The ecology and evolution of microsporidian parasites

J. E. SMITH

Institute of Integrative and Comparative Biology, Faculty of Biological Sciences, The University of Leeds, Leeds LS2 9JT, UK

(*Received 13 August 2009; revised 9 November 2009; accepted 9 November 2009*)

SUMMARY

The phylum Microspora is ancient and diverse and affects a wide range of hosts. There is unusually high use of vertical transmission and this has significant consequences for transmission and pathogenicity. Vertical transmission is associated with low pathogenesis but nevertheless can have significant impact through associated traits such as sex ratio distortion. The majority of microsporidia have mixed transmission cycles and it is not clear whether they are able to modify their phenotype according to environmental circumstances. There is a great need to understand the mechanisms controlling transmission and one of the first challenges for the genomics era is to find genes associated with life cycle stages. Similarly we cannot currently predict the ease with which these parasites might switch between host groups. Phylogenetic analysis suggests that there are strong relationships between Microsporidia and their hosts. However closer typing of parasite isolates, in relation to host range and disease phenotype, is required to assess future environmental risk from these pathogens.

Key words: Microsporidia, Microspora, vertical transmission, ecology, evolution.

THE PHYLUM MICROSPORA: AN ANCIENT AND DIVERSE GROUP

Microsporidia are very common parasites which are widespread among animal hosts. They are best known as parasites of insects but are not restricted to this host group and are in fact found across a vast range of host taxa including, protists, bryozoa, nematodes, oligochaete worms, insects, fish and mammals including humans (Becnel and Andreadis, 2001; Canning *et al*. 2002; Lom and Nilsen, 2003; Didier, 2005; Morris *et al*. 2005; Fokin *et al*. 2008; Troemel *et al*. 2008). This widespread distribution in nature is consistent with an ancient origin for the phylum Microspora. Phylogenetic analysis places the group as early branching eukaryotes. Microsporidia have 16S rather than 18S ribosomes and lack 5·8S ribosomal RNA and on this basis were originally considered to be primitive eukaryotes (Curgy *et al*. 1980). Although this assertion was initially supported by molecular phylogeny of SSUrDNA (Vossbrink *et al*. 1987) subsequent analysis, based on multiple genes, placed the group closer to the fungi (Hirt *et al*. 1999; Keeling *et al*. 2000; James *et al*. 2006). Recently Lee *et al*. (2008) compared both the identity and synteny of multiple loci across the genomes of microsporidian and fungal species and concluded that the Microspora are highly derived fungi descended from a zygomycete ancestor.

The diversity of the phylum indicated by phylogenetic analysis is currently based on analysis of SSUrDNA with approximately 200 sequences available from microsporidia infecting a wide range of hosts. Reconstruction of the phylogeny (Fig. 1) indicates the presence of five major, deep-rooted clades each of which shows considerable divergence (Terry *et al*. 2004; Vossbrinck and Debrunner-Vossbrinck, 2005). The level of variation in ribosomal RNA sequence across the phylum is very high (Keeling and Fast, 2002) and analysis of sequence divergence reveals major insertions and deletions within each clade. Within each major lineage SSU rRNA is sufficiently polymorphic to discriminate between microsporidian genera, as for example in clade IV where the mammalian infective genus *Encephalitozoon* is very clearly separated from related genera such as *Ordospora, Enterocytozoon* and *Vitaforma* and *Endoreticulatus* and *Nosema* (Fig. 1). Similarly, clear separation of taxa is seen within Clade I which mainly contains parasites of Diptera (Vossbrinck *et al*. 2004) and Clade III, which is dominated by species infecting fish (Lom and Nilsen, 2003).

The high divergence apparent from phylogenetic analysis is echoed in the size and structure of microsporidian genomes. *Encephalitozoon cuniculi*, the first microsporidian parasite to be sequenced, famously has the smallest genome of any eukaryote with eleven chromosomes of 2–300 kb and a total genome size of 2·9 Mb (Katinka *et al*. 2001). There is evidence that the reduced genome size is associated

Corresponding author: Judith E. Smith, Institute of Integrative and Comparative Biology, Miall Building, Faculty of Biological Sciences, The University of Leeds, Leeds LS2 9JT, UK Tel: 0044 (0) 113 343 2892. E-mail: j.e.smith@leeds.ac.uk

doi:10.1017/S0031182009991818

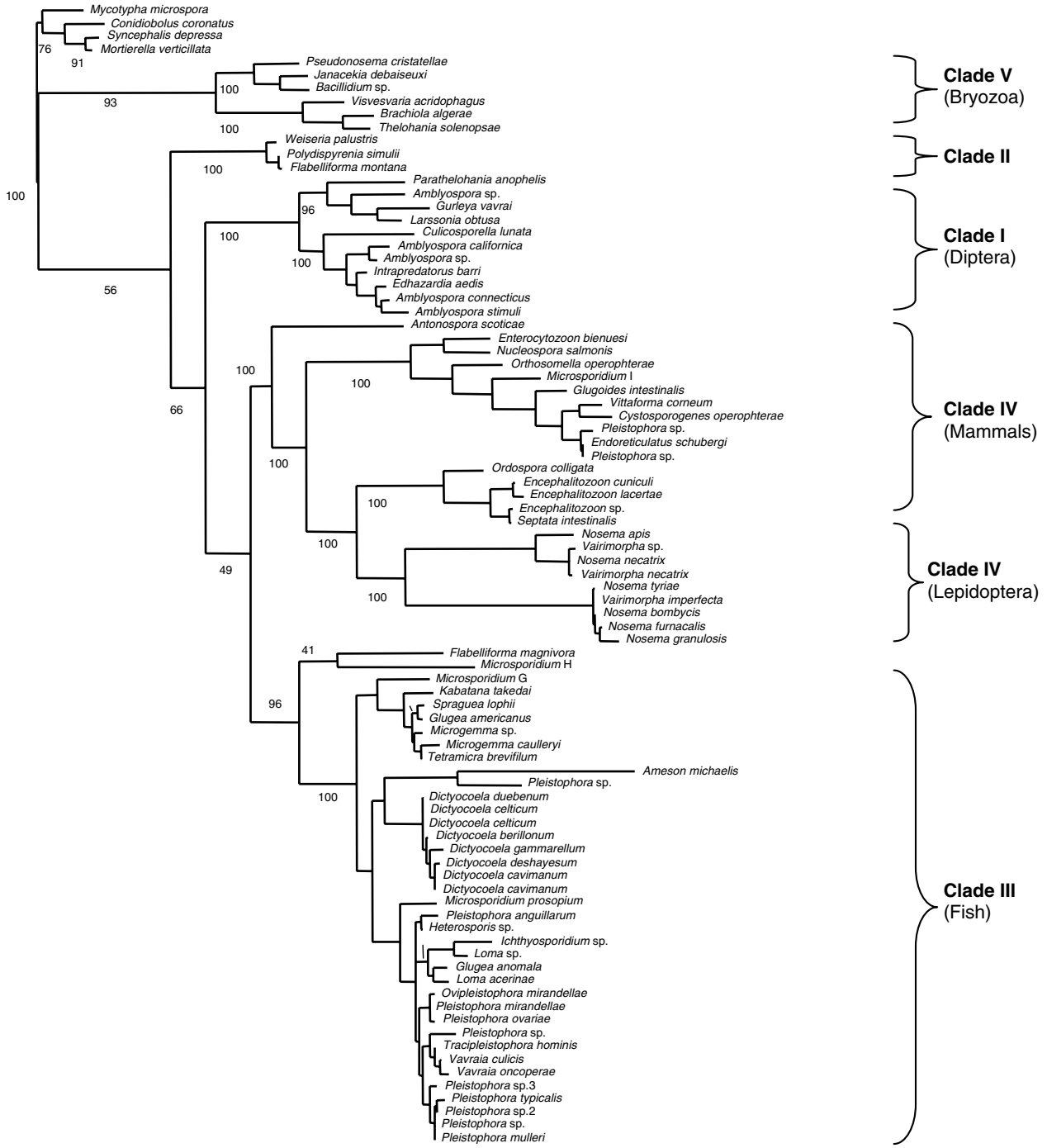

Fig. 1. Microsporidian phylogenetic tree. The tree is based on around 800 bp 800 bp SSU rDNA sequences were used to reconstruct a phylogenetic tree of 315 microsporidian parasites with 4 species of fungi representing Chytridiomycota, Zygomycota, Ascomycota as outgroups. MrBayes v 3.1.1 (Huelsenbeck and Ronquist, 2001) was used to infer these Bayesian analyses over 8,000,000 generations, and nodal support was assessed by posterior probabilities estimated from the final 75% (60,000) sampled trees.

with loss of metabolic function. *E. cuniculi* has only 1,997 predicted proteins and lacks many enzymes, such as those involved in the tricarboxylic acid cycle, or in *de novo* synthesis of amino acids, purines and pyrimidines. *Encephalitozoon* is not, however, a typical representative of the phylum and other microsporidia have substantially larger genome sizes. The mammal-infective species *Enterocytozoon bieneusi* has a predicted genome size of 6 Mb predicted to encode 3,800 genes (Akiyoshi *et al.* 2009), *Paranosema locusta* (synonymous with *Antonospora*/*Nosema locusta*) is estimated at 5·4 Mb (Streett, 1984). The genome sizes of several parasites from the *Nosema*/*Vairimorpha* clade have been estimated by pulsed field methodology to be around 10 Mb (Malone and McIvor, 1993) while *Nosema bombycis*

https://doi.org/10.1017/S0031182009991818 Published online by Cambridge University Press

at 15·6 Mb (Xu *et al*. 2006) and *Brachiola algerae* at approximately 23 Mb (Belkorchia *et al*. 2008) have much larger genomes. One of the fascinating aspects of microsporidian genomes is their high degree of compaction. This was first noted in *Encephalitozoon* where gene prediction models suggested there were no introns and many overlapping transcripts (Katinka *et al*. 2001). This unusual feature is seen in other microsporidia with small genomes and may a be general characteristic of compacted eukaryotic genomes (Williams *et al*. 2005). Emerging evidence indicates that multigene transcripts are not common among microsporidia with larger genomes (Belkorchia *et al*. 2008, Corradi *et al*. 2008, Gill *et al*. 2008).

Microsporidia exhibit some morphological traits that are consistent with their fungal origins, such as the formation of an intranuclear spindle during mitosis (Bigliardi *et al*. 1998) and the chitin-rich wall of the spore stage: however, their cellular structure is unusual. There are two basic life-cycle stages, the proliferative stage, the intracellular meront which lies directly within the host cell cytoplasm and spore stage which mediates transmission (Dunn *et al*. 2001). The meront is adapted to scavenge nutrients directly from the host cell: it lies directly in the host cell cytoplasm and thus has direct access to nutrients. Microsporidia have a vestigial mitochondrial organelle, or mitosome, which does not have support oxidative phosphorylation but retains a role in iron sulphur cluster assembly (Williams *et al*. 2002, Goldberg *et al*. 2008). The parasites scavenge ATP from the host cell through the activity of nucleotide transporters (Tsaousis *et al*. 2008) and close interactions between the meront plasma membrane and host cell mitochondria may enhance this energy scavenging (Terry *et al*. 1997). In species such as *Encephalitozoon*, meronts divide within a parasitophorous vacuole. Evidence suggests that this vacuolar membrane is of host origin and, similar to the vertebrate pathogen *Toxoplasma gondii*, is porous to small molecules (Ronnebaumer *et al*. 2008). Interaction with mitochondria is preserved and they are tethered to the vacuolar membrane (Scanlon *et al*. 2004).

The spore stage is highly adapted for transmission and has an elaborate structure designed to deliver the parasite directly into the host cell cytoplasm (Dissanaike and Canning, 1957; Vavra, 1976). The nucleus and cytoplasm, which is densely packed with ribosomes, are enclosed within by multilayered endosprore and exospore walls. The anterior section of the spore contains a membranous organelle, the polaroplast and the hollow coiled polar filament attached via an anchoring disc to the spore wall (Sinden and Canning, 1974). Once triggered to hatch, the spore absorbs water and the polaroplast swells, increasing pressure in the cell which causes the polar filament to shoot out to penetrating the host cell membrane and allowing the sporoplasm to enter the cytoplasm of the host cell (Undeen and Vandermeer, 1994; Xu and Weiss, 2008). The spore, with its tough external wall and specialised 'injection mechanism', appears highly adapted for survival and dissemination in the extracellular environment but, in fact, certain spore types are also adapted to mediate cell to cell transmission within the host (Iwano and Kurtti, 1995; Terry *et al*. 1999).

The core life-cycle stages, the meront and spore found in all microsporidian parasites but both the structure of these stages and the way the complexity of the parasite life cycle may vary. For example, the spore can vary massively in size and structure, i.e. *Bacillidium vesiculoformis* produces rod-like spores $12{\cdot}2 \times 1{\cdot}3\ \mu$m while the mollusc-infective species *Steinhausia mytilovum* produces spherical spores of approximately $1{\cdot}5\ \mu$m in diameter (Sagrista *et al*. 1998; Morris *et al*. 2005). The fine structure of the spore, including the presence of a monokaryotic or diplokaryotic nucleus, the organisation of endospore and exospore walls, the morphology of the polaroplast and the structure and number of coils in the polar filament all show many differences (Vavra and Larsson, 2001). These phenotypic traits have been the basis for taxonomic classification of the phylum Microspora, together with information on the life cycle and on the host species infected. The complexity of the life cycle varies greatly: among the most straightforward examples is the parasite *Brachiola algerae*, (synonymous with *Nosema algerae*) which was first isolated from mosquitoes, but has the potential to infect humans. Diplokaryotic meronts divide directly in host cell cytoplasm and enter disporous sporogony, leading to the production of a single diplokaryotic spore type. Multiplication of the parasite is rapid and asynchronous causing severe disruption of infected muscle cells (Vavra and Undeen, 1970; Cali *et al*. 2004). The most complex life cycle is potentially that of *Eharzardia aedis* which has four different sporulation sequences (Becnel *et al*. 1989; Johnson *et al*. 1997).

In terms of alpha taxonomy, approximately 140 genera of microsporidia have been described (Sprague *et al*. 1992) on the basis of morphological data. There are ongoing efforts to resolve the relationships between molecular phylogeny and alpha taxonomy. These studies suggest that while phenotypic data are often support phylogenetic relationships they are insufficient for taxonomic assignment. An example of this is seen in polyphyletic origins of parasites assigned to the genus *Nosema*. Many parasites were designated to this genus on the basis of structural similarity to the type species *N. bombycis*, which falls into Clade IV of the phylum, but it is clear from molecular studies that this phenotype is also widespread in Clade V. Three branches can be resolved in clade V one of which contains *Brachiola algerae*, originally *N. algerae* (Vavra and

Undeen, 1970), together with the crustacean parasite *Fibrillonosema crangonycis* (Slothauber-Galbreath *et al*. 2004). A second branch contains the orthoptera-infective parasites *Paranosema locusta*, originally *Nosema locusta* (Canning, 1953), and *Paranosema whiteii* (Soklova *et al*. 2003, 2005) while the third branch also contains *Nosema*-like parasites infecting bryozoans, designated *Pseudonosema*, *Trichonosema* and *Bryonosema* (Canning *et al*. 2002). Although the systematics of phylum Microspora is under active review, additional sequence data from new host groups continues to support division of the phylum into five major clades (Terry *et al*. 2004; Vossbrinck and Debrunner-Vossbrinck, 2005), while integration of morphological and molecular data adds to the resolution of these lineages.

In conclusion, the phylum Microspora contains a group of unusual and highly derived fungi that they have a range of unusual structural adaptations and high dependency on their hosts. They are highly diverse with significant differences in their structure, life cycle, genome organisation and cellular biology. Their presence as pathogens across all animal taxa is consistent with ancient origins and makes them an excellent model to study the host parasite evolution and transmission.

FROM PASTEUR TO POLLINATORS: TRANSMISSION AND DISEASE IN BENEFICIAL INSECTS

The first microsporidian parasite to be described was *Nosema bombycis* the causative agent of pebrine disease in the silkworm *Bombyx mori*. Pasteur's experiments noted the presence of 'corpuscules' in the egg, the first description of the parasite linked with Vertical Transmission (VT) (Pasteur, 1870). The life cycle of the parasite is described from both *in vitro* and *in vivo* studies and is relatively straightforward (Ishihara, 1969; Iwano and Ishihara, 1991). In the silkworm larva, infection begins when spores infect the midgut epithelium, the diplokaryotic meront divides directly in the cytoplasm and generates sporonts which undergo disporoblastic development to produce spores. Two types of spores are formed, those with a long polar filament with thirteen coils, which are destined to transmit the infection between hosts and those with a short polar filament of only three coils which are responsible for disseminating infection from the midgut to muscle and other tissues. The parasite has a mixed transmission strategy with and disease can be spread horizontally between larvae during sericulture (Ishihara and Fujiwara, 1965) but can also be transmitted vertically from the adult silkworm to the egg (Han and Watanabe, 1988). Infection of larvae is most severe in the first second and fifth instars and can cause significant mortality in production systems, but larvae infected in late instars are likely to progress to adults and transmit the disease vertically. This mixed strategy both maximises transmission between individuals in phase of population growth and enables to shift to next generation in a discontinuous production system.

The impact of *Nosema* infection in sericulture is massive as the profit margin is tied to the number of cycles of production and repeated crashes can decimate productivity. Serious steps are taken to control the infection in the industry by trying to eradicate vertical transmission. Silkworm eggs are produced centrally by crossing different genetic stocks of *Bombyx*. In the initial cross adult females are screened for the presence of parasites and any eggs from infected batches are discarded, thus providing 'sterile' material for the regional sericulture industry (Hatakeyama and Hyasake, 2003; Liu *et al*. 2004). Clearly outbreaks do still occur and these could be due either to vertical transmission through low level contamination of eggs, below the limit of detection of current diagnostic methods, or horizontally through introduction of parasites deposited on Mulberry leaves by indigenous host species.

There are many unresolved questions about the diversity and host range of microsporidia causing pebrine disease. It is uncontroversial that the type species for the genus *Nosema bombycis* is responsible for much of the disease seen in sericulture, but it has been shown that a number of lepidopteran species are susceptible to this species (Kashkarova and Khakhanov, 1980) raising the possibility that reservoirs of disease may exist in wild hosts. It has also been noted that there is genetic diversity among parasites causing the disease, (Rao *et al*. 2005, 2007). The genus *Nosema* is subdivided into two clades, the 'true Nosemas', including *Nosema bombycis*, and the Vairimorpha clade (Baker *et al*. 1994). Members of the Vairimorpha clade have a second developmental cycle in which haploid octospores are produced within a sporophorous vesicle (Vavra *et al*. 2006). Studies on disease phenotype and the phylogenetic relationships of silkworm isolates reveal that both the virulence and the capacity for vertical transmission may vary and parasites from either of the two *Nosema* clades can be responsible for disease (Rao *et al*. 2005, 2007).

Microsporidian infection is also of concern in other beneficial insects, particularly pollinator species. There are three species of high importance *Nosema apis*, *N. ceranae* and *N. bombi*, all of which fall into the *Nosema/Vairimorpha* clade. *N. apis* has long been known as a parasite of the honey bee *Apis mellifora* (Fantham and Porter, 1912). The parasite is primarily transmitted horizontally through ingestion of spores that establish infection in the midgut (Fries, 1989, 1992). The severity of infection clearly varies but the parasite infects both workers and queens (Webster *et al*. 2004) and can cause mortality of adult bees (Malone and Giacon, 1996) and regression in the ovaries of queens (Liu, 1992). In social insects,

https://doi.org/10.1017/S0031182009991818 Published online by Cambridge University Press

such as bees, infection of the queen is critical in disseminating infection both within the colony and to new colonies during reproductive swarming (Czekonska, 2000; Fries and Camazine, 2001). There is no evidence of transovarial transmission in infected queens (Webster *et al*. 2008) but their role in infecting new colonies which might be regarded as a form of vertical transmission. A second species, *N. ceranae*, originally from the Asian honey bee *Apis ceranae* (Fries *et al*. 1996), has recently spread into *Apis mellifera* and appears to be more pathogenic than *N. apis* (Klee *et al*. 2007, Paxton *et al*. 2007). This parasite has been proposed as a cause of colony collapse disorder (Higes *et al*. 2008) but this syndrome, which is currently so damaging to honey bee populations, is likely to have multifactorial origins (Cox-Foster *et al*. 2007; Watanabe, 2008).

It is not only the honey bee which is affected by *Nosema* parasites since commercially-reared and wild bumblebees are also affected. *N. bombi* causus disseminated infection in the host (Fries *et al*. 2001) and is widespread in *Bombus* spp. (Larsson, 2007). Experimental studies show that the parasite can be highly detrimental causing mortality in workers and almost complete loss reproductive capacity in infected gynes (Otti and Schmid-Hempel, 2007). Such high virulence might be deemed to lead to extinction of the parasite but it seems that parasite virulence may vary between different bumble bee species sustaining the parasite in the environment (Rutrecht and Brown, 2009). There is some evidence to suggest that *N. bombi* is transovarially transmitted but the importance of this in disease epidemiology is not fully understood (Rutrecht and Brown, 2007).

The key challenges in microsporidiosis of beneficial insects lie in understanding the role or vertical and horizontal transmission in relation to disease pathogenesis. Control of vertical transmission should focus on screening of reproductive females. This is well established in the sericulture industry but less rigorously applied in managed pollinator species. Control of horizontal transmission currently relies on the use of antifungal agents such as fumagillin (Katznelson and Jamieson, 1952; Pajuelo *et al*. 2008) which continues to be used despite its potential genotoxic effects to (Stanimirovic *et al*. 2007). Greater understanding of disease epidemiology and identification of reservoirs of infection are essential to improve disease management and prevent damaging spillover into wild populations (Otterstatter and Thompson, 2008).

MANIPULATING MORTALITY: TRANSMISSION AND DISEASE IN PEST SPECIES

While in beneficial insect species our focus is on control of microsporidiosis in pest species the perspective is different. The high virulence associated with some microsporidian infections has long been cited as a useful trait in biological control. There are several instances where this has been successfully employed. *Nosema pyracusta* is a parasite of the corn borer (*Ostrinia nubilalis*). Transovarial transmission is critical to epidemiology of this parasite as *Ostrinia* has discontinuous generations and overwinters as a fifth instar larva. The parasite is found in the larval gonad and infects the developing oocysts produced by the resulting adult (Sajap and Lewis, 1988). The parasite has significant effects on the development and survival of larvae and on the reproductive success of adults (Sajap and Lewis, 1992) and its influence is maintained by further horizontal transmission between hosts (Andreadis, 1987). The role of *N. pyracusta* in biocontrol is well established (Lewis *et al*. 2009) but the use of microsporidia more widely has also been considered for many other pest species. Among the Lepidoptera *Endoreticulatus schubergi*, *N. lymantriae* and *Vairimorpha disparis* have been proposed for control of the Gypsy moth *Lymantria dispar* (Goetz and Hoch, 2008, 2009), *Vairimorpha ephestiae* for the wax moth *Galleria mellonella* (Vorontsova *et al*. 2004) and *Vairimorpha necatrix* for the tomato moth *Lacanobia oleracea* (Down *et al*. 2004). *Paranosema locusta* used for control of locust and other orthopteran pests (Lomer *et al*. 2001; Tounou *et al*. 2008) and *Thelohania solenopsae* has been proposed for control of the fire ant *Solenopsis invicta* (Fuxa *et al*. 2005).

In employing microsporidia for biological control one clear aim is to maximize the impact of the parasite on the target population. Although efficient horizontal transmission is an important characteristic here, in the majority of the cases cited vertical transmission is critical for the introduction and maintenance of the parasite in the environment and for perpetuation of disease across generations of hosts (Sajap and Lewis, 1988; Raina *et al*. 1995; Briano *et al*. 1996; Goetz and Hoch, 2008). In biocontrol it is also important to ensure that the control agent does not have detrimental effects on non-target species. Solter, Maddox and McManus (1997) tested the host range of microsporidia isolated from European populations of the Gypsy moth in a range of American lepidopteran species and found that the majority of isolates had broad host specificity when spores were fed directly to larvae. Similarly Microsporidia isolated from US hosts were infective to the Gypsy moth (Solter and Maddox, 1998). However, although direct feeding resulted in transmission, few isolates were sustained by horizontal transmission in non-native hosts, implying that artificial infection experiments could not be extrapolated to evaluate environmental transmission risks (Solter and Maddox, 1998; Solter, 2006). *Paranosema locusta*, used for control of locusts, is now known to occur in over 120 species of orthoptera (Lange, 2005), so consideration of impacts on non-target species is clearly appropriate.

PARASITES WHICH DICTATE HOST SEX: TRANSMISSION AND REPRODUCTIVE MANIPULATION

In some cases vertical transmission has been associated with reproductive manipulation of the host (Dunn and Smith, 2001). Bacterial endosimbionts are known to induce a range of such manipulations including cytoplasmic incompatibility, parthenogenesis and sex ratio distortion (SRD) (Duron *et al*. 2008) but the microsporidia are the only group of eukaryotes in which SRD has been unequivocally demonstrated (Dunn, Terry and Smith, 2001). The first SRD microsporidian described was *Nosema granulosis*, a parasite which is closely related to *N. bombycis* but infects the amphipod crustacean *Gammarus duebeni*. *N. granulosis* had a high rate of transovarial transmission (91%) and was estimated to feminise 66% of offspring leading to a highly female biased sex ratio (72–82%). The parasite had no impact on the survival of offspring although infected females were smaller than uninfected females (Terry *et al*. 1998). The parasite life cycle, like that of *N. bombycis*, consists of a single cycle of meronts producing diplokaryotic spores, but the spore is unusual in having a short polar filament, similar to autoinfection spores (Terry *et al*. 1999). On closer examination, many adaptations can be found for efficient transovarial transmission. In the adult female host, parasites are at low density and via EM were concentrated in the follicle cells surrounding the ovary. The parasite life cycle appears to be coordinated by host endocrine cues and sporulation is triggered in synchrony with the reproductive cycle producing spores which germinate to infect the oocytes (Terry *et al*. 1997). The embryo has a relatively low burden of parasites and these are associated with host mitochondria and microtubules and segregate to a subset of cells during embryogenesis (Terry *et al*. 1999*a*; Weedall *et al*. 2006). The molecular mechanism of feminisation is currently unclear but it is likely that the parasite causes endocrine interference. In crustaceans, sexual differentiation controlled by the androgenic gland which forms and a masculinising hormone (AGH). Studies have shown that this gland does not mature in infected females and intersexes and no hormone is produced (Rodgers-Gray *et al*. 2004).

Although *N. granulosis* was the first feminising microsporidian to be studied there is accumulating evidence for a relationship between vertical transmission and sex ratio distortion in amphipod hosts. Terry *et al*. (2004) surveyed 17 amphipod species discovering eight species of microsporidia four of which were associated with SRD in one or more hosts. Supportive evidence for sex ratio distortion has come from breeding experiments which show that shown that *Dictyocoela duebenum* feminises *Gammarus duebeni* (Dunn *et al*. 2006) and from field studies which indicate an association between sex ratio distortion and infection with *Fibrillonosema crangonycis* in *Crangonyx pseudogracilis* (Slothauber-Galbreath *et al*. 2004), for two species of *Dictyocoela* in *Gammarus roeseli* (Haine *et al*. 2004) and for a novel, clade V, microsporidian in *Corophium volutatur* (Mautner *et al*. 2007). These data suggest the SRD phenotype may be widespread but it is notable that it occurs in amphipods and not in insects where the mechanism of sexual differentiation is different.

Nosema granulosis and other SRD microsporidia have very efficient vertical transmission but the question arises as to whether they could sustain themselves in the population with no horizontal transmission. The low burden and limited disease pathogenesis would limit the detrimental effects of the infection, but in fact there is evidence that the parasite may be able to offset these negative effects by conferring survival advantage to infected offspring (Haine *et al*. 2007). It has also been suggested that a bias in the sex ratio towards females enhances the growth rate of infected host populations and that this might confer advantages in colonisation of new habitats (Slothauber-Galbreath *et al*. 2004, 2009).

LIFE CYCLE COMPLEXITY AND THE ROLE OF VERTICAL TRANSMISSION

The life cycles of microsporidian parasites can be quite complex and elaborate involving several sporulation cycles in multiple hosts. Complex life cycles are common among the Amblyosporidae which form clade I of the phylum Microspora and largely infect dipteran host species, including mosquitoes (Vossbrinck *et al*. 2004). With *Edharzardia aedis*, mosquito larvae are infected by ingesting uninucleate spores, which penetrate the midgut and undergo shizogony before differentiating into gametes, which fuse to produce binucleate spores within four days of infection. These binucleate spores are responsible for disseminating the infection from the midgut but infection is limited and does not kill the developing host. When the host reaches the adult stage and takes a blood meal the parasite is stimulated to produce a second binucleate spore that is responsible for transovarial transmission to the next generation of larvae. In these larvae there a two separate sporulation sequences, one leading to the formation of meiospores, the second leading to the production of large numbers of uninucleate spores (Becnel *et al*. 1989; Johnson *et al*. 1997). In this host-parasite system there is a strong relationship between infection route and disease: larvae that have acquired the infection transovarially die while those that are horizontally infected survive. In several species there is an alternation of hosts with the uninucleate spore stages being found within a copepod rather than in the mosquito (Andreadis, 1985) parasites, such as *Amblyospora connecticus* in

the saltmarsh mosquito, *Aedes cantator* (Andreadis, 1990), *Amblyospora albifasciati* in the neotropical mosquito, *Aedes albifasciatus* (Micieli *et al*. 2000), or *Amblyospora camposi* in the bromeliad-inhabiting mosquito, *Culex renatoi* (Micieli *et al*. 2007). In these systems the vertical transmission cycle is constrained and occurs in a single species of mosquito but a single copepod species can harbour multiple microsporidian parasites.

In the Amblyosporidae we see both vertical and horizontal cycles employed to ensure transmission of parasites between alternate hosts. It is possible that there are many other examples of complex life cycles across the phylum Microspora, as there are many cases where our understanding of transmission is very basic. With the advent of improved molecular systematics, it is now possible to match parasite sequences retrieved from different hosts allowing reconstruction of life cycles. For example, a recent analysis of *Pleistophora* sequences revealed likely overlap between *P. mulleri*, found in the amphipod *Gammarus duebeni* and *P. typicalis* from the cottoid fish *Myoxocephalus scorpius* leading to the proposal that these represent alternate hosts (Ironside *et al*. 2008).

ADAPTIVE TRANSMISSION STRATEGIES: GENETICS *VS* ENVIRONMENTAL CUES

A survey of the phylum Microspora shows that both vertical and horizontal transmission are extensively used by microsporidian parasites (refer to Fig. 1). Terry *et al*. (2004) proposed that vertical transmission was an ancestral trait which occurred throughout the phylum. While horizontal transmission is well reported, vertical transmission requires screening of reproductive tissue and eggs so is likely to be underestimated. In fact, in addition to the well studied examples discussed above we find evidence of vertical transmission strategies in many host taxa. The mammal-infective *Encephalitozoon* species are zoonotic parasites horizontally transmitted between hosts (Didier, 2005), but there are reports that transplacental transmission can occur in some host species (Baneaux and Pognan, 2003; Webster *et al*. 2008). A number of microsporidia infecting fish are also thought to be vertically transmitted including *Oviepleistophora ovale* in the golden shiner *Notemigonus crysoleucas* (Phelps and Goodwin, 2008) and *Pseudoloma neurophilia* in the zebrafish, *Danio rerio* (Kent and Bishop-Stewart, 2003). Screening of the aquatic snail *Bulinus globosus* also revealed the presence of Microsporidia in the gonad and egg sacs (McClymont *et al*. 2005) while a parasite morphologically described as *Steinhausia* sp. in the marine bivalve *Eurhomalea lenticularis* was transoverially transmitted to 88% of oocytes (Olivares, 2005).

The majority of Microsporidia use a combination of vertical and horizontal transmission in their life cycles. This presents a conflict for the parasite as adaptations that favour the horizontal route would be very different from those that supported vertical transmission (Dunn and Smith, 2001). Horizontal transmission would be enhanced by the production and dissemination of large numbers of infective stages and this is likely to be associated with severe pathogenesis or with the death of infected hosts. In contrast, vertical transmission would favour low parasite burden to ensure the hosts survival and reproductive manipulation to increase the frequency of transmission to the next generation. Strong selective pressures might, for example lead to parasites with high or sole VT strategies which had lost the genes required for horizontal transmission. Similarly parasites could be selected for high horizontal transmission. There is evidence to suggest that this might happen: for example, *N. granulosis* is a transovarially transmitted feminising parasite and artificial infection experiments show that horizontal transmission is inefficient (Dunn *et al*. 1993) and the non-random association with host mitochondrial haplotypes argues that it is little horizontal transmission in the field (Ironside *et al*. 2003). On the other hand, *Vairimorpha disparis*, proposed for control of the Gypsy moth, has no vertical transmission and spores can survive through the winter in cadavers and horizontal transmission among larvae is efficient (Goertz and Hoch, 2008). The majority of microsporidian species use both routes and must therefore have complex mechanisms to control the phenotype.

One possibility is that the phenotype is primarily determined by host and parasite genetic factors, in which case we would predict fixed relationships between parasite strain diversity and transmission route. An alternative hypothesis is that control of the parasite phenotype and life cycle could be largely epigenetic and the relevant phenotype would be induced by environmental conditions. These might include external signals such as temperature, pH and salinity or host-related factors such as endocrine signals, the immune response, and nutritional or physiological status. It is important to understand the relative importance of genetic and environmental factors as this has significant implications for disease ecology.

It is currently difficult to discriminate between 'genetic' and 'environmental' effects but evidence from experimental studies gives support to both. There clearly are intrinsic differences between microsporidian isolates as revealed by comparison of disease phenotype in a single host, such as the silkworm (Rao *et al*. 2007) or the Gypsy moth (Solter and Maddox, 1998; Goetz and Hoch, 2009). There is also evidence that the parasite phenotype varies according to host species (Solter, 1997). In terms of environmental factors, Vizoso and Ebert (2005) found evidence of phenotypic plasticity in studies of

the infection route of *Octosporea bayeri* in *Daphnia magna*. This research demonstrated a trade-off between spore production (HT trait) and the production of infected host offspring (VT trait). There is also evidence that temperature affects the dynamics of infection and the disease phenotype: for example, *N. ceranae* can complete its life cycle over a much wider temperature range (25–37 °C) than *N. apis* (Martin-Fernandez *et al*. 2009).

ECOLOGICAL AND EVOLUTIONARY INTERACTIONS AND EMERGING DISEASE RISK

Given the widespread use of VT among microsporidian parasites it is interesting to question to whether it influences the evolutionary relationships between these parasites and their hosts (Terry *et al*. 2004). A high degree of adaptation is required to mediate transovarial or transplacental transmission and this might require specific molecular interactions. In addition, vertical transmission leads to clonal segregation of the parasite within a subset of hosts and this could potentially drive co-speciation. If the influence of vertical transmission is strong we would predict close association between host and parasite taxa and closely tied to their hosts and lineage switching between unrelated taxa would be rare.

We can evaluate the relationships between microsporidia and their hosts on an 'ecological' time-scale through studies of trans-continental invasion. The amphipod *Crangonyx pseudogracilus* was introduced to Europe from North America over seventy years ago (Crawford, 1937) and has since spread throughout European waterways. In its native range this species harbours at least seven different species of microsporidia that do not overlap parasites found in European amphipod species. During invasion, a subset of these parasites has been introduced but one species *Fibrillonosema crangonycis* dominates in the invasive range (Slothauber-Galbreath *et al*. 2004, 2009). Interestingly, this species is a vertically transmitted SRD parasite and it is proposed that it may have contributed to the success of the invasion through overproduction of females and enhanced population growth rate. While the amphipod is sympatric with many European amphipod species there has been no transfer of *Fibrillonosema* to these native amphipods, nor have there been any introductions of microsporidia from European amphipods into populations of *Crangonyx* (Terry *et al*. 2004; Slothauber-Galbreath *et al*. 2009). Evidence from other crustacean invasions is less easy to interpret, the pontocaspian gammarid *Dikerogammarus villosus* has spread across Western Europe from its Ponto-Caspian origins over the last ninety years. Characterization of its parasite fauna (Wattier *et al*. 2007) reveals one dominant species, *Microsporidium* sp. *D*, which was abundant across the invasive range and may have been introduced with source populations together with three further parasites which were very rare and were similar but not identical to *N. granulosis* and *Dictyocoela* spp. found in European amphipods (Haine *et al*. 2004; Terry *et al*. 2004). It is not clear whether these parasites have been acquired from sympatric amphipod species or are close relatives derived from Ponto-Caspian *Dikerogammarus*. Studies reveal that populations of the invasive signal crayfish *Pacifastacus leniusculus* and the native white clawed crayfish *Austropotamobius pallipes* in the UK are both infected with *Thelohania contejeani* (Dunn *et al*. 2009). However, it is difficult to map the origins of this microsporidian parasite, which may have cosmopolitan distribution among crayfish species (Lom *et al*. 2001). Attempts have also been made to analyse the likelihood of switching in Lepidopteran hosts. Solter and Maddox (1998) reported that the Gypsy moth had failed to acquire microsporidia from sympatric Lepidoptera in its invasive North American range. They also found that microsporidia infecting the Gypsy moth in its native range were distinct from species affecting sympatric Lepidoptera (Solter *et al*. 2000). These studies imply that microsporidia are 'ecologically segregated' within the environment and that transfer between host taxa is rare (Solter, 2006). Some caution should be exercised in interpreting these data as the isolates retrieved from *Lymantria* were very closely related to those from sympatric hosts and a number were able to cross-infect when tested in bioassay (Solter *et al*. 2000). Taking these ecological studies at face value, there is very little evidence for host switching in microsporidian parasites, consistent with the concept that these parasites are closely linked to their hosts. Our understanding is however very incomplete. Only a handful of studies have employed molecular methods to identify microsporidian species and these rely on the use of ribosomal DNA as a phylogenetic tool. This means that we can only evaluate host parasite associations at a relatively high taxonomic level.

It is also possible to consider interactions between microsporidia and their hosts on an evolutionary time-scale. One of the striking facts about the large-scale phylogenetic analysis of the phylum Microspora is that there are quite strong links between parasite lineages and host groups (Fig. 1). For example, clade V, which is often basal to the tree contains parasites such as *Bacillidium* and *Pseudonosema* and found in primitive animals such as bryozoans and oligochetes (Canning *et al*. 2002; Morris *et al*. 2005). The majority of Microsporidia in Clade I fall within the Amblosporidae which infect dipteran hosts and within this further separate into species infecting Anopholine or Culicine mosquitoes (Vossbrinck *et al*. 2004), Clade III consists mainly of parasites such as *Loma*, *Glugea* and *Pleistophora* which infect fish (Lom and Nilson, 2003) while the majority of

https://doi.org/10.1017/S0031182009991818 Published online by Cambridge University Press

mammal-infective species are found in Clade IV. These deep associations between host and parasite taxa are not perfect but they are very strong. There are two possible interpretations of this pattern. One is that there are many undiscovered species which will ultimately break up these relationships. The second possibility is that there are emerging strong relationships and that the divisions seen at the level of the deep branches reflect the evolutionary associations between ancestral microsporidia and their hosts. One group of hosts, the amphipod crustacea does not follow this pattern. Microsporidian from amphipods are distributed through multiple branches of the tree and this might imply that the early radiation of the parasites occurred in this host group.

If we move from the deep relationships to the tips of the tree it is possible to see detailed interactions between Microsporidia genera and groups of hosts. For example, the emerging genus *Dictyocoela* is present across many species of amphipods and the divergence of this parasite genus may relate to that of its host. Our ability to test this relationship is currently limited by the resolution of our current, single locus, marker and there is a need to identify polymorphic loci and develop multilocus typing methods in order to analyse these relationships fully. Similar issues arise over the relationships of *Nosema* parasites with lepidopteron and non-lepidopteron hosts, It is currently unclear whether this group consists of generalist parasites which have overlapping host ranges or of many closely related species each of which has a unique interaction with its host. Much more work is needed to test host-parasite associations and demonstrate whether they reflect co-speciation or extensive lineage switching driven by ecological interaction.

These studies of the interaction between microsporidia and their hosts are of great importance in assessing the impact these parasites might have. Since the advent of AIDS, microsporidiosis has been considered an emerging disease of humans, as patients became susceptible to a range of mammal-infective (e.g. *Encephalitozoon* spp. and *Vittaforma corneum*) and generalist (e.g. *Brachiola algerae*) parasites (Didier, 2005). Disease emergence is not simply a human health problem, as illustrated by the case of *Nosema ceranae* where the penetration of a parasite species into a new host background may have significant ecosystem consequences.

CONCLUSIONS

The phylum Microspora is ancient and diverse and affects a wide range of hosts. There is unusually high use of vertical transmission and this has significant consequences for transmission and pathogenicity. A high VT parasite would be cryptic with little patent disease but might nevertheless create significant impacts upon its host. Vertical transmission is adapted to fulfil different roles, including overwintering survival, transmission between discontinuous generations and transmission to alternate hosts. The majority of microsporidia have mixed transmission cycles and it is not clear whether they could switch and modify their phenotype according to environmental circumstances. There is a great need to understand the mechanisms controlling transmission and one of the first challenges for the genomics era is to find genes associated with life cycle stages. Similarly, we cannot currently predict the ease with which these parasites might switch between host groups. Phylogenetic analysis suggests that there are strong relationships between Microsporidia and their host but multilocus typing data is urgently needed for close identification of parasite isolates in relation to host range and disease phenotype and to assess future environmental risk from these pathogens.

ACKNOWLEDGEMENTS

I gratefully acknowledge the support of my research collaborators at the University of Leeds for discussions on this topic and thank Prof Geoff Hide for his encouragement in producing this manuscript.

REFERENCES

Andreadis, T. G. (1985). Experimental transmission of a microsporidian pathogen from mosquitoes to an alternate copepod host. *Proceedings of the National Academy of Sciences, USA* **82**, 5574–5577.

Andreadis, T. G. (1987). Horizontal transmission of *Nosema pyrausta* (Microsporidia: Nosematidae) in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Environmental Entomology* **16**, 1124–1129.

Andreadis, T. G. (1990). Epizootiology of *Amblyospora connecticus* (Microsporida) in field populations of the saltmarsh mosquito, *Aedes cantator*, and the cyclopoid copepod, *Acantocyclops vernalis*. *Journal of Protozoology* **37**, 174–182.

Akiyoshi, D. E., Morrison, H. G., Lei, S., Feng, X., Zhang, Q., Corradi, N., Mayanja, H., Tumwine, J. K., Keeling, P. J., Weiss, L. M. and Tzipori, S. (2009). Genomic survey of the non-cultivatable opportunistic human pathogen, *Enterocytozoon bieneusi*. *PLoS Pathogens* **5**, e1000261.

Baker, M. D., Vossbrinck, C. R., Maddox, J. V. and Undeen, A. H. (1994). Phylogenetic relationships among *Vairimorpha* and *Nosema* species (Microspora) based on ribosomal RNA sequences. *Journal of Invertebrate Pathology* **61**, 100–106.

Baneux, J. R. and Pognan, F. (2003). *In utero* transmission of *Encephalitozoon cuniculi* strain type I in rabbits. *Laboratory Animal* **37**, 132–138.

Becnel, J. J. and Andreadis, T. G. (2001). Microsporidia in Insects. In *Microsporidia and Microsporidiosis* (ed. Wittner, M. and Weiss, L. M.) pp. 447–501. ASM press, Washington DC.

Becnel, J. J., Sprague, V., Fukada, T. and Hazard, E. (1989). Development of *Edharzardia aedis* (Kudo, 1930)

n.g.n. comb. (Microsporidia:Amblyosoridae) in the mosquito *Aedes aegypti* (L.) (Diptera:Culicidae). *Journal of Protozoology* **36**, 119–130.

Belkorchia, A., Biderre, C., Militon, C., Polonais, V., Wincker, P., Jubin, C., Delbac, F., Peyretaillade, E. and Peyret, P. (2008). *In vitro* propagation of the microsporidian pathogen *Brachiola algerae* and studies of its chromosome and ribosomal DNA organization in the context of the complete genome sequencing project. *Parasitology International* **57**, 62–71.

Bigliardi, E., Riparbelli, M. G., Selmi, M. G., Lanzarini, P., Corona, S., Gatti, S., Scaglia, M. and L. Sacchi, L. (1998). Mechanisms of microsporidial cell division: ultrastructural study on *Encephalitozoon hellem*. *Journal of Eukaryotic Microbiology* **45**, 347–351.

Briano, J. S., Patterson, R. S., Becnel, J. J. and Cordo, H. A. (1996). The black imported fire ant, *Solenopsis richteri*, infected with *Thelohania solenopsae*: Intracolonial prevalence of infection and evidence for transovarial transmission. *Journal of Invertebrate Pathology* **67**, 178–179.

Cali, A., Weiss, L. M. and Takvorian, P. M. (2004). An analysis of the microsporidian genus Brachiola, with comparisons of human and insect isolates of *Brachiola algerae*. *Journal of Eukaryotic Microbiology* **51**, 678–685.

Canning, E. U. (1953). A new microsporidian *Nosema locusta* n. sp. from the fat body of the African migratory locust, *Locusta migratoria migrattoroides* R and F. *Parasitology* **43**, 297–290.

Canning, E. U., Refardt, D., Vossbrinck, C. R., Okamura, B. and Curry, A. (2002). New diplokaryotic microsporidia (Phylum Microsporidia) from freshwater bryozoans (Bryozoa, Phylactolaemata). *European Journal of Protistology* **38** 247–265.

Corradi, N., Gangaeva, A. and Keeling, P. J. (2008). Comparative profiling of overlapping transcription in the compacted genomes of microsporidia *Antonospora locustae* and *Encephalitozoon cuniculi*. *Genomics* **91**, 388–393.

Cox-Foster, D. L., Conlan, S., Holmes, E. C., Palacios, G., Evans, J. D., Moran, N. A., Quan, P.-L., Briese, T., Hornig, M., Geiser, D. M., Martinson, V., Vanengelsdorp, D., Kalkstein, A. L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchison, S. K., Simons, J. F., Egholm, M., Pettis, J. S. and Lipkin, W. I. (2007). A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* **318**, 283–287.

Crawford, G. I. (1937). An amphipod, *Eucrangonyx gracilis* S.I. Smith, new to Britain. *Nature* **139**, 327.

Curgy, J. J., Vavra, J. and Vivares, C. (1980). Presence of Ribosomal-RNAs with prokaryotic properties in microsporidia, eukaryotic origins. *Biologie Cellulaire* **38**, 49–51.

Czekonska, K. (2000). The influence of *Nosema apis* on young honeybee queens and transmission of the disease from queens to workers. *Apidologie* **31**, 701–706.

Didier, E. S. (2005), Microsporidiosis: An emerging and opportunistic infection in humans and animals. *Acta Tropica* **94**, 61–76.

Dissanaike, A. S. and Canning, E. U. (1957). The mode of emergence of the protoplasm in Microsporidia and its relation to the structure of the spore. *Parasitology* **47**, 92–99.

Down, R. E., Bell, H. A., Kirkbride-Smith, A. E. and Edwards, J. P. (2004). The pathogenicity of *Vairimorpha necatrix* (Microspora: Microsporidia) against the tomato moth, *Lacanobia oleracea* (Lepidoptera: Noctuidae) and its potential use for the control of lepidopteran glasshouse pests. *Pest Management Science* **60**, 755–764.

Dunn, A. M., Adams, J. and Smith, J. E. (1993). Transovarial transmission and sex ratio distortion by a microsporidian parasite in a shrimp. *Journal of Invertebrate Pathology* **61**, 248–252.

Dunn, A. M., Hogg, J. C. and Hatcher, M. J. (2006). Transmission and burden and the impact of temperature on two species of vertically transmitted microsporidia. *International Journal for Parasitology* **36**, 409–414.

Dunn, A. M. and Smith, J. E. (2001). Microsporidian life cycles and diversity: the relationship between virulence and transmission. *Microbes and Infection* **3**, 381–388.

Dunn, A. M., Terry, R. S. and Smith, J. E. (2001). Transovarial transmission in the Microsporidia. *Advances in Parasitology* **48**, 57–101.

Dunn, J. C., McClymont, H. E., Christmas, M. and Dunn, A. M. (2009). Competition and parasitism in the native White Clawed Crayfish *Austropotamobius pallipes* and the invasive Signal Crayfish *Pacifastacus leniusculus* in the UK. *Biological Invasions* **11**, 315–324.

Duron, O., Bouchon, D., Boutin, S., Bellamy, L., Zhou, L., Engelstädter, J. and Hurst, G. D. D. (2008). The diversity of reproductive parasites among arthropods: Wolbachia do not walk alone. *BMC Biology* **6**, 27.

Fantham, H. B. and Porter, A. (1912). Microsporidiosis, a protozoal disease of bees due to *Nosema apis* and popularly known as the Isle of Wight Disease. *Annals of Tropical Medcine and Parasitology* **6**, 145–60.

Fokin, S. I., Di Giuseppe, G., Erra, F. and Dini, F. (2008). *Euplotespora binucleata* n. gen., n. sp (Protozoa: Microsporidia), a parasite infecting the hypotrichous ciliate *Euplotes woodruffi*, with observations on microsporidian infections in Ciliophora. *Journal of Eukaryotic Microbiology* **55**, 214–228.

Fries, I. (1989). Observations on the development and transmission of *Nosema apis* Z. in the ventriculus of the honeybee. *Journal of Apiculture Research* **28**, 107–117.

Fries, I. and Camazine, S. (2001). Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. *Apidologie* **32**, 199–214.

Fries, I., De Ruijter, A., Paxton, R. J., Da Silva, A. J., Slemenda, S. B. and Pieniazek, N. J. (2001). Molecular characterization of *Nosema bombi* (Microsporidia: Nosematidae) and a note on its sites of infection in *Bombus terrestris* (Hymenoptera: Apoidea). *Journal of Apiculture Research* **40**, 91–96.

Fries, I., Feng, F., Da Silva, A., Slemenda, S. B. and Pieniazek, N. J. (1996). *Nosema ceranae* n. sp. (Microspora, Nosemaditae), morphological and molecular characterisation of a microsporidian parasite of the Asian honey bee *Apis cerana* (Hymenoptera, Apidae). *European Journal of Protistology* **32**, 356–365.

Fries, I., Granados, R. R. and Morse, R. A. (1992). Intracellular germination of *Nosema apis* Z. *Apidologie* **23**, 61–70.

Fuxa, J. R., Milks, M. L., Sokolova, Y. Y. and Richter, A. R. (2005). Interaction of an entomopathogen with an insect social form: an epizootic of *Thelohania solenopsae* (Microsporidia) in a population of the red imported fire ant, *Solenopsis invicta*. *Journal of Invertebrate Pathology* **88**, 79–82.

Gill, E. E., Becnel, J. J. and Fast, N. M. (2008). ESTs from the microsporidian *Edhazardia aedis*. *BMC Genomics* **9**, 296.

Goertz, D. and Hoch, G. (2008). Vertical transmission and overwintering of microsporidia in the gypsy moth, *Lymantria dispar*. *Journal of Invertebrate Pathology* **99**, 43–48.

Goertz, D. and Hoch, G. (2009). Three microsporidian pathogens infecting *Lymantria dispar* larvae do not differ in their success in horizontal transmission. *Journal of Applied Entomology* **133**, 568–570.

Goldberg, A. V., Molik, S., Tsaousis, A. D., Neumann, K., Kuhnke, G., Delbac, F., Vivares, C. P., Hirt, R. P., Lill, R. and Embley, T. M. (2008). Localization and functionality of microsporidian iron–sulphur cluster assembly proteins. *Nature* **452**, 624–628.

Haine, E. R., Brondani, E., Hume, K. D., Perrot-Minnot, M.-J., Gaillard, M. and Rigaud, T. (2004). Coexistence of three microsporidia parasites in populations of the freshwater amphipod *Gammarus roeseli*: evidence for vertical transmission and positive effect on reproduction. *International Journal for Parasitology* **34**, 1137–1146.

Haine, E. R., Motreuil, S. and Rigaud, T. (2007). Infection by a vertically-transmitted microsporidian parasite is associated with a female-biased sex ratio and survival advantage in the amphipod *Gammarus roeseli*. *Parasitology* **134**, 1363–1367.

Han, M.-S. and Watanabe, H. (1988). Transovarial transmission of two microsporidia in the silkworm *Bombyx mori* and disease occurance in the progeny population. *Journal of Invertebrate Pathology* **51**, 41–45.

Hatakeyama, Y. and Hayasaka, S. (2003). A new method of pebrine inspection of silkworm egg using multiprimer PCR. *Journal of Invertebrate Pathology* **82**, 148–151.

Higes, M., Martín-Hernández, R., Botías, C., Bailón, E. G., González-Porto, A. V., Barrios, L., Del Nozal, M. J., Bernal, J. L., Jiménez, J. J., Palencia, P. G. and Meana, A. (2008). How natural infection by *Nosema ceranae* causes honeybee colony collapse. *Environmental Microbiology* **10**, 2659–2669.

Hirt, R. P., John, M., Logsdon, J., Healy, B., Dorey, M. W., Doolittle, W. F. and Embley, T. M. (1999). Microsporidia are related to Fungi: evidence from the largest subunit of RNA polymerase II and other proteins. *Proceedings of the National Acadamy of Sciences, USA* **96**, 580–585.

Ironside, J., Dunn, A. M., Rollinson, D. R. and Smith, J. E. (2003). Association with host mitochondrial haplotypes suggests that feminizing microsporidia lack horizontal transmission. *Journal of Evolutionary Biology* **16**, 1077–1083.

Ironside, J. E., Wilkinson, T. J. and Rock, J. (2008). Distribution and host range of the microsporidian *Pleistophora mulleri*. *Journal of Eukaryotic Microbiology* **55**, 355–362.

Ishihara, R. (1969). Life cycle of *Nosema bombycis* as revealed in tissue culture cells of *Bombyx mori*. *Journal of Invertebrate Pathology* **14**, 316–320.

Ishihara, R. and Fujiwava, T. (1965). The spread of pebrine within a colony of the Silkworm, *Bombyx mori* (Linnaeus). *Journal of Invertebrate Pathology* **7**, 126–131.

Iwano, H. and Ishihara, R. (1991). Dimorphic development of *Nosema bombycis* spores in the gut epithelium of the larvae of the silkworm *Bombyx mori*. *Journal of Sericulture Science Japan* **60**, 249–256.

Iwano, H. and Kurtti, T. J. (1995). Identification and Isolation of Dimorphic Spores from *Nosema furnacalis* (Microspora: Nosematidae). *Journal of Invertebrate Pathology* **65**, 230–236.

James, T. Y., Kauff, F., Schoch, C. L., Matheny, P. B., Hofstetter, V., Cox, C. J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., Lumbsch, H. T., Rauhut, A., Reeb, V., Arnold, A. E., Amtoft, A., Stajich, J. E., Hosaka, K., Sung, G. H., Johnson, D., O'Rourke, B., Crockett, M., Binder, M., Curtis, J. M., Slot, J. C., Wang, Z., Wilson, A. W., Schüssler, A., Longcore, J. E., O'Donnell, K., Mozley-Standridge, S., Porter, D., Letcher, P. M., Powell, M. J., Taylor, J. W., White, M. M., Griffith, G. W., Davies, D. R., Humber, R. A., Morton, J. B., Sugiyama, J., Rossman, A. Y., Rogers, J. D., Pfister, D. H., Hewitt, D., Hansen, K., Hambleton, S., Shoemaker, R. A., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Spotts, R. A., Serdani, M., Crous, P. W., Hughes, K. W., Matsuura, K., Langer, E., Langer, G., Untereiner, W. A., Lücking, R., Büdel, B., Geiser, D. M., Aptroot, A., Diederich, P., Schmitt, I., Schultz, M., Yahr, R., Hibbett, D. S., Lutzoni, F., McLaughlin, D. J., Spatafora, J. W. and Vilgalys, R. (2006). Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* **443**, 818–822.

Johnson, M. A., Becnel, J. J. and Undeen, A. H. (1997). A new sporulation sequence in *Edhazardia aedis* (Microsporidia: Culicosporidae), a parasite of the mosquito *Aedes aegypti* (Diptera: Culicidae). *Journal of Invertebrate Pathology* **70**, 69–75.

Kashkarova, L. F. and Khakhanov, A. I. (1980). Range of hosts of the agent of Microsporidiosis *Nosema bombycis* of the Chinese silkworm. *Parazitologiya* (St. Petersburg) **14** 164–167.

Katinka, M. D., Duprat, S., Cornillot, E., Metenier, G., Thomarat, F., Prensier, G., Barbe, V. A., Peyretaillade, E., Brottier, P., Wincker, P., Delbac, F., El Alaoui, H., Peyret, P., Saurin, W., Gouy, M., Weissenbach, J. and Vivares, C. P. (2001). Genome sequence and gene compaction of the eukaryote parasite *Encephalitozoon cuniculi*. *Nature* **414**, 450–453.

Katznelson, H. and Jamieson, C. A. (1952). Control of *Nosema* disease in honey bees with fumagillin. *Science* **115**, 70–71.

Keeling, P. J. and Fast, N. M. (2002). Microsporidia: Biology and evolution of highly reduced intracellular parasites. *Annual Review of Microbiology* **56**, 93–116.

Keeling, P. J., Luker, M. A. and Palmer, J. D. (2000). Evidence from beta-tubulin phylogeny that

Microsporidia evolved from the Fungi. *Molecular Biology and Evolution* **17**, 23–31.

Kent, M. L. and Bishop-Stewart, J. K. (2003). Transmission and tissue distribution of *Pseudoloma neurophilia* (Microsporidia) of zebrafish, *Danio rerio* (Hamilton). *Journal of Fish Diseases* **26**, 423–426.

Klee, J., Besana, A. M., Genersch, E., Gisder, S., Nanetti, A., Tam, D. Q., Chinh, T. X., Puerta, F., Ruz, J. M., Kryger, P., Message, D., Hatjina, F., Korpela, S., Fries, I. and Paxton, R. J. (2007). Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. *Journal of Invertebrate Pathology* **96**, 1–10.

Lange, C. E. (2005). The host and geographical range of the grasshopper pathogen *Paranosema* (*Nosema*) *locustae* revisited. *Journal of Orthopteran Research* **14**, 137–141.

Larsson, J. I. R. (2007). Cytological variation and pathogenicity of the bumble bee parasite *Nosema bombi* (Microspora, Nosematidae). *Journal of Invertebrate Pathology* **94**, 1–11.

Lee, S. C., Corradi, N., Byrnes, E. J., Torres-Martinez, S., Dietrich, F. H., Keeling, P. J. and Heitmam, J. (2008). Microsporidia evolved from ancestral sexual fungi. *Current Biology* **18**, 1675–1679.

Lewis, L. C., Bruck, D. J., Prasifka, J. R. and Raun, E. S. (2009). *Nosema pyrausta*: Its biology, history, and potential role in a landscape of transgenic insecticidal crops. *Biological Control* **48**, 223–231.

Liu, T. P. (1992). Oocytes degeneration in the queen honey bee after infection by *Nosema apis*. *Tissue and Cell* **24**, 131–138.

Liu, J. P., Cao, Y., Smith, J. E. and Xu, X. (2004). Studies of the application of PCR molecular diagnosis to silkworms with simulated pebrine disease. *Scientia Agricultura Sinica* **37**, 1925–1931.

Lom, J. and Nilsen, F. (2003). Fish microsporidia: fine structural diversity and phylogeny. *International Journal for Parasitology* **33**, 107–127.

Lom, J., Nilsen, F. and Dykova, I. (2001). *Thelohania contejeani* Henneguy, 1892: dimorphic life cycle and taxonomic affinities, as indicated by ultrastructural and molecular study. *Parasitology Research* **87**, 860–872.

Lomer, C. L., Bateman, P. P., Johnson, D. L., Langewald, J. and Thomas, M. B. (2001). Biological control of locusts and grasshoppers. *Annual Review of Entomology* **46**, 667–702.

Malone, L. A. and Giacon, H. A. (1996). Effects of *Nosema apis* Zander on inbred New Zealand honey bees (*Apis mellifera ligustica* L). *Apidologie* **27**, 479–486.

Malone, L. A. and McIvor, C. A. (1993). Pulsed-field electrophoresis of DNA from four microsporidian isolates. *Journal of Invertebrate Pathology* **61**, 203–205.

Martin-Hernandez, R., Meana, A., Garcia-Palencia, P., Marin, P., Botias, C., Garido-Bailon, E., Barrios, L. and Higes, M. (2009). Effect of temperature on the biotic potential of Honeybee microsporidia. *Applied and Environmental Microbiology* **75**, 2554–2557.

Mautner, S. I., Cook, K. A., Forbes, M. R., McCurdy, D. G. and Dunn, A. M. (2007). Evidence for sex ratio distortion by a new microsporidian parasite of a Corophiid amphipod. *Parasitology* **134**, 1567–1573.

McClymont, E. H., Dunn, A. M., Terry, R. S., Rollinson, D., Littlewood, D. T. J. and Smith, J. E. (2005). Molecular data suggest that microsporidian parasites in freshwater snails are diverse. *International Journal for Parasitology* **35**, 1071–1078.

Micieli, M. V., Garcia, J. J. and Becnel, J. J. (2000). Horizontal transmission of *Amblyospora albifasciati* García and Becnel, 1994 (Microsporidia: Amblyosporidae), to a copepod intermediate host and the neotropical mosquito *Aedes albifasciatus* (Macquart, 1837). *Journal of Invertebrate Pathology* **75**, 76–83.

Micieli, M. V., Marti, G. A., Garcia, J. J., Tranchida, M. C. and Becnel, J. J. (2007). Epizootiological studies of *Amblyospora camposi* (Microsporidia: Amblyosporidae) in *Culex renatoi* (Diptera: Culicidae) and *Paracyclops fimbriatus fimbriatus* (Copepoda: Cyclopidae) in a bromeliad habitat. *Journal of Invertebrate Pathology* **194**, 31–37.

Morris, D. J., Terry, R. S., Ferguson, K. D., Smith, J. and Adams, A. (2005). Ultrastructural and molecular characterization of *Bacillidium vesiculoformis* n. sp. (Microspora: Mrazekiidae) in the freshwater oligochaete *Nais simplex* (Oligochaeta: Naididae). *Parasitology* **30**, 31–45.

Olivares, C. A. (2005). Evidence of a parasite protist in *Eurhomalea lenticularis* (Sowerby, 1835) (Mollusca: Bivalvia): A case of intraoocytarian parasitism. *Journal of Natural History* **39**, 2073–2082.

Otti, O. and Schmid-Hempel, P. (2007). *Nosema bombi*: A pollinator parasite with detrimental fitness effects. *Journal of Invertebrate Pathology* **96**, 118–124.

Otterstatter, M. C. and Thomson, J. D. (2008). Does pathogen spillover from commercially reared bumble bees threaten wild pollinators? *PloS One* **3**, 1–9.

Pasteur, L. (1870). *Etudes sur la maladie des vers à soie, moyen pratique assuré de la combattre et d'en prévenir le retour*. Paris, Gauthier-Villars, 327 pp.

Pajuelo, A. G., Torres, C. and Bermejo, F. J. O. (2008). Colony losses: a double blind trial on the influence of supplementary protein nutrition and preventative treatment with fumagillin against *Nosema ceranae*. *Journal of Apiculture Research* **47**, 84–86.

Paxton, R. J., Klee, J., Korpela, S. and Fries, I. (2007). *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. *Apidologie* **38**, 558–565.

Phelps, N. B. D. and Goodwin, A. E. (2008). Vertical transmission of *Ovipleistophora ovariae* (Microspora) within the eggs of the golden shiner. *Journal of Aquatic Animal Health* **20**, 45–53.

Raina, S. K., Das, S., Rai, M. M. and Khurad, A. M. (1995). Transovarial transmission of *Nosema locustae* (Microsporida: Nosematidae) in the migratory locust *Locusta migratoria migratorioides*. *Parasitology Research* **81**, 38–44.

Rao, S. N., Nath, B. S., Bhuvaneswari, G. and Urs, S. R. (2007). Genetic diversity and phylogenetic relationships among microsporidia infecting the silkworm, *Bombyx mori*, using random amplification of polymorphic DNA: Morphological and ultrastructural characterization. *Journal of Invertebrate Pathology* **96**, 193–204.

Rao, S. N., Nath, B. S. and Saratchandra, B. (2005). Characterization and phylogenetic relationships among microsporidia infecting silkworm, *Bombyx mori*, using

inter simple sequence repeat (ISSR) and small subunit rRNA (SSU-rRNA) sequence analysis. *Genome* **48**, 355–366.

Rodgers-Gray, T. P., Smith, J. E., Ashcroft, A. E., Isaac, R. E. and Dunn, A. M. (2004). Mechanisms of parasite-induced sex reversal in *Gammarus duebeni*. *International Journal for Parasitology* **34**, 747–753.

Ronnebaumer, K., Gross, U. and Bonhe, W. (2008). The nascent parasitophorous vacuole membrane of *Encephalitozoon cuniculi* is formed by host cell lipids and contains pores which allow nutrient uptake. *Eukaryotic Cell* **7**, 1001–1008.

Rutrecht, S. T. and Brown, M. J. F. (2007). Within colony dynamics *of Nosema bombi* infections: disease establishment, epidemiology and potential vertical transmission. *Apidologie* **39**, 504–514.

Rutrecht, S. T. and Brown, M. J. F. (2009). Differential virulence in a multiple-host parasite of bumble bees: resolving the paradox of parasite survival. *OIKOS* **118**, 941–949.

Sagrista, E., Bozzo, M. G., Bigas, M., Poquet, M. and Durfort, M. (1998). Developmental cycle and ultrastructure of *Steinhausia mytilovum*, a microsporidian parasite of oocytes of the mussel, *Mytilus galloprovincialis* (Mollusca, Bivalvia). *European Journal of Protistology* **34**, 58–68.

Sajap, A. S. and Lewis, L. C. (1988). Histopathology of transovarial transmission of *Nosema pyrausta* in the European corn borer, *Ostrinia nubilalis*. *Journal of Invertebrate Pathology* **52**, 147–153.

Sajap, A. S. and Lewis, L. C. (1992). Chronology of infection of European corn borer (Lepidoptera: Pyralidae) with the microsporidium *Nosema pyrausta*: effect on development and vertical transmission. *Environmental Entomology* **21**, 178–182.

Scanlon, M., Leitch, G. J., Visvesvara, G. S. and Shaw, A. P. (2004). Relationship between the host cell mitochondria and the parasitophorous vacuole in cells infected with *Encephalitozoon* Microsporidia. *Journal of Eukaryotic Microbiology* **51**, 81–87.

Sinden, R. E. and Canning, E. U. (1974). The ultrastructure of the spore of *Nosema algerae* (Protozoa, Microsporida), in relation to the hatching mechanism of Microsporidian spores. *Journal of General Microbiology* **85**, 350–357.

Slothouber-Galbreath, J. G. N., Smith, J. E., Becnel, J. J., Butlin, R. J. and Dunn, A. M. (2009). Reduction in post-invasion genetic diversity in *Crangonyx pseudogracilis* (Amphipoda: Crustacea): a genetic bottleneck or the work of hitchhiking vertically transmitted microparasites? *Biological Invasions*, online doi: 10.1007/s10530-009-9442-3.

Slothouber-Galbreath, J. G. M., Smith, J. E., Terry, R. S., Becnel, J. J. and Dunn, A. M. (2004). Invasion success of *Fibrillanosema crangonycis*, n.sp., n.g.: a novel vertically transmitted microsporidian parasite from the invasive amphipod host *Crangonyx pseudogracilis*. *International Journal for Parasitology* **34**, 235–244.

Solter, L. F. (2006). Transmission as a predictor of ecological host specificity with a focus on vertical transmission of Microsporidia. *Journal of Invertebrate Pathology* **92**, 132–140.

Solter, L. F. and Maddox, J. V. (1998). Physiological host specificity of microsporidia as an indicator of ecological host specificity. *Journal of Invertebrate Pathology* **71**, 207–216.

Solter, L. F., Maddox, J. V. and McManus, M. L. (1997). Host specificity of Microsporidia (Protista: Microspora) from European populations of *Lymantria dispar* (Lepidoptera: Lymantriidae) to indigenous North American Lepidoptera. *Journal of Invertebrate Pathology* **69**, 135–150.

Solter, L. F., Pilarska, D. K. and Vossbrinck, C. F. (2000). Host specificity of microsporidia pathogenic to forest Lepidoptera. *Biological Control* **19**, 48–56.

Sokolova, Y. Y., Dolgikh, V. V., Morzhina, E. V., Nassonova, E. S., Issi, I. V., Terry, R. S., Ironside, J. E., Smith, J. E. and Vossbrinck, C. R. (2003). Establishment of the new genus *Paranosema* based on the ultrastructure and molecular phylogeny of the type species *Paranosema grylli* Gen. Nov., Comb. Nov. (Sokolova, Selezniov, Dolgikh, Issi 1994), from the cricket *Gryllus bimaculatus* Deg. *Journal of Invertebrate Pathology* **84**, 159–172.

Soklova, Y. Y., Issi, I. V., Morzina, E. V., Tokarev, Y. S. and Vossbrink, C. R. (2005). Ultrastructural analysis supports transferring *Nosema whitei* Weiser 1953 to the genus *Paranosema* and creation a new combination, *Paranosema whitei*. *Journal of Invertebrate Pathology* **90**, 122–126.

Sprague, V., Becnel, J. J. and Hazard, E. I. (1992). Taxonomy of phylum Microspora. *Critical Reviews in Microbiology* **18**, 285–395.

Stanimirovic, Z., Stevanovic, J., Bajic, V. and Radovic, I. (2007). Evaluation of genotoxic effects of fumagillin by cytogenetic tests *in vivo*. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis* **628**, 1–10.

Streett, D. A. (1994). Analysis of *Nosema locustae* (microsporidia:Nosematidae) chromosomal DNA with pulsed-field gel electrophoresis. *Journal of Invertebrate Pathology* **63**, 301–303.

Terry, R. S., Dunn, A. M. and Smith, J. E. (1997). Cellular distribution of a feminizing microsporidian parasite: A strategy for transovarial transmission. *Parasitology* **115**, 157–163.

Terry, R. S., Dunn, A. M. and Smith, J. E. (1999). Segregation of a cytoplasmic parasite during host cell mitosis. *Parasitology* **118**, 43–48.

Terry, R. S., Smith, J. E., Bouchon, D., Rigaud, T., Duncanson, P., Sharpe, R. G. and Dunn, A. M. (1999). Ultrastructural characterisation and molecular taxonomic identification of *Nosema granulosis* n. sp., a transovarially transmitted feminising (TTF) microsporidium. *Journal of Eukaryotic Microbiology* **46**, 492–499.

Terry, R. S., Smith, J. E. and Dunn, A. M. (1998). Impact of a novel feminizing microsporidian parasite on its crustacean host. *Journal of Eukaryotic Microbiology* **45**, 497–501.

Terry, R. S., Smith, J. E., Sharpe, R. G., Rigaud, T., Littlewood, D. T., Ironside, J. E., Rollinson, D., Bouchon, D., MacNeil, C., Dick, J. T. and Dunn, A. M. (2004). Widespread vertical transmission and associated host sex-ratio distortion within the eukaryotic phylum Microspora. *Proceedings of the Royal Society Series B, Biological Sciences* **271**, 1783–1789.

Tounou, A. K., Kooyman, C., Douro-Kplndou, O. K. and Poehling, H.-M. (2008). Interaction between *Paranosema locustae* and *Metarhizium anisopliae* var. acridum, two pathogens of the desert locust, *Schistocerca gregaria* under laboratory conditions. *Journal of Invertebrate Pathology* **97**, 203–210.

Troemel, E. R., Felix, M.-A., Whiteman, N. K., Barriere, A. and Asubel, F. M. (2008). Microsporidia are natural intracellular parasites of the nematode *Caenorhabditis elegans*. *PLoS Biology* **6**, 2736–52.

Tsaousis, A. D., Kunji, E. R., Goldberg, A. V., Lucocq, J. M., Hirt, R. P. and Embley, T. M. (2008). A novel route for ATP acquisition by the remnant mitochondria of *Encephalitozoon cuniculi*. *Nature* **453**, 553–556.

Undeen, A. H. and Vandermeer, R. K. (1994). Conversion of intrasporal trehalose into reducing sugars during germination of *Nosema algerae* (Protista: Microspora) spores—a quantitative study. *Journal of Eukaryotic Microbiology* **41**, 129–132.

Vavra, J. and Undeen, A. H. (1970). *Nosema algerae* n. sp. (Cnidospora, Microsporidia) a pathogen in a laboratory colony of *Anopheles Stephensi* Liston (Diptera:Culicidae). *Journal of Protozoology* **17**, 240–249.

Vavra, J. (1976). Structure of the Microsporidia. In *Comparative Pathobiology* (ed. Bulla, L. A. and Cheng, T. C.), pp. 1–85, Vol. 1, Plenum Press, New York, NY.

Vavra, J., Hylis, M., Vossbrinck, C. R., Pilarska, D. K., Weiser, J., McManus, M. L., Hoch, G. and Solter, L. F. (2006). *Vairimorpha disparis* n. comb. (Microsporidia: Burenellidae): A redescription and taxonomic revision of *Thelohania disparis* timofejeva 1956, a microsporidian parasite of the gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). *Journal of Eukaryotic Microbiology* **53**, 292–304.

Vavra, J. and Larsson, J. I. R. (2001). In *Microsporidia and Microsporidiosis* (ed. Wittner, M. and Weiss, L. M.), pp. 447–501, 7–84. ASM press, Washington DC.

Vizoso, D. B. and Ebert, D. (2005). Phenotypic plasticity of host-parasite interactions in response to the route of infection. *Journal of Evolutionary Biology* **18**, 911–921.

Vorontsova, L., Tokarev, S., Sokolova, Y. Y. and Glupov, V. V. (2004). Microsporidiosis in the wax moth *Galleria mellonella* (Lepidoptera: Pyralidae) caused by *Vairimorpha ephestiae* (Microsporidia: Burenellidae). *Parazitologiia* **38**, 239–50.

Vossbrinck, C. R., Maddox, J. V., Friedman, S., Debruner-Vossbrink, B. A., Vossbrinck, C. R., Andreadis, T. G., Vavra, J. and Becnel, J. J. (2004). Molecular phylogeny and evolution of mosquito parasitic Microsporidia (Microsporidia: Amblyosporidae). *Journal of Eukaryotic Microbiology* **51**, 88–95.

Vossbrinck, C. R., Maddox, T. J., Friedman, S., Debrunner-Vossbrinck, B. A. and Woese, C. R. (1987). Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. *Nature* **326**, 411–414.

Vossbrinck, C. R. and Debrunner-Vossbrinck, B. A. (2005). Molecular phylogeny of the microsporidia: ecological, ultrastructural and taxonomic considerations. *Folia Parasitologica* **52**, 131–142.

Watanabe, M. E. (2008). Colony collapse disorder: Many suspects, no smoking gun. *Bioscience* **58**, 384–388.

Wattier, R. A., Haine, E. R., Beguet, J., Martin, G., Bollache, L., Muskó, I. B., Platvoet, D. and Rigaud, T. (2007). No genetic bottleneck or associated microparasite loss in invasive populations of a freshwater amphipod. *Oikos* **116**, 1941–1953.

Webster, J. D., Miller, M. A. and Vemulapalli, R. (2008). *Encephalitozoon cuniculi*-associated placentitis and perinatal death in an alpaca (*Lama pacos*). *Veterinary Parasitology* **45**, 255–259.

Webster, T. C., Pomper, K. W., Hunt, G., Thacker, E. M. and Jones, S. C. (2004). *Nosema apis* infection in worker and queen *Apis mellifera*. *Apidologie* **35**, 49–54.

Webster, T. C., Thacker, E. M., Pomper, K., Lowe, J. and Hunt, G. (2008). *Nosema apis* infection in honey bee (*Apis mellifera*) queens. *Journal of Apiculture Research* **47**, 53–57.

Weedall, R. T., Robinson, M., Smith, J. E. and Dunn, A. M. (2006). Distribution of two vertically transmitted, feminising microsporidia during host embryogenesis. *International Journal for Parasitology* **36**, 749–756.

Williams, B. A., Hirt, R. P., Lucocq, J. M. and Embley, T. M. (2002). A mitochondrial remnant in the microsporidian *Trachipleistophora hominis*. *Nature* **418**, 865–869.

Williams, B. A., Slamovits, C. H., Patron, N. J., Fast, N. M. and Keeling, P. J. (2005). A high frequency of overlapping gene expression in compacted eukaryotic genomes. *Proceedings of the National Academy of Sciences, USA* **102**, 10936–10941.

Woese, C. R. (1987). Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. *Nature* **326**, 411–414.

Xu, J., Pan, G., Fang, L., Li, J., Tian, X., Li, T., Zhou, Z. and Xiang, Z. (2006). The varying microsporidian genome: Existence of long-terminal repeat retrotransposon in domesticated silkworm parasite *Nosema bombycis*. *International Journal for Parasitology* **36**, 1049–1056.

Xu, Y. and Weiss, L. M. (2008). The microsporidian polar tube: A highly specialised invasion organelle. *International Journal for Parasitology* **35**, 941–953.