
10 Symbiosis of Microsporidia and Insects

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CONTENTS

Introduction	145
Phylogenetic Position and Genomic Properties	146
Ancient Eukaryotes	146
Not-So-Ancient Eukaryotes	147
Comparisons with the Fungi	147
The Microsporidia: Degenerate Eukaryotes	148
Genomic Data	148
Aspects of Intracellular Life	150
Macroevolutionary Variation and Patterns of Transmission	152
Spores and the Polar Filament	152
Intrahost Transmission	153
Vertical Transmission	154
Mixed Patterns of Vertical and Horizontal Transmission	155
Where Vertical Transmission Dominates	156
Microevolutionary Variation	156
Adaptation to Altered Conditions of Transmission	157
Intrahost Competition	157
Local Adaptation	158
Genetic Variation	158
Host Responses	159
Concluding Remarks	160
Acknowledgments	160
References	161

INTRODUCTION

A symbiotic relationship is one in which two dissimilar organisms live in close association with one another. This does not imply that they necessarily live together in harmony; symbiosis also applies to host–parasite relationships. The relationships formed by the microsporidia are extreme forms of symbiosis: these unicellular eukaryotes have become obligate intracellular parasites that cannot live independently outside of their host-cell environment.

In essence, microsporidia infect host cells, exploit what is there to replicate within them, produce spores, and then transmit themselves to other cells either within the same host or to a new host. However, the diversity of ways that they achieve this and the diversity of host species

they exploit indicate the number of the evolutionary pathways these organisms have managed to successfully negotiate in adapting to the needs of their own life histories and those of their hosts. The course of their evolution toward an obligate intracellular life style is marked by several departures from what is considered to be a “standard” eukaryotic cell. Furthermore, their transition to a parasitic life style is associated with a unique manner of infecting host cells that separates them from other eukaryotes.

The aim of this chapter is to illustrate aspects of the symbiotic relationship between the microsporidia and their hosts. Most examples are drawn from relationships involving invertebrate hosts, with additional material taken from those that infect vertebrates. The bias toward invertebrate host material is appropriate as the microsporidia have been described mainly as pathogens of insects. Indeed, the first species to be described, *Nosema bombycis* (Naegeli, 1857), was from an insect. This species was later shown by Pasteur (1870) to be the causative agent of pébrine, a disease responsible for serious economic harm to the commercial silk industry at the time; a related species, *N. apis*, still causes similar problems for commercial honey producers today. In fact, a substantial proportion of our knowledge about the basic biology and ecology of the microsporidia is based on studies related to their potential for controlling insects of either a medical or economic importance (Sweeney and Becnel, 1991). However, much of our knowledge about what goes on at the molecular level has been derived from studies on the microsporidia, which have been found to be important opportunist pathogens of immunocompromised humans.

PHYLOGENETIC POSITION AND GENOMIC PROPERTIES

The microsporidia have occupied the attention of taxonomists and systematists for some time and have been subject to several reclassifications at all levels of organization, from the species level up to that of their phylum’s affinity with other eukaryotes. In this section we discuss their phylogenetic position and properties of their genome that reveal valuable information about the changes that have occurred during their transition to an intracellular and parasitic life style.

ANCIENT EUKARYOTES

Early studies on the morphological characteristics of microsporidian cells found that they lacked several components of a “standard” eukaryotic cell, e.g., the organelles of mitochondria and peroxisomes, suggesting they were primitive and had branched off the eukaryote lineage before their acquisition (see Table 10.1 for further details). The interpretation of a distant origin was

TABLE 10.1
Cytological Structures
Considered “Standard” for
Eukaryote Cells That Are Absent
in Microsporidian Cells

Mitochondria
Centrioles
Flagella
Peroxisomes
Hydrogenosomes
Glycosomes
Nutrient storage granules
A conventional Golgi apparatus
80 S. ribosomes

supported by molecular data showing that microsporidia possessed not the typical 80S ribosomes of eukaryotes but rather the 70S ribosomes more representative of the prokaryotes (Ishihara and Hayashi, 1968). The structure of their 5.8S rRNA was also viewed as prokaryote-like (Vossbrinck and Woese, 1986). Phylogenetic studies based on small and large subunit rRNA, elongation factors EF-1 α and EF-2, and isoleucyl-tRNA synthetase also tended to place the microsporidia near the base of the eukaryotic tree, reinforcing the notion of their ancient origin (Vossbrinck et al., 1987; Keeling and McFadden, 1998).

NOT-SO-ANCIENT EUKARYOTES

This picture began to change as evidence started to accumulate in favor of a much more recent origin of the microsporidia; there was also evidence suggesting that the traits considered primitive were more likely to be derived characteristics. For example, their amitochondrial origin was strongly challenged when genes coding for heat-shock protein 70 (hsp70) were detected in two species of microsporidia (Germot et al., 1997; Hirt et al., 1997). These genes were once part of a mitochondrial genome and are likely to have been transferred and incorporated into the genome of their host during the transition from symbiont to fully-fledged organelle. Their presence reveals that the ancestors of the microsporidia had mitochondria. The prokaryote-like 5.8S rRNA could also be interpreted as a derived state due to deletions in rRNA (Cavalier-Smith, 1993).

The basal phylogenetic placement of the microsporidia in the studies above is likely to be an artifact due to the high degree of divergence in their sequences and a rapid rate of evolution, which creates long branch lengths on phylogenetic trees (Keeling and McFadden, 1998). Long branches tend to cluster together, whether related or not, thereby increasing the probability that microsporidia and other long-branched eukaryotes will be found at the base of a phylogenetic tree and artificially clustered with long-branched prokaryotes (Keeling and McFadden, 1998).

As molecular data continue to accumulate, it appears that the microsporidia are located in the crown of the eukaryotes and closely related to the fungi, if not directly descended from them. Evidence for this is found in the similarities between the hsp70 sequence data of the microsporidia and the fungi (Hirt et al., 1999). Furthermore, data from a number of structural proteins, such as the α - and β -tubulins, place the microsporidia as a clearly defined lineage within the main fungal radiation (Keeling et al., 2000). A detailed treatment of the molecular data available on the microsporidia can be found in Weiss and Vossbrinck (1999).

COMPARISONS WITH THE FUNGI

Fungi constitute an extremely large and diverse group of heterotrophic organisms devoid of chlorophyll that have a cell wall, are nonmotile (some species have motile reproductive cells), and reproduce by means of a tremendous variety of spore types (Alexopoulos et al., 1996). Fungi are usually filamentous and multicellular, with glycogen as the primary carbohydrate-storage product (trehalose in yeast and lichens). Obligate parasitic fungi infect plants, animals, and, in some cases, even other fungi.

The microsporidia are a large group of strictly obligate, intracellular parasites that infect most animal groups (from protists to humans) but are not known to infect plants or fungi (Becnel and Andreadis, 1999; Vávra and Larsson, 1999). Only the spores of microsporidia are walled, and all spores contain large amounts of trehalose. Vegetative growth is by nonmotile amoeba-like stages (often multinucleate) with simple plasma membranes. Although variable in some respects, all microsporidian spores are definitively and uniquely characterized by a coiled polar filament. At germination, the polar filament is inverted to become a tube for transport of the sporoplasm into the host cell.

Recent interest in comparisons of microsporidia and fungi is the result of molecular evidence that places microsporidia as a sister group of the fungi (Keeling et al., 2000). However, these

comparisons are confounded by widespread convergence and rapid divergence of these groups. Therefore, the following table is an attempt, in a very general way, to examine some of the similarities and differences between microsporidia and fungi related to their basic biology and characteristics that are more closely aligned with parasitism (Table 10.2). It is hoped that this will stimulate investigations on both groups to help clarify the many points where information is unclear or lacking.

THE MICROSPORIDIA: DEGENERATE EUKARYOTES

Microsporidia were previously considered to be primitive eukaryotes partly because they lacked several “standard” components of a eukaryotic cell (see Table 10.1). Phylogenetic data provide a means to refute this possibility. Furthermore, this pattern of degeneration is widely observed in other organisms, eukaryotic and prokaryotic, that have made the transition to either a mutualistic or parasitic intracellular life style (Andersson and Kurland, 1998; Moran and Wernegreen, 2000). In essence, selection acts to eliminate any redundancy caused by overlapping functions between the two organisms, leaving only genes that serve some essential function. In the case of parasitic relationships, these genes will include those responsible for ensuring the parasite’s transmission among its hosts.

GENOMIC DATA

The impressive reductionism in the physical components of microsporidian cells is matched by that of their genomes. Microsporidia possess some of the smallest eukaryotic genomes known, currently ranging from 2.3 to 19.5 megabases (Mb), and show great economy in their genetic material relative to free-living organisms. Much of the data on this topic has been derived from studies on *Encephalitozoon cuniculi*, an important opportunist pathogen of immunocompromised humans whose entire genome has recently been sequenced (Katinka et al., 2001).

The haploid genome of *E. cuniculi* consists of 2.9 Mb and is considerably smaller than some prokaryotic genomes; for example, estimates for strains of the bacterium *Escherichia coli* vary from 4.6 to 5.6 Mb. Furthermore, comparably compact genomes are found in related species that also infect humans and other mammals — *E. hellem* 2.5 Mb, *E. intestinalis* 2.3 Mb (data taken from Méténier and Vivarès, 2001).

The genome of *E. cuniculi* itself is made from 11 linear chromosomes that range in length from 217 to 315 kilobases and harbor a total of approximately 2000 protein-coding genes (Katinka et al., 2001). Important features that reveal its compactness include the paucity of duplicated genes and lack of simple sequence repeats, minisatellite arrays, and known transposable elements in its chromosome cores. Its intergenic spacing regions are short, having a mean and minimum length of 129 and 29 base pairs, respectively. As a consequence, protein-coding DNA sequences occupy approximately 90% of the chromosome cores. Its rRNA operons are unusually short, due to deletions in transcribed spacers and coding regions. Interestingly, there is evidence that the 16S rRNA is shortened from early to late diverging species of *Amblyospora* and relatives (Baker et al., 1997).

There is also evidence of extensive reduction in the size of individual genes, as reflected in the number of amino acids forming their proteins. The genome-wide extent of these reductions was shown by a comparison of the lengths of 350 homologous proteins from *E. cuniculi* and those of another eukaryote that had been entirely sequenced — that of the free-living yeast *Saccharomyces cerevisiae*. Roughly 85% of the *E. cuniculi* proteins were shorter, and on average 15% shorter (Katinka et al., 2001). For example, genes for the receptor proteins of kinesin and SRP are up to one third shorter than those known for other eukaryotes (Biderre et al., 1999).

Future developments in the field of comparative genomics promise to be revealing, both with respect to other eukaryotes and within the microsporidia: the few data that currently exist suggest that the genomes of microsporidia infecting invertebrates are larger and vary considerably in size

TABLE 10.2
A Comparison of Major Features of the Microsporidia and Fungi

Characteristics	Microsporidia	Fungi
Cytological Structures		
Mitochondria	Not demonstrated	Present
Perioxosomes	Absent	Present
Lysosomes	Absent	Present
Paramural Bodies	Present	Present
Golgi	Lack stacked cisternae	Variable, some stacked, some unstacked
Endoplasmic reticulum	Smooth and rough	Smooth and rough
Ribosomes	Prokaryote-size (70S) consisting of large (23S) and small (16S) subunits; lack 5.8S but homolog found at beginning of 23S subunit	Typical eukaryotic size of 80S
Cytoskeleton	Microtubules, keratin filaments, probably actin and intermediate filaments	Microtubules, actin, and intermediate filaments
Centrioles	Absent, spindle attachment to "spindle plaque;" small "polar bodies" located near the spindle plaque	Present only in Chytridiomycota (composed of nine triplets), replaced by spindle pole bodies (SPBs) in most true fungi. Multivesicular bodies associated with the SPB
Physiology		
Trehalose	Present in large amounts in spores of most species examined and is perhaps involved in germination	A reserve disaccharide of fungi (esp. yeast and lichens)
Chitin	Present in endospore	Present in cell and spore walls
Glucans	?	Present
Motion		
Flagella	Absent	Present only in the Chytridiomycota
Invasion		
Resting stage (spore)	Polymorphic, uninucleate, and binucleate spores. Some species produce only one type of spore, some produce as many as four types	Sexually (oospores, zygosporoes, ascospores, basidiospores) and asexually (conidia or sporangiospores) are produced, all uninucleate. Species with one up to four types of spores
Spore wall Mechanism	Exospore (which can be layered) and endospore Typically, spore germination occurs in the gut by eversion (under tremendous pressure) of the polar filament which becomes a hollow tube for transfer of the sporoplasm to the cytoplasm of a host cell	Variable, two to five layers Spores attach to surface and produce a penetration germ tube that gains access through enzymatic and mechanical activity
Replication		
Nuclear arrangement	Uninucleate and diplokaryotic (stable arrangement of paired haploid nuclei)	Mostly uninucleate, some with a dikaryon (two unpaired haploid nuclei)
Mitosis	Intranuclear, nuclear envelopes do not break down during division	Intranuclear
Meiosis	Occurs in both uninucleate (diploid) and diplokaryotic nuclei (each diploid), synaptonemal complexes of typical eukaryotic type	Occurs only in uninucleate nuclei (diploid). Not present in imperfect fungi

(continued)

TABLE 10.2 (CONTINUED)
A Comparison of Major Features of the Microsporidia and Fungi

Characteristics	Microsporidia	Fungi
Vegetative growth	Binary fission and multiple fission of wall-less plasmodia	Walled hyphae develop by apical growth, in yeast by budding or binary fission
Sporulation	Varies from bisporous sporogony that produces two spores from each sporont to polysporous sporogony producing many spores; can involve meiosis or nuclear dissociation of diplokarya	Very variable
Reproduction		
Asexual species	Two basic forms: one uninucleate (haploid) throughout development and produces uninucleate spores, the other diplokaryotic (diploid) throughout development and produces diplokaryotic spores	Uninucleate (haploid) throughout development producing uninucleate spores
Sexual species	Typically an alternation of uninucleate development producing haploid spores (some involving meiosis) and diplokaryotic development producing diplokaryotic spores; some involve an obligate intermediate host	Alternation of haploid and diploid cell states, usually included meiosis and produces only uninucleate spores; some involve an intermediate host
Others Features		
Habitat	Obligatory parasites	Facultative and obligatory parasites
Hosts	Only animals, protists to humans	Plants, animals, and other fungi
Tissues	Intracellular, mainly in cytoplasm; many species tissue specific with some causing systemic infections	Exocellular in pathogenic associations
Pathogens	None known	Infected by viruses and other fungi
Drug reaction	Some fungicides and antibiotics effective	Fungicides

relative to *E. cuniculi* and its close relatives, e.g., *N. locustae* 5.4 Mb, *N. pyrausta* 10.5 Mb, and *N. bombycis* 15.3 Mb (data taken from Méténier and Vivarès, 2001). Whether or not these differences are due mainly to protein-coding sequences of DNA remains to be established. Identifying matching or mismatching genes from different genomes should shed light on some of the functional constraints faced by different species.

To summarize, several organelles that characterize a “standard” eukaryotic cell are absent in the microsporidia. Contrary to being a primitive state due to an early separation from the eukaryotic lineage, it now seems likely that these organelles were lost at a much later date and during their transition toward a parasitic intracellular life style. This loss of organelles is paralleled by the loss of nonessential genetic material leading to some microsporidia possessing some of the most compact eukaryotic genomes known. Current phylogenetic estimates place the microsporidia as being closely related to the fungi, if not derived from within them. These two groups share some characteristics but not others.

ASPECTS OF INTRACELLULAR LIFE

Almost all species of microsporidia undergo their growth and production of spores within the host cell’s cytoplasm. The only exceptions to this pattern are the few species that develop within the nuclear cavity of their host cell, e.g., *Nucleospora* spp. (Elston et al., 1987). No microsporidia

develop outside of their host cells. This pattern strongly suggests that these parasites require their host environment for the uptake of energy and nutrients. This suspicion is reinforced by data from *E. cuniculi* that show it possesses few genes for amino-acid biosynthesis and those required for the tricarboxylic-acid cycle, the respiratory electron-transport chain, and fatty-acid synthesis have not been detected (Méténier and Vivarès, 2001).

Cytological evidence shows that the initial developmental stages (meronts) can take resources directly from the host's cytoplasm by endocytosis (Vávra and Maddox, 1976). However, this cannot be the case in the later developmental stages of many species, as a structure forms around the developing parasite and physically separates it from the host's cytoplasm (forming a parasitophorous vacuole). Depending on the relationship involved, this structure may be secreted by the host, the parasite, or both (Cali and Takvorian, 1999). The functional significance of these differences is unknown.

A conspicuous feature observed in ultrastructural studies that implicates the host cell as a provider of resources is the physical attraction of host organelles, notably mitochondria and endoplasmic reticulum, to the interface of the structure enveloping the developing parasite (Figure 10.1). This physical proximity suggests the parasite is exploiting its host cell's resources. Furthermore, there are documented cases of gap junctions being formed with host mitochondria (e.g., Terry et al., 1999). These intercellular channels provide the means for the exchange of ions and macromolecules from host to parasite, e.g., iron, fatty acids, cholesterol, and ATP. The parasite's potential for energy uptake is further supported by the identification of four ADP/ATP carrier protein genes in *E. cuniculi* (Vivarès and Méténier, 2001). These carriers are homologs to those found in other obligate intracellular organisms known to import host ATP, e.g., *Rickettsia* and *Plasmodium*.

The microsporidia, however, are probably not entirely dependent on host ATP for their source of energy. In particular, the use of trehalose for ATP production, via glycolysis, is thought to be of importance (Weidner et al., 1999). Insect fat body cells are potentially rich sources of this resource; they have a central role in an insect's metabolism because they convert sugars into carbohydrates (glucose and trehalose) and lipids. The parasite's use of trehalose would correlate with the frequent observation of reduced amounts of granular carbohydrates in the cytoplasm of infected host cells (Weiser, 1976). Infected cells also often show reduced amounts of lipids. The microsporidia cannot be directly metabolizing this resource themselves, as that would require oxidative respiration by mitochondria. However, as insects cannot convert lipids back into carbohydrates, they must instead pass via the tricarboxylic-acid cycle within mitochondria. It is at this point that the microsporidia can exploit this source of the host's energy reserves.

Much still remains to be discovered about the metabolism of the microsporidia. For example, what are the parasite's protein requirements and where do they come from? Do they, as some

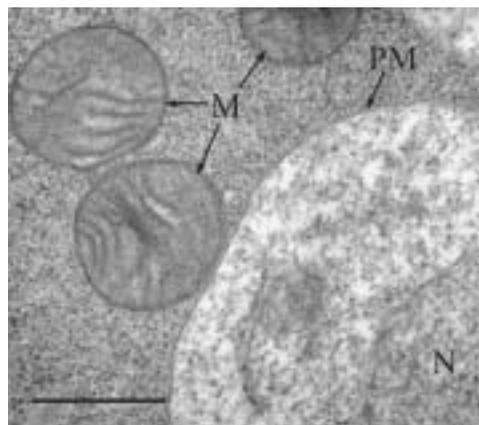


FIGURE 10.1 Several host mitochondria near the plasma membrane of a uninucleate (N) meront. Bar = 1 μ m.

authors suggest (Scanlon et al., 2000), manipulate activity of the host cell via polyamines secreted into its cytoplasm? Perhaps most intriguingly, it seems possible that the microsporidia have a unique form of energy metabolism catabolized by different enzymes than those of other amitochondrial eukaryotes (Vivarès and Méténier, 2001).

To summarize, the evidence suggests that microsporidia depend entirely on their host cells for their metabolic requirements. Resource uptake may be partially achieved by endocytosis, but a more important mechanism is probably by direct importation across the cell membrane from host organelles recruited into close physical proximity to the parasite or its surrounding parasitophorous vacuole. The exploitation of host-derived ATP and trehalose seems likely, as less is currently known about how other metabolic requirements are satisfied. Progress in this area can be expected as further elements arise from the decoding of the *E. cuniculi* genome: to date, only approximately half of its 2000 protein-coding genes have been assigned a putative functional role.

MACROEVOLUTIONARY VARIATION AND PATTERNS OF TRANSMISSION

Despite the simplicity of the generic microsporidian life cycle outlined in the introduction, the diversity of ways that this has been adapted is impressive. Currently there are some 143 described genera of microsporidia, encompassing well over 1000 species. Almost half of these genera, 69, have an insect as the type host (Becnel and Andreadis, 1999). In this section, we focus on a limited range of examples to illustrate some of this diversity and adaptations these parasites have made to enhance their transmission success, both within and among individual hosts. However, first we will briefly describe the unique apparatus that enables them to infect host cells and that provides the basis for classifying the microsporidia apart from other eukaryotes.

SPORES AND THE POLAR FILAMENT

Like many other organisms, microsporidia produce spores for protecting themselves against harsh environmental conditions. These spores are protein-walled and typically small in size, varying from 1 to 10 μm in length depending on the species involved. Some species show adaptations to increase their contact with hosts, e.g., appendages to aid spore floatation for those infecting aquatic larvae (Vávra and Maddox, 1976), but none possesses its own means of propulsion. Species that infect terrestrial hosts typically produce spores that can withstand prolonged periods of desiccation and cold temperatures, while those from aquatic hosts may become inviable after only a few hours of being dried or held at 4°C (Undeen and Becnel, 1992). Up to four different types of spores may be produced during the course of a life cycle, with each type having a specific role in the transmission of the parasite (Johnson et al., 1997). However, the defining feature of the microsporidia is not their spores but the polar filament apparatus they enclose.

The polar filament is effectively a hollow tube anchored at the anterior of the spore and coiled up inside it (Figure 10.2). The number of coils varies among species and spore types, but when fully unfurled it can easily reach beyond a dozen times the length of the spore itself (Figure 10.3). When the appropriate environmental conditions trigger germination, this filament begins to evert itself from its point of attachment, turning inside out as it extends into the environment outside the spore. This unravelling is explosive, having been recorded with a mean acceleration and velocity of 500 $\mu\text{m}/\text{sec}^{-2}$ and 105 $\mu\text{m}/\text{sec}^{-1}$, respectively (Frixione et al., 1992). In many species, the force for this propulsion is thought to derive from an osmotic process increasing the turgor within the cell. Once externalized, the polar filament becomes known as the polar tube. It is thought that this tube is capable of piercing adjacent host cells and entering directly into their cytoplasm. As pressure continues to develop within the spore, the nucleus and other contents get forced down the polar tube, distending it as they go, until they pass out of the end of the tube. If the polar tube has pierced a host cell, these contents (the sporoplasm) are injected directly into the host cell's cytoplasm.

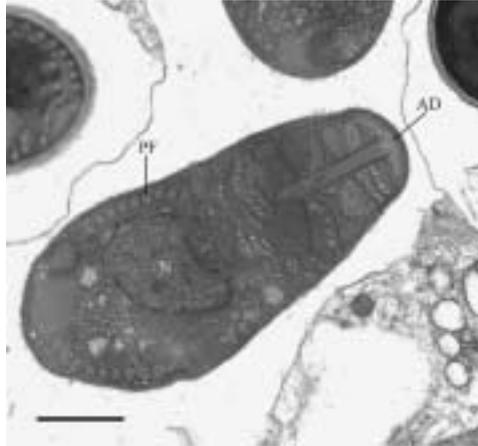


FIGURE 10.2 Immature spore of *Hazardia miller* showing the anchoring disc (AD) of the coiled polar filament (PF) and the single nucleus. Bar = 1 μ m.

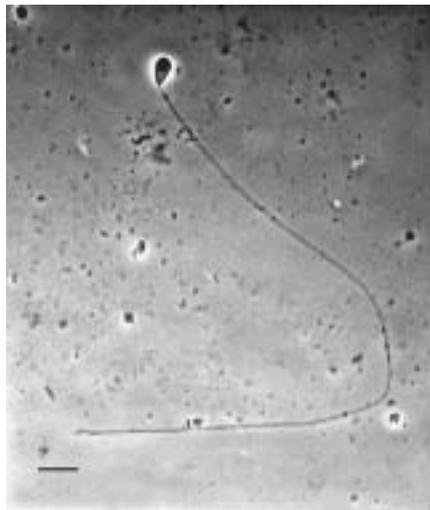


FIGURE 10.3 Germinated spore of *E. aedis* with the polar tube fully everted. Bar = 10 μ m.

From this point, the parasite's intracellular development can begin. No other eukaryotic parasite possesses a similar means of entering its host cell that combines elements of a harpoon and a hypodermic needle.

Many details about the processes of spore formation, germination, and host-cell infection await clarification (Vávra and Larsson, 1999). Rapid progress in this area is predicted as the spores and their polar tubes are the likely targets for chemotherapeutic intervention and are under intensive study.

INTRAHOST TRANSMISSION

The predominant route of infection for most microsporidia is probably via spores ingested from the host's environment along with its food. This also means that epithelial cells lining the host's gut are the tissues most directly exposed to the risk of infection.

For microsporidia with a relatively simple life cycle, infection of a host gut cell is followed by a succession of developmental stages, during which proliferation occurs, before spores are produced.

The accumulation of spores leads to rupturing of the host cell and the dissemination of spores into the gut lumen. From here they may infect other gut cells or get passed out into the environment with feces and thus become available for horizontal transmission to other hosts. Such infections are usually chronic and have a debilitating effect on the host's fecundity and longevity. An example of such a microsporidian, discussed elsewhere in this chapter, is *Glugoides intestinalis* (Larsson et al., 1996).

Rather than completing their whole life cycle within a single cell of the host's gut epithelium, some microsporidia show intrahost transmission and spread themselves away from the original site of infection to other tissues within the host. Various mechanisms by which this happens have been proposed, but the most clearly documented is via the production of "early" spores (Iwano and Kurtti, 1995). These spores are often formed within 48 to 72 h post infection and are morphologically different from the spores responsible for the initial infection via the environment — "environmental" or "late" spores. In particular, they have shorter polar tubes and thinner spore walls, suggesting they are more economical to manufacture both in terms of time and material. The functional role of these spores is to infect secondary cells within the same host, a process also known as "autoinfection." This may occur by the *in situ* germination of spores and the inoculation of their contents into neighboring cells. Alternatively, ruptured gut cells may disseminate these spores into the host's hemocoel and provide a means of infecting more distant tissues.

There are several reasons to consider why intrahost transmission might be advantageous. It increases the number of foci of infection and potentially the number of spores produced. A related advantage is that it may permit the parasite to maintain its presence by escaping from epithelial gut cells likely to be shed or digested during a moult or metamorphosis (Andreadis, 1985b). Transmission within the host can also give access to other tissues of a superior nutritional value for the parasite's growth, e.g., fat-body cells. However, migration away from epithelial cells can also physically limit the opportunities for subsequent transmission to other hosts via spores shed into the alimentary canal. The microsporidia seem to have found two main ways of solving this problem.

The first is to exploit the host's inner tissues for a significant production of spores, causing widespread pathology and provoking the host's death. These spores are subsequently released into the environment as the host cadaver decomposes. In examples of such cases, the host's fat body can literally be converted into a sack containing millions of spores (Becnel and Andreadis, 1999).

An alternative solution is not to seek horizontal transmission from infected individuals themselves but instead to do so from their offspring.

VERTICAL TRANSMISSION

Many microsporidia show a pattern of intrahost transmission that brings them into close physical proximity with the host's reproductive tissues and from where cells of the developing offspring can be infected. This may be achieved directly by spores circulating in the host hemocoel or indirectly via passage in mobile host cells that migrate toward reproductive tissues, e.g., oenocytes (Becnel et al., 1989).

There are at least two important consequences of adopting this transmission behavior. First, a parasite's transmission success relies on the reproductive success of its host. This constrains the negative effects that the parasite can have on its host's reproduction if it is not to adversely effect its own transmission success. The second important constraint associated with vertical transmission is that a host offspring's cytoplasm is uniparentally inherited from its mother. Thus, only females provide the physical means by which their offspring can be directly infected, with males effectively being a dead end for vertical transmission. Despite these constraints, the use of vertical transmission is phylogenetically widespread among the microsporidia (Dunn et al., 2001). We will focus on two contrasting examples of adaptation to these constraints.

MIXED PATTERNS OF VERTICAL AND HORIZONTAL TRANSMISSION

Our first example involves microsporidia of the family Amblyosporidae that predominantly infect Dipteran hosts, most notably mosquitoes. Host larvae are horizontally infected when they ingest spores from their aquatic environment. Although not documented for all species, it is likely that they all produce “early” spores, enabling migration away from the gut epithelium (Johnson et al., 1997). Circulating oenocytes appear to be targeted by these spores (Becnel et al., 1989). Within these cells a third type of spore is produced. These spores are responsible for vertical transmission when they germinate in proximity to a female host’s reproductive tissues. In some relationships, the production and germination of these spores is arrested until the female mosquito host provides hormonal cues that she is in the process of maturing a clutch of eggs (Hall and Washino, 1986). This mechanism helps to limit the energetic drain and physical damage that continued development and spore production would otherwise have on the host prior to its reproduction.

The exploitation of the vertically infected larvae by this family of microsporidia is remarkable (Kellen et al., 1965). In the most straightforward cases, all of a female’s offspring are likely to be killed late in their juvenile development by a significant production of spores in their fat-body cells. This fourth type of spore (a meiospore) is infectious to an obligate intermediate host, usually a microcrustacean (Andreadis, 1985a). It is the spores released upon the death of the intermediate host that are responsible for horizontal transmission to mosquito larvae. In some relationships, however, only vertically infected male larvae are killed by the production of meiospores, whereas infected females experience “benign” infections, become adults, and transmit the parasite to their own offspring. Intermediate relationships also exist in which varying proportions of female larvae are killed (Kellen et al., 1965). This “late male killing” behavior is reportedly unique to these mosquito–microsporidian relationships and contrasts with “early male killing” behavior shown by several other vertically transmitted parasites in which infected males are killed at an early stage in their development (Hurst, 1991). The essential difference between these two types of parasites is that the microsporidia are more adept at exploiting male hosts for horizontal transmission, whereas the others are limited to increasing their success by vertical transmission alone, e.g., by freeing infected females from resource competition with their brothers (Hurst and Majerus, 1993).

The reasons for this diversity in the sex-specific development of these microsporidia (mechanism unknown) are thought to depend on the ecological conditions of particular relationships and the relative success to be derived from exploiting host females for vertical or horizontal transmission (Hurst, 1991; Agnew and Koella, 1999a). The “sparing” of females for vertical transmission is more likely to be favored in conditions where hosts occupy ephemeral habitats or where the presence of intermediate hosts is not assured. Vertical transmission in these cases has the advantage of enabling the parasite to disperse with its host into the temporally available sites where spores released from male larvae may have the opportunity to infect other host lineages.

An interesting member of this family of microsporidia is *Edhazardia aedis*, as it has no requirement for an intermediate host to complete its life cycle, and the developmental sequence leading to meiospore production is abortive and rarely completed (Becnel et al., 1989). This may reflect an adaptation to a habitat change by its host, the yellow fever mosquito *Aedes aegypti*, to ovipositing in urban habitats that lack the presence of intermediate hosts. Furthermore, it does not rely on a host cue for the production and germination of its vertically transmitting spores. In certain circumstances, this can lead to these spores germinating and for the developmental sequence leading to the production of horizontally transmitting spores occurring in the infected individual rather than in the next generation after vertical transmission. Consequently, although the parasite undergoes the same developmental cycle, its observed sequence of transmission may be horizontal-vertical or horizontal-horizontal (Becnel et al., 1989). The former sequence of transmission is more likely in environmental conditions that favor fast larval growth, while slow larval growth conditions favor the latter (Agnew and Koella, 1999b). These patterns of host exploitation may be adaptive. Fast larval growth is associated with the emergence of large and fecund female mosquitoes likely to

offer the parasite substantial vertical-transmission success. In contrast, slow larval growth rates lead to the emergence of much smaller females that potentially offer much less vertical-transmission success. Prolonged larval growth also increases the potential for the parasite to complete its life cycle and kill its host before emerging, thereby granting the possibility of exploiting both males and females for horizontal transmission (Agnew and Koella, 1999b). While this pattern of development is not without risk to the parasite's own transmission success (Koella and Agnew, 1997), it appears to be adapted to maximizing its vertical- or horizontal-transmission success as a function of its host's life-history traits given the temporally unpredictable and spatially heterogeneous environments occupied by its host.

Further examples of the variation in life cycles of microsporidia that infect mosquitoes or insects in general can be found in Becnel (1994) and Becnel and Andreadis (1999), respectively.

WHERE VERTICAL TRANSMISSION DOMINATES

Our second example of adaptation by the microsporidia to the constraints imposed by vertical transmission is centered on those that are almost exclusively transmitted by this means, with horizontal transmission probably being a rare event.

Some microsporidia only known to have vertical transmission and that infect crustaceans of the genus *Gammarus* have been found to cause very little tissue pathology and no negative effects on their host's growth, reproduction, or longevity (Bulnheim and Vávra, 1968). This pattern fits expectations that vertical transmission will not have an adverse influence on the host's reproductive success. However, a lack of pathology does not indicate that these parasites have a limited effect on their hosts. In contrast, these microsporidia share a trait with several other vertically transmitted pathogens in that they actively augment their possibilities for cytoplasmic inheritance by manipulating the functional sex ratio of their hosts toward females (Dunn et al., 2001). In doing so, these infected matriline are likely to increase in frequency relative to those producing offspring in a 50:50 sex ratio and will help maintain the parasite's presence in its host population.

To summarize, the microsporidia that infect insects are diverse. They produce a range of spore types that have different functional roles in either transmitting the parasite among different cells within an individual host or among different host individuals. The parasite's migration to inner tissues is associated with either development leading to an important production of spores, host death, and horizontal transmission or a developmental sequence leading to vertical transmission. The ways in which vertically infected offspring are exploited vary across different relationships. These differences appear to reflect adaptations by the microsporidia to gain transmission success as a function of the possibilities offered by the host and its ecological context.

MICROEVOLUTIONARY VARIATION

Thus far we have concentrated on variation among different microsporidia. In this section, we focus on cases of evolutionary change within a species. In particular, attention will center on traits linked with a change in the parasite's virulence.

An organism's fitness can be defined as a composite function of how its life-history traits interact with the its environment (Stearns, 1992). Various life-history traits are often correlated with each other to some extent due to the pleiotropic action of individual genes influencing the expression of more than one trait. Of particular relevance are negative pleiotropic interactions such that an increase in fitness due to the increased expression of one trait may be annulled or even reversed by its effect on a correlated trait. In natural conditions, selection pressures may arrive from a variety of sources to keep in check the overexpression of negatively correlated traits and to help maintain genetic diversity for trait values.

Experimentally accentuating particular sources of selection while controlling for others can provide a useful means by which to identify which traits harbor genetic diversity and how they

are correlated with other traits. Serial passage experiments have been particularly useful in this context (Ebert, 1998). In such cases, parasites are usually grown under well-defined conditions where their passage from one environment to the next is performed by the experimenter, e.g., by the injection or transfer of infected material. This latter condition has the effect of reducing constraints acting on the parasite's transmission success among hosts while also offering a selective advantage to genotypes that are proportionately well represented in the parasite population at the time of transfer. Thus, parasites are likely to find themselves in a changed environment with fewer counterbalancing sources of selection due to environmental heterogeneity and where selection pressure is concentrated more on their growth rates than on their ability to come into contact and infect other hosts via the environment.

ADAPTATION TO ALTERED CONDITIONS OF TRANSMISSION

An example of a serial-transfer experiment involving a microsporidian is provided by a study of *N. furnacalis* maintained in cell-culture conditions (Kurtti et al., 1994). After 77 generations of serial passage via infected cells, the parasite showed faster rates of spread than it did after only four generations of culture. This change in growth rate was correlated with an increase in the production of "early" spores, used for autoinfection within host tissues, relative to that for the "late" or "environmental" type of spore used for transmission among hosts via the environment. Furthermore, these "environmental" spores showed reduced infection success and virulence when subsequently exposed to their natural host, the European corn borer *Ostrina furnacalis*. Thus, these results show the parasite's life-history traits adapting to the environmental conditions of serial culture transfer in such a way as to increase their rate of spread through host tissue cells; these changes were negatively correlated with their ability to exploit their original host via the use of their "environmental" spores. Such tradeoffs between the ability to exploit individual hosts and transmit among them have been reported for a number of disease agents and is thought to play a role in limiting the evolution of increased virulence in natural conditions (Ebert, 1998).

Another indication that microsporidia can change their relative investment in different spore types can be drawn from an artificial selection experiment on the vertical transmission success of *Amblyospora dyxenoides* in its mosquito host *Culex annulirostris* (Sweeney et al., 1989). This parasite would normally kill the majority of its host's vertically infected offspring with the production of meiospores destined for horizontal transmission to an intermediate host. However, the spores are not produced in some vertically infected larvae, which experience "benign" infections, and females can vertically transmit the infection to a second generation of larvae. At the beginning of the experiment, 10% of the clutches of vertically infected larvae showed "benign" infections in which there was no meiospore production. This figure increased to 70% after nine generations of selection for vertical transmission. The possibility that these results were influenced by changes in the host cannot be excluded, as they would also have been selected during the course of the experiment. Nonetheless, the results are interesting in view of current estimations placing the Amblyosporidae at a basal position within the microsporidian phylogeny and thus being representative of the life histories from which other genera were derived (Baker et al., 1997).

INTRAHOST COMPETITION

The idea that virulence increases due to intrahost competition among parasites has experimental support from the relationship between *Glugoides intestinalis* and its water flea host *Daphnia magna* (Ebert and Mangin, 1997). Replicated lines of the host-parasite relationships were subjected to a regime of either high or low rates of host mortality for a period of 14 months. It was predicted that higher virulence would evolve in the high-mortality treatment due to the need for faster average rates of host exploitation. The opposite trend was observed, with virulence being higher in the low host-mortality environment. It appears that the low-host-mortality environment increased host

exposure to infection and the probability of their cells receiving a double infection. The consequence of this was to place parasites in direct competition with each other for the resources of the host cell. Indeed, the size of the sporophorous vesicles containing the parasite's spores was smaller in doubly vs. singly infected gut cells, indicating host resource limitation. When tested in standardized conditions, parasites from the low-host-mortality treatment showed faster rates of growth and produced larger sporophorous vesicles. The hosts exposed to these parasites were also killed earlier and with a higher sporeload, indicating that intrahost competition had resulted in the selection for more virulent parasites. Interestingly, although the more virulent strains produced more spores, they had a lower degree of transmission success for a standard infective dose. This suggests a potential tradeoff between virulence and transmission success that may prevent the escalation of virulence in the absence of intrahost competition.

LOCAL ADAPTATION

A further aspect of serial-passage experiments is that pathogens are often exposed to limited genetic diversity of the host during the course of selection (Ebert, 1998). This increases the possibility of their evolving to exploit a particular host genotype, a process known as local adaptation. Evidence that this occurs in natural conditions was shown in an experiment involving strains of *G. intestinalis* isolated from geographically different populations of *D. magna* (Ebert, 1994b). This cyclically parthenogenetic host has the advantage of using mainly clonal reproduction during the course of a breeding season, hence increasing the temporal possibility of parasites encountering the same host genotype within a season. The experiment found for several traits (infection success, probability of killing host, number and transmission success of spores produced) that the different strains of parasite tested were best adapted to exploiting the sympatric host population they were regularly exposed to rather than hosts of unfamiliar genotypes. While not demonstrating local adaptation, other studies have found heterogeneous results when crossing different strains of host and microsporidian in standardized conditions (Ebert, 1994a; Schmid-Hempel and Loosli, 1998), giving rise to the possibility that genotype-by-genotype interactions exist in other relationships involving microsporidia, though this has not always been found to be the case (Woyciechowski and Krol, 2001).

GENETIC VARIATION

A key element in determining the rates of evolutionary change in microsporidian populations will be the amount of genetic variation present. The potential for coevolutionary interactions with some invertebrate hosts may be increased in cases where both have life cycles of a similar duration (Koella et al., 1998). However, very little is currently known with certainty as to how much variation exists or how it is maintained. Some of the studies cited above suggest that individual populations harbor enough variation to respond to experimental selection pressure and that interpopulation variation exists. Genetic diversity at the molecular level has been most intensively studied for species that infect humans. Variation in the number of ITS copies from strains originating from different host reservoirs has been found for some species (Mathis et al., 1999), and nucleotide heterogeneity exists in others (Rinder et al., 1997). How representative these data may be is very unclear. Meiosis has not been reported from these microsporidia, whereas it has from those of invertebrates (Becnel and Andreadis, 1999), prompting the suggestion that this function was lost during the course of their genome compaction (Vivarès and Méténier, 2001). Even where meiosis exists, the opportunity for outcrossing is possible only when individual host cells are infected by more than one strain, for which no data from natural conditions is available. Perhaps the only certainty is that many species can exploit individual hosts for the production of millions of spores, prompting expectations that mutation will result in a certain level of genetic variation.

One type of microevolutionary change that has not yet been reported is that of the evolution of drug resistance. Several benzimidazoles with antimicrosporidial activity are used in the clinical treatment of infections (Blanshard et al., 1992). Widespread use of such drugs will create the

selection conditions for this type of response, though this may be countered if it is traded off with transmission success or if humans form only a limited fraction of the mammalian hosts these species exploit.

To summarize, only a few studies have demonstrated evidence of microevolutionary change either within or among populations of microsporidia. The results obtained have tested theoretical predictions and shed valuable empirical light on factors influencing evolution in host–parasite relationships. However, our ability to address their evolution in the context of population genetics is currently negligible for want of appropriate data.

HOST RESPONSES

The first general line of defense likely to be encountered by microsporidia that infect insects is that of the peritrophic membrane (or matrix) lining the host's alimentary canal. This saves epithelial cells from abrasion during food passage and provides a barrier to help physically separate them from parasites ingested with their diet. It seems plausible that the spatial chasm this creates between spores enmeshed in the membrane and living cells of the host may have had a role in the evolution of the spectacular polar-tube-invasion apparatus of the microsporidia.

There is little or no evidence to show that invertebrate hosts have an immune response that can suppress or clear a microsporidian infection once it has successfully reached a host cell's cytoplasm. Spores can potentially be encapsulated once within the host's hemocoel (Vávra and Undeen, 1970). However, such host responses are rarely reported. Even if this response is possible, it will be useful only if spores are encapsulated before discharging their contents into another cell. Furthermore, this response can do nothing to prevent infection if spores germinate *in situ* and directly inoculate their sporoplasm into a neighboring cell, thus without being exposed to the host's hemocoel.

Hosts may be able to reduce the costs of parasitism by means other than an immune response. One example is provided by an experiment involving crickets infected with *N. acridophagus* (Boorstein and Ewald, 1987). When given the choice, infected hosts preferentially moved to hotter rather than cooler environments and did so more than uninfected controls. When hosts were not given the choice, those that were maintained in the hotter environments suffered less from the costs of parasitism than those kept in cooler conditions. Thus, the infected hosts displayed an example of "behavioral fever" by choosing a temperature that had a more adverse effect on their infection than on themselves. We note parenthetically that there do not appear to be any examples of microsporidia specifically manipulating their host's behavior to promote their own transmission success.

The scope for infected hosts to change their environment or behavior to reduce the costs of parasitism may be fairly limited. A more general response predicted by evolutionary theory is that hosts can achieve this by altering their life-history traits. In particular, infected hosts are expected to lessen the costs they experience by bringing forward their reproductive schedule (Minchella, 1985). In essence, infected hosts should reproduce as much as they can while they can, even if this reallocation of resources would be otherwise detrimental to their fitness if uninfected. Several insect hosts have responded to parasitism in this manner, including the mosquito *Culex pipiens* when infected by *Vavraia culicis*. When infected as larvae, females brought forward their age at pupation relative to control females, even though this was at the expense of their adult size and potential fecundity (Agnew et al., 1999). In different experimental conditions, Reynolds (1970) found no difference in ages at pupation of infected and uninfected females but an increased egg production by infected females in their early adult life. Though the responses to *V. culicis* by the two populations of *C. pipiens* were different, they both result in bringing forward the host's reproductive schedule.

A possible example of a life-history response was observed in a seminatural field experiment where bumblebee colonies became naturally infected with *N. bombi* (Imhoof and Schmid-Hempel, 1999). These colonies showed an increased production of sexual offspring, particularly males,

relative to uninfected colonies. A particular advantage of producing males is that, even if they become infected while within the colony, they mate soon after leaving it and cannot paternally transfer infection to their offspring. In contrast, infected females (future queens) must overwinter before founding a new colony (prolonging her exposure to the parasite's costs) and a colony she is likely to contaminate via spores in her feces.

The production of male offspring can potentially be a useful device against maternally transmitted parasites that have a negative effect on a female's fitness: a female's sons, even if infected themselves, cannot transmit the infection to the next generation, and hence their descendants will be purged of the infection. Mangin et al. (1995) reported the fitness costs for *D. magna* of being infected by *Flabelliforma magnivora* (then described as *Tuzetia* sp.). This microsporidian has a vertical-transmission success of approximately 100% but a negative effect on female fecundity of 30 to 60% and no direct horizontal transmission among individuals of its host species. Without competition with uninfected hosts, infected *Daphnia* can be maintained despite the high virulence of the parasite. However, if faced with competition from uninfected clones, both infected hosts and parasites go extinct rapidly (Ebert et al., 2000). The potential of sexual reproduction to purge infection from part of the descending lineage via a generation including male offspring provides a means for the host to avoid this fate.

To summarize, the first line of defense for insects against microsporidian infections arriving from the external environment is probably to reduce the proximity between ingested spores and epithelial cells lining the gut. Once the parasite has established its presence in host cells, this class of hosts seems to have limited means with which to directly combat the infection immunologically. As a consequence, hosts need to resort to alternative forms of response to limit the costs of an infection that cannot be suppressed or cleared. Bringing forward investment in reproductive success provides a solution to this problem for some hosts, while increased investment in the production of the sex less affected by the costs of parasitism may work for hosts able to manipulate the sex ratio of their offspring.

CONCLUDING REMARKS

Microsporidia are obligate symbionts. The evolutionary transition to endosymbiosis is marked by a general loss of cytological complexity and reduction in the size of their genomes that is matched by few other eukaryotes. The adaptation to a parasitic lifestyle is particularly identifiable by their polar filaments, which provide a unique means to infect host cells. Despite this highly specialized life style, they have been highly successful, being among the most common parasites of arthropods as well as many other taxa including humans. The spectrum of their known transmission behavior ranges from purely vertical to purely horizontal, including examples with intermediate patterns of investment in both modes of transmission and where an intermediate host may be involved. The spores produced for different types of transmission are usually specific to their tasks and destined to transmit the parasite either within the same host or to different host individuals. Field and laboratory data show that they are capable of adapting to the particular conditions or constraints provided by their host and its environment and that they can do so rapidly. Progress at the molecular level has recently provided the means to surmount many of the technical problems posed by their small physical size and intracellular lifestyle. These developments are throwing valuable new light on many aspects of their biology and helping to put their diversity and evolutionary origin in perspective.

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