andrena scotica* (Fries *et al.,* 1999), with potentially profound effects on its host (Paxton *et al.,* 1997). That *N. ceranae* has now been detected in Argentinean

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Bombus spp. (Plischuk *et al.,* 2009) is clearly cause for concern because, though world crop yields of pollinator-dependent crops have not decreased over the past 45 years, our dependence on these crops has increased over the same period (Aizen *et al.,* 2008). We are more reliant than ever on the ecosystem service of pollination, yet the threats posed by EIDs on bees, the major pollinators of the Angiosperma, have never been greater.

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