



Entomopathogenic Protozoa

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Summary

Entomopathogenic infections by protozoans predominantly, the microsporidians, are of paramount significance in being ineradicable that caused subtle pathologies to induce reproductive productivity decline and shortening of life spans. These have been considered incredibly fit as long-term regulators of pests for effective checks of disease outbreaks. Protozoans are an important constituent of biopesticides with a narrow target range, hence used for a specific problem; their slow-action characteristics rejuvenate suppression of pests with limited field persistence, and devoid of residues. The detection and diagnostic potential for the length of survival of spores in the habitat has been facilitated by the application of advanced biochemical techniques. The critical inputs regulate mutualism after invasion of microsporidia into intracellular environment of the host-cell and influence protein synthesis, growth and endomitosis during merogony. A determined impact of lessening noxious pest hosts with the assistance of non-chemical application techniques in the field attributed commercial significance to the protists (microsporidia) and a variety of other protozoa. Vertical transmission comes into effect once the microsporidians invade gonads of insect pests, followed by the contamination of eggs. Therefore, not only that spores adhere to eggshell surface, and egg contents comprise spores and developmental stages yet the larvae hatched from internally contaminated eggs are often not infected. Microsporidia with reduced mitochondria, that is mitosomes, are *sans* mitochondrial genome, and the energy for their functioning is extracted from the host's intracellular environment. Mitosomes, devoid of cristae, might be dependent on ATP produced by glycolytic pathway because oxidative phosphorylation was dysfunctional. Pest suppression characteristics of biocontrol agent are a factor dependent on host's ecology.

Keywords

Entomopathogenic · Protozoa · Microsporidia · Biocontrol · Molecular · Strategy · Transovarial transmission · Environmental

8.1 Introduction

The protozoans belonging to certain phyla are pathogenic to insects. Some of these insects are important pests of various agricultural crops. Thus, these protozoans may act as biocontrol agents and play a significant role to regulate the population of insect pests. The protozoans, unicellular eukaryotes, particularly under the Sub Kingdom Protozoa, viz., neogregarines and microsporidia, are relatively more important in this context. The potential of protozoans to invade the body of their hosts in huge numbers, and kill them by inducing dysfunction of organs or depleting the host of essential reserves, has earned these organisms a unique position in their own way. The refinement of the concept of management of pests incorporating conservation efforts in coherence with refurbishing enhanced populations of the existing natural control agents has been of primary interest in the application of protozoans in the biocontrol of insects. The issues of application of protozoan organisms as important elements of economic entomology have been frequently reviewed (Brooks 1974, 1979; McLaughlin 1971, 1973; Tanada 1976). Initially it was believed that the actions by protozoans were neither rapid nor useful for a broader range of effectivity. The slower-paced performance of the pathogenic protozoan forms deprived them of their penetrative capabilities to cause mortality into higher abundance of host pest on their own. Their effective use is, therefore, implied in high threshold pest populations to cause damage or else when they are required as a component of pathogen-plus-chemical formulation or in a multiple pathogen combination to act upon pests. In addition, in *Lambornella clarki* Corliss and Coats, an endoparasitic ciliate of the tree-hole mosquito, *Aedes sierrensis* (Ludlow), exhibited remarkable potential as a biocontrol agent to keep effective check on it, to prevent outbreak of dangerous diseases. The armament of cuticular cysts is deployed by the ciliates of the genus *Lambornella* that penetrate to cause damage by attaching to the cuticle of culicine mosquitoes, particularly tree-hole breeding species, for a remarkable mosquito control. Clark and Brandl (1976) gave an illustrated account of the mechanism of dissolution of the cuticle of larval mosquitoes to facilitate penetration into epidermis for its arrival in haemocoel. Two mosquito-associated species have been reported, viz., *Lambornella stegomyiae* Keilin and *L. clarki* Corliss & Coats. Egeter et al. (1986) and Washburn and Anderson (1986) also emphasized its effective role as a potential biocontrol agent of the container breeding mosquitoes.

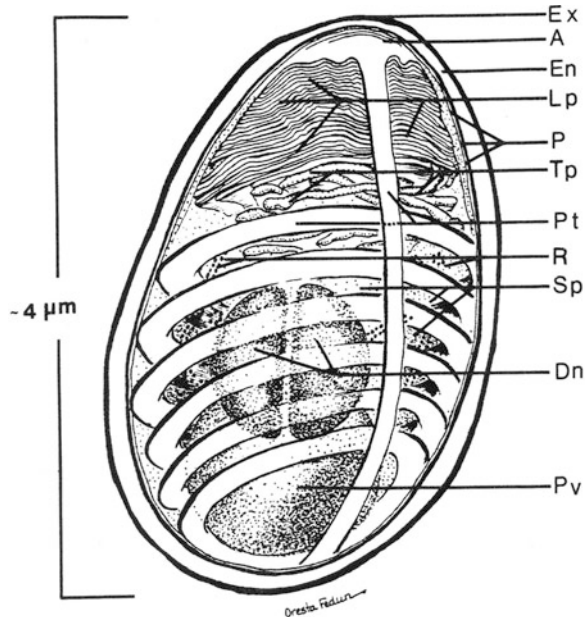
The typical cyst forming ability of this endoparasitic ciliate comes in handy for it to survive over the years to pass over unfavourable conditions. The property of resistance to the cystic framework is the typical characteristic of such protozoans that equipped these pathogenic organisms with appreciable resistance against desiccation. This unique ability of the cysts to withstand adversities during the stages of development enabled the organism encased within, to overwinter or cross over to the next weather circle stage of the following year. Taking a cue out of such apparent characteristics of the cysts of this endoparasite, Anderson et al. (1986a, b) advocated field trials and perused in vitro methods to determine the efficacy and feasibility of their use in field experiments. The association of a variety of protozoans, particularly microsporidians, with mosquitoes and other arthropods of medicinal significance, is

known (Lacey and Undeen 1986). The exposition of their practical utility has been restricted because of their complex life cycle, as well as in vivo technology to be employed for maintenance. The application of novel inoculative technique to ensure the enhanced productivity oriented release highlighted its functional significance in the dynamics of mosquitoes, under specific habitat conditions. The report of Flint and Dreistadt (1998) compiled microorganisms, particularly entomophilic protozoans as pathogens in coordination with a variety of bacteria, fungi, nematodes and viruses that possessed the natural potential to infect and kill the hosts if the congregated pest populations flourished under conditions of high humidity. Certain protozoans, viz. the microsporidia, *Nosema pyrausta* (Fig. 8.5) and *Vairimorpha necatrix* (Fig. 8.6), are the typical examples that demonstrated regulation of lepidopteran pest populations. The abundance of pest populations would be monitored the moderate to low pathogenicity because the inverse relationship between their pathogenicity and prevalence was obvious. While *V. necatrix* operated through the insect gut wall invasion as well as disruption due to the bacterial septicaemia. Though this ensured survival and propagation of larval populations, yet adulthood was never attained. Thus, this microsporidian could rarely register its occurrence in natural populations as it was deprived of the transovarial transmission. Under the circumstances, therefore, its potential as an important constituent of being a microbial pesticide, on a short-term control operation, has been realized. On the other hand, the natural companion in the lepidopteran pests, *N. pyrausta* (Paillot 1927) that parasitized a single host, that is, the European corn borer, with acquired lower pathogenicity in the stressful environment, succeeded to kill larvae. Resultantly, therefore, the adulthood was achieved by most survivors but whose power to reproduce was adversely affected and length of life curtailed. This microsporidian was encountered with higher prevalence in natural precincts, equipped with the virtues of being transmitted transovarially. In such ways though not being an organism that showed severe pathogenicity, it still has been attributed a significant constituent of the mechanism regulating natural pest populations.

8.2 Protozoan Assessment as Biocontrol Agent

The microsporidia (Fig. 8.1) of various protozoan organisms encountered in a variety of insects in terrestrial as well as aquatic ecosystems have been summarized in Table 8.1. The occurrence of microsporidia in the insect pests has been listed by insect host taxonomic order in Table 8.2. The insect orderwise enumeration of microsporidian fauna is presented in Table 8.3. The reported species of insects frequently occurring in the insect pests in the forest areas have been compiled in Table 8.4. The common as well as specific names of insect pests, that occurred in forests and microsporidia harboured by them are listed in Table 8.5. The precise details of spore wall proteins of microsporidia and their subcellular location are summarily presented in Table 8.6.

Fig. 8.1 Internal structure (Diagrammatic) of a microsporidian spore (Courtesy: Cali and Takvorian, 2014) (Reprinted from Han et al. 2020) (A, Anchoring disc; Dn, Diplokaryon; En, Endospore; Ex, Exospore; Lp, Lamellar Ploroplast; P, Unit membrane; Pt, Polar tube; Pv, Posterior vacuole; R, Ribosome; Sp, Sporoplasm; Tp, Tubular Ploroplast)



8.2.1 Phylum Ciliophora

Though not recognized as obligate endoparasitic forms, a few strains, like *T. pyriformis* of genus *Tetrahymena* spp. that otherwise are free-living forms, possessed the traits of parasitism. Corliss and Coats (1976) enlisted parasites of genus *Tetrahymena* Furgason and *Lambornella* Keilin of the haemocoel of culicine mosquitoes. The mortality-induced infectivity in the haemocoel of mosquito hosts by both *Tetrahymena* and *Lambornella* (Table 8.1) was taken into account, although the exact data on the rates of mortality were evading.

The evidence of culturability of *Tetrahymena* (Table 8.1) and *Lambornella* being available, the findings of Clark and Brandl (1976) to conclude prevalence of *L. clarki* in *Aedes sierrensis* to the level of 50% strengthened the contrary view of low control potential of ciliophorans. This was because of lower infectivity or abstained natural epizootics of ciliates underlined by McLaughlin (1971) and Henry (1981) that could respond to the likelihood of decimation of infected lot of individuals in the population due to severe pathogenicity. WHO released the document WHO/VBC/81.803 briefing the details of results to give evidence.

8.2.2 Phylum Sarcomastigophora

The evidence is in favour of the spread of these parasitic protozoa due to the faecal ingestion, which is not necessarily associated with the formation of cysts. But the

Table 8.1 Insect pest hosts and protozoan organisms harboured by them

| Sl. No. | Protozoan organism (s) | Insect pest(s) | Site of infection | Author(s) ^a |
|---------|--|---|---|--|
| 1. | Gregarine, <i>Dirofilaria immitis</i> | <i>A. aegypti</i> and other culicine mosquitoes | Malpighian capsules | Sneller (1979) |
| 2. | <i>Mattesia grandis</i> McLaughlin | Cotton boll weevil, <i>Anthonomis grandis</i> | Gonads | McLaughlin (1971) |
| 3. | <i>Mattesia trogodermae</i> (Canning) | Stored grain pest, <i>Trogoderma glabrum</i> | Pheromones as tools | Shapas et al. (1977) |
| 4. | <i>Adelina tribolii</i> and <i>Farino cystistribolii</i> (in formulations) | Flour beetles, <i>Tribolium</i> spp. | Pheromones as tools | Shapas et al. (1977) |
| 5. | <i>Lambornella clarki</i> Corliss and coats, an endoparasitic ciliate | Tree hole mosquito, <i>Aedes sierrensis</i> (Ludlow) | Encysted stages | Wallace (1979) |
| 6. | <i>Lambornella</i> | Culicine mosquitoes | The cuticle of culicine mosquitoes | Wallace (1979) |
| 7. | Microsporidia of <i>Vairimor</i> | Lepidopteran host populations | – | – |
| 8. | <i>Tetrahymena</i> Furgason and <i>Lambornella</i> Keilin | Culicine mosquitoes | Haemocoel | Corliss and Coats (1976) |
| 9. | <i>L. clarki</i> | <i>Aedes sierrensis</i> | – | Clark and Brandl (1976) |
| 10. | <i>Nosema pyrausta</i> (Paillot) | European corn borers, <i>Ostrinia nubilalis</i> | Malpighian tubules | Introduced into the United States of America in 1917 |
| 11. | Cyst forming protozoans of the genus <i>Lambornella</i> , free-swimming ciliates | The mosquito hosts through culture invasion into the aptly suited | Encysted stages | Wallace (1979) |
| 12. | <i>N. Pyrausta</i> | In larvae of <i>O. Nubilalis</i> | – | – |
| 13. | <i>Nosema</i> sp. | Borer population | – | – |
| 14. | <i>N. Pyrausta</i> | <i>O. nubilalis</i> | – | Kramer (1959a, 1959b) |
| 15. | Invasive spores of microsporidian organisms | In larvae, pupae as well as adult moths | In the yolk accompanying embryonated oocytes in the ovary | Zimmack and Brindley (1957) |

(continued)

Table 8.1 (continued)

| Sl. No. | Protozoan organism (s) | Insect pest(s) | Site of infection | Author(s) ^a |
|---------|--|--|---|--|
| 16. | Larval infestation of the dimorphic Diptera <i>Amblyospora</i> and <i>Thelohania</i> | – | – | Hazard and Oldacre (1976) |
| 17. | <i>N. heliothidis</i> (Lutz and Splendore) | Corn earworms, <i>Heliothiszea</i> | Larval tissues | Gaugler and Brooks (1975) |
| 18. | <i>N. fumiferanae</i> (Thomson) | Spruce budworm, <i>Choristoneura fumiferana</i> | In the actively feeding larvae post-emergence | Thomson (1958) |
| 19. | 3 species of microsporidia | Winter moth, <i>Operophtera brumata</i> (L.) (Canning & Barker, 1981) | Newly laid, non-embryonated eggs (Canning & Barker 1981; On the surface of egg shells & inside egg contents (Kramer 1959a; Brooks 1968) | Canning (1982) |
| 20. | <i>V. Necatrix</i> | Seasonal pests; young larvae | Gut, malpighian tubules & salivary or silk glands | – |
| 21. | The ciliate, <i>Lambornella</i> | Mosquitoes | Cuticle | Wallace (1979) |
| 22. | Trypanosomatid flagellates | Insects | Contaminated faecal material | Wallace (1979) |
| 23. | A large variety of protozoans | Insects | | Fine (1981) |
| 24. | <i>Vairimorpha necatrix</i> (Kramer); <i>N. pyrausta</i> and <i>V. necatrix</i> (Kramer) | Army worms, <i>Mythimna Mythimna (Pseudaletia) unipuncta</i> (Haworth) | a microbial insecticide | Tanada and Chang (1962); Tanada (1964) |
| 25. | <i>Vairimorpha necatrix</i> (Kramer) | Forest insects; several major agricultural pests | A phytophagous Lepidoptera | Maddox et al. (1981); Maddox et al. (1981) |

^aReferences are species descriptions or research reports. (Reprinted from publications by various authors)

possibility of their being pathogenic is a rarer one. Therefore, in a review published by Wallace (1979), a large number of flagellates belonging to the group of kinetoplasic flagellates, commonly being harboured by a variety of invertebrates, including arthropods, were not considered to be biocontrol organisms.

Table 8.2 Genera of microsporidian type species isolated from insects; listed by insect host taxonomic order

| Insect order | Microsporidian genus | Insect order | Microsporidian genus |
|--------------|----------------------|---------------|----------------------|
| Collembola | Auraspora | Diptera | Tricornia |
| Diptera | Aedispora | | Tubilinosema |
| | Amblyospora | | Vavraia |
| | Andreanna | | Weiseria |
| | Anisofilariata | | Pegmatheca |
| | Bohuslva | | Pernicivesicula |
| | Campanulospora | | Pilosporella |
| | Caudospora | | Polydispyrenia |
| | Chapmanium | | Ringueletium |
| | Coccospora | | Scipionospora |
| | Crepidulospora | | Semenovaia |
| | Crispospora | | Senoma |
| | Cristulospora | | Simuliospora |
| | Culicospora | | Spherospora |
| | Culicosporella | | Spiroglugea |
| | Cylindrospora | | Striatospora |
| | Dimeiospora | | Systemostrema |
| | Edhazardia | | Tabanispora |
| | Evlachovaia | | Toxoglugea |
| | Flabelliforma | | Toxospora |
| | Golbergia | | Trichoctosporea |
| | Hazardia | Coleoptera | Anncaliia |
| | Helmichia | | Canngia |
| | Hessea | | Chytridiopsis |
| | Hirsutusporos | | Endoreticulatus |
| | Hyalinocysta | | Ovavesicula |
| | Intrapredatorus | Ephemeroptera | Mitoplastophora |
| | Janacekia | | Pankovaia |
| | Krishtalia | | Stempellia |
| | Merocinta | | Telomyxa |
| | Napamichum | | Trichoduboscqia |
| | Neoperezia | Hemiptera | Becnelia |
| | Octosporaea | Hymenoptera | Antonospora |
| | Octotetraspora | | Burenella |
| | Parapleistophora | | Kneallhazia |
| | Parastempellia | Isoptera | Duboscquia |
| | Parathelohania | | |

Table 8.3 The total number of identified microsporidian genera distributed along various orders of insects

| Sl. No. | Insect order | Microsporidian genera | Insect order | Microsporidian genera totals |
|---------|-----------------------------------|------------------------|---------------|------------------------------|
| 1. | | <i>Cystosporogenes</i> | Collembola | 1 |
| 2. | <i>Archips cerasivoranus</i> | <i>Larssoniella</i> | Diptera | 57 |
| 3. | <i>Choristoneura con flictana</i> | <i>Nosema</i> | Coleoptera | 5 |
| 4. | <i>Choristoneura fumiferana</i> | <i>Orthosomella</i> | Ephemeroptera | 5 |
| | | <i>Vairimorpha</i> | Hemiptera | 1 |
| | | <i>Nudispora</i> | Hymenoptera | 3 |
| | | <i>Resiomeria</i> | Isoptera | 1 |
| 5. | <i>Dendroctonus species</i> | <i>Heterovesicula</i> | Lepidoptera | 5 |
| | | <i>Johenrea</i> | Odonata | 2 |
| | | <i>Liebermannia</i> | Orthoptera | 4 |
| 6. | <i>Euproctis chrysorrhoea</i> | <i>Paranosema</i> | Syphonaptera | 2 |
| | | <i>Nolleria</i> | Thysanura | 1 |
| | | <i>Pulicispora</i> | Trichoptera | 3 |
| 7. | <i>Hyphantria cunea</i> | <i>Buxtehudea</i> | | |
| | | <i>Episeptum</i> | Total | 90 |
| | | <i>Issia</i> | | |
| 8. | <i>Ips</i> spp. | <i>Tardivesicula</i> | | |

^aReferences are species descriptions or research reports. (Reprinted from Solter, L.F., Becnel, J.J. & Oiy, D.H., 2012. Microsporidian Entomopathogens, DOI: <https://doi.org/10.1016/B978-0-12-384,984-7.00007-5>)

8.2.3 Phylum Apicomplexa

At least three modes were employed to explore the role of gregarines (sub-class Gregarina) and some adeleine coccidia (sub-class Coccidia, sub-order Adeleina) of the Apicomplexa in biological control of insects.

Laboratory Experiments

A gregarine, *D. immitis* exhibited the potential to damage Malpighian tubules of *A. aegypti* (Table 8.1) and other culicine mosquitoes in the laboratory experiments conducted by Sneller (1979).

Field Trials

Sneller (1979) also reported parasitization by *D. immitis* and damage in process, of the Malpighian tubules, by this gregarine. Similarly, the neogregarines and adeleine coccidia, the common intracellular parasitic forms sustained one or more cycles of

Table 8.4 Partial list of microsporidia infecting forest insect pests

| Sl. No. | Host species | Microsporidian species | References ^a |
|---------|----------------------------------|---|---------------------------------|
| 1. | <i>Agrilus anxius</i> | <i>Cystosporogenes</i> sp. | Kyei-Poku et al. (2011) |
| 2. | <i>Archips cerasivoranus</i> | <i>Endoreticulatus (Pleistophora) schubergi</i> | Wilson and Burke (1978) |
| 3. | <i>Choristoneura conflictana</i> | <i>Nosema thomsoni</i> | Wilson and Burke (1971) |
| 4. | <i>Choristoneura fumiferana</i> | <i>Cystosporogenes</i> sp. (<i>legeri</i> ?) | Van Frankenhuyzen et al. (2004) |
| | | <i>Endoreticulatus (Pleistophora) schubergi</i> | Wilson (1975) |
| | | <i>Nosema fumiferanae</i> | Thompson (1955) |
| | | <i>Thelohania</i> sp. | Wilson (1975) |
| 5. | <i>Dendroctonus species</i> | <i>Nosema dendroctoni</i> | Weiser (1970) |
| | | <i>Chytridiopsis typographi</i> | Knell and Allen (1978) |
| | | <i>Unikaryon minutum</i> | |
| 6. | <i>Euproctis chrysorrhoea</i> | <i>Nosema chrysorrhoeae</i> | Hylis et al. (2006) |
| | | <i>Nosema kovacevici</i> | Purrini and Weiser (1975) |
| | | <i>Endoreticulatus</i> sp. | Purrini (1975) |
| 7. | <i>Hyphantria cunea</i> | <i>Endoreticulatus schubergi hyphantriae</i> | Weiser (1971) |
| | | <i>Nosema</i> sp. (<i>bombycis</i> -type) | |
| | | <i>Vairimorpha</i> sp. | – |
| 8. | <i>Ips</i> spp. | <i>Chytridiopsis typographi</i> | Purrini and Weiser (1985) |
| | | <i>Larsoniella duplicate</i> | Weiser et al. (2006) |
| | | <i>Nosema typographi</i> | Weiser et al. (1997) |
| | | <i>Unikaryon montanum</i> | Wegensteiner et al. (1996) |
| 9. | <i>Lymantria dispar</i> | <i>Endoreticulatus schubergi</i> | McManus and Solter (2003) |
| | | <i>Nosema lymantriae</i> | |
| | | <i>Nosema serbica</i> | |
| | | <i>Vairimorpha disparis</i> | |
| 10. | <i>Malacosoma americanum</i> | <i>Nosema</i> sp. (<i>bombycis</i> -type) | Weiser and Veber (1975) |
| | <i>Malacosoma disstria</i> | <i>Nosema disstriae</i> | Thomson (1959) |
| | <i>Operophtera brumata</i> | <i>Cystosporogenes operophterae</i> | Canning et al. (1983) |
| 11. | <i>Pristiphora erichsoni</i> | <i>Thelohania pristiphorae</i> | Smirnov (1967) |
| | <i>Tmicus piniperda</i> | <i>Caningia tomici</i> | Kohlmayr et al. (2003) |
| | <i>Tortrix viridana</i> | <i>Osema tortricis</i> | Franz and Huger (1971) |

^aReferences are species descriptions or research reports. (Reprinted from Solter, L.F., Becnely, J.J. & Oiy, D.H., 2012. Microsporidian Entomopathogens, DOI: <https://doi.org/10.1016/B978-0-12-384,984-7.00007-5>)

Table 8.5 Partial list of forest insects from which microsporidia have been reported

| Sl. No. | Host species | | References ^a |
|---------|--------------------------|---------------------------------|---|
| | Common name | Specific name | |
| 1. | Eastern tent caterpillar | <i>Malacosoma americanum</i> | – |
| 2. | Fall webworm | <i>Hyphantria cunea</i> | Weiser and Veber (1975) Nordin and Maddox (1974) |
| 3. | Forest tent caterpillar | <i>Malacosoma disstria</i> | Thomson (1959) |
| 4. | Green tortrix | <i>Tortrix viridana</i> | Lipa (1976), Franz and Huger (1971) |
| 5. | Larch sawfly | <i>Pristiphora erichsoni</i> | Smirnoff (1966), Quednau (1968) |
| 6. | Large aspen tortrix | <i>Choristoneura confictana</i> | Wilson and Burke (1971) |
| 7. | Spruce bedworm | <i>Choristoneura fumiferana</i> | Thomson (1958) |
| 8. | Uglynest caterpillar | <i>Archips cerasivoranus</i> | Wilson and Burke (1978) |
| 9. | Winter moth | <i>Operophtera brumata</i> | Canning et al. (1983) |

^aReferences are species descriptions or research reports. (Reprinted from (Reprinted from Maddox et al. 1998. In: McManus, M.L. & Liebhold, A.M. (Eds.)0.1998. Proceedings: Population Dynamics, Impacts, & Integrated Management of Forest Defoliating Insects. USDA Forest Service General Technical Report NE-247, p.187–197)

schizogony, to produce them in enormous numbers. The destructive abilities of the latter and excessive consumption of reserves in the host body led to the greater number of death of host bodies, with decline in reproductive ability in the colonies of insects maintained in the laboratory. McLaughlin (1971) highlighted success of propagation of *Mattesia grandis* McLaughlin (Table 8.1), a parasite of cotton boll weevil, *Anthonomus grandis* as a biocontrol agent of insect pest, in field trials. Their application was successful on their engagement as supplement in the role of feed stimulant.

Stored Grain Pests

The use of pheromones in the stored grain pest, *Trogoderma glabrum*, which lured insects as a tool (Shapas et al. 1977), has partially resolved the problem of outreach of infective spores of *Mattesia trogodermae* (Canning). Several other protozoan mixed formulations have also been applied to utilize *Adelina tribolii* and *Farino cystistribolii* against flour beetles, *Tribolium* spp.

Table 8.6 The identified spore wall proteins of microsporidia (Reprinted from Han et al. 2020)

| | Protein | Subcellular location | Function domain | Mw (kDa) | Amino acids/ GenBank ID | References (as in) |
|-------------------------------------|--------------------|---|-------------------------------------|--------------|--|---|
| <i>Encephalitozoon cuniculi</i> | EcSWP1 | Exospore | – | 45.9 | 450aa ECU10_1660 | Bohne et al. (2000) |
| | EcEnP1 | Endospore | HBM | 40.6 | 357a ECU10_0820 | Peuvel-Fanget et al. (2006) |
| | EcEnP2/ EcSWP3 | Endospore | Transmembrane | 22.5 | 221 aaECUO1_1270 | Peuvel-Fanget et al. (2006), Xu et al. (2006) |
| | EcCDA | Endospore and plasma membrane | Glycoside hydrolase and deacetylase | 28.1 | 254aa ECU11_0510 | Brosson et al. (2005) |
| <i>Encephalitozoon intestinalis</i> | EiSWP1 | Exospore | – | 41.5 | 388aa AF355750.1 | Hayman et al. (2001) |
| | EiSWP2 | Exospore | – | 107.2 | 1002aa AF355749.1a | Hayman et al. (2001) |
| | EiEnP1 | Exospore and endospore and polar membrane layer | HBM | 39.1 | 348aa EF539266 | Southern et al. (2007) |
| <i>Encephalitozoon hellem</i> | EhSWP1a EhSWP1b | Exospore Exospore | – | 54.9 57.9 | 509aa F18770923 533aa F18770924 | Pollonais et al. (2010) |
| | NbSWP5 | Exospore and endospore | – | 20.3 | 186aa EF683105 | Lie et al. (2012) |
| <i>Nosema bombycis</i> | NbSWP7 | Exospore and polar tube | – | 32.8 | 287aa EOB13707.1 | Yang et al. (2015) |
| | NbSWP9 | Exospore, endospore and polar tube | Transmembrane helix region (TMHMM) | 42.8 | 367aa EOB13793.1 | Yang et al. (2014) |
| | NbSWP11 | Exospore and endospore | DnaJ domain | 52.3 | 446aa EF683111 | Yang et al. (2014) |

| | | | | | | |
|------------------------------------|----------|---|---------------------|------|----------------------|-------------------------|
| | NbSWP12 | Exospore and endospore | BAR-2 domain | 44.0 | 221aa EOFB683112 | Chen et al. (2013) |
| | NbSWP16 | Exospore | HBM | 44 | 221aa EOB14338 | Wang et al. (2015) |
| | NbSWP25 | Endospore | HBM | 30.7 | 268aa EF683102 | Wu et al. (2009) |
| | NbSWP26 | Exospore, endospore and plasma membrane | HBM | 25.7 | 223aa EU677842 | Li et al. (2009) |
| | NbSWP30 | Endospore | - | 32.1 | 278aa EU683101 | Wu et al. (2008) |
| | NbSWP32 | Exospore | - | 37.4 | 316aa | Wu et al. (2008) |
| | EOB14572 | Endospore and pollen tube | Four tandem repeats | 37.0 | 316aa NBO_24g0018 | Wang et al. (2015) |
| <i>Enterocytozoon hepatopenaei</i> | EHSWP1 | Endospore and exospore | HBM, Bar-2 | 27.0 | 228aa MGO15710 | Jarcentak et al. (2018) |
| <i>Antonospora</i> | AlocSWP2 | Endospore and exospore | GPI, HBM | 25 | 222aa | Chen et al. (2013) |

8.2.4 Phylum Microspora

Microsporidia are among the largest group of successful biocontrol organisms under Protozoa. The widely spread out resistant spores initiate infections into pest populations. The spores hatch after ingestion, and penetration (Fig. 8.2) of its polar filament into a gut epithelial cell occurred, pushing through the peritrophic membrane barrier. However, the cytoplasm of the host cell received inoculation of sporoplasm in cases where polar filament was longer enough to have reached up to the cytoplasm of the host cell after passing through the gut wall and penetrating haemocytes directly (Malone 1990). The spores were resultantly produced by sporogony that was preceded by frequently occurring binary or multiple fissions, after initial invasion by the tube of polar filament. It is also known that the fatality, as well as the decline in reproductive potential of the host, and their life span dawned upon because the tissues of the host were replaced by the invasive infective spores in heavier numbers (Fig. 8.3).

It has been emphasized that the negativity features of greater time lag between initiation of infection by a microsporidia and the resultant tissue destruction, which might extend to several weeks, affected the potential of these infective agents that are detrimental to pest, to facilitate their usefulness in vector control. However, the

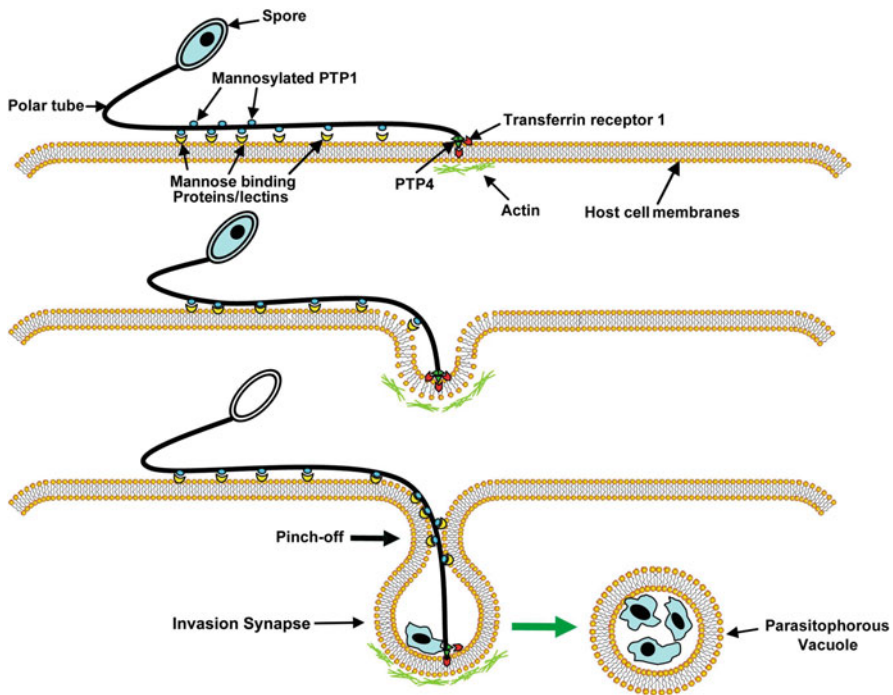


Fig. 8.2 Invasion by microsporidia into a cell of the host (a model) (Reprinted from Han et al. 2020)

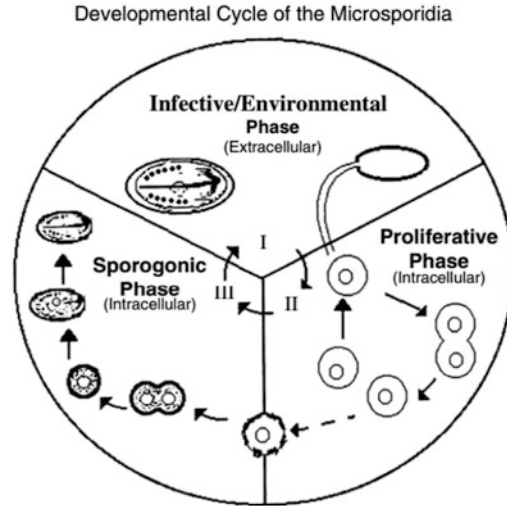


Fig. 8.3 A typical microsporidial developmental cycle (Fig. 8.3) can be divided into three phases. Phase I, the infective/environmental phase, is the only extracellular part of the cycle. It is represented by mature spores shed into the environment from previously infected hosts. Under appropriate conditions, the spores germinate (e.g., if the spores are ingested by an appropriate host). They are activated by the digestive tract environment; this results in the explosive expulsion of the polar filament (which 'everts' becoming a hollow tube). If the polar tube pierces a host cell, the spore contents, the sporoplasm, are injected into it and phase II begins. Phase II is the proliferative phase, the first phase of intracellular development. During this part of the microsporidian life cycle, organisms are usually in direct contact with the host cell cytoplasm or in a parasitophorous vacuole as they increase in number. The transition to phase III, the sporogonic phase, represents the organisms' commitment to spore formation. In many life cycles, this is morphologically indicated by parasite secretions through the plasmalemma producing a 'thickened' membrane (many also form a surrounding sporophorous vesicle, SPOV). The number of cell divisions that follow varies, depending on the genus in question, and results in sporoblast cells that develop into spores (Reprinted from Cali et al. 1999)

characteristic of transovarial transmission negates this ineptitude to overcome by attaining of the retention of the potential of spread full to capacity of the infective microsporidia.

The activated transferability, sustainability of infective stages, and severe ferocity of invasive penetrative capability equip a biocontrol agent to achieve success. A long-term strategic management scheme encompassed the manipulation of several alternative methods. These included field trials to test the methods to ensure availability of bulk volumes of microsporidia and neogregarines, incorporating tests for ensuring their safety during the process of production, as well as testing of bait formulations to suit appropriate mass production. This has resulted into the registration of a microsporidia as a microbial pesticide with the United States Environmental Protection Agency.

A lot of efforts have been put in to explore novel methods of introduction, keeping an eye on the evaluation of their pathogenicity and the potential of their wider spread. While, on the other hand, the groups like ciliates, flagellates, amoebae and the less pathogenic Apicomplexa have been under focus to investigate their prevalence during random surveys as well as the descriptions of their newer taxa, if any.

The concerns have been expressed to verify the viability of cyst formation activity under experimental conditions (Fig. 8.4), though the cyst forming protozoans of the genus *Lambornella*, the free-swimming ciliates were amply encountered in natural conditions. This is yet to be tested whether *Lambornella* organisms could be cultured in the laboratory, and promoted for invasion into the aptly suited mosquito hosts, under natural habitats, by forming cuticular cysts. Therefore, most of the studies illustrating appropriate protozoan organisms for biocontrol of insects depended heavily on microsporidians and neogregarines.

8.3 Pathogenicity

The manifestation of pathogenic effects of the cysts (Fig. 8.4) harboured by pests was illustrated by the declining pest population growth emanated as a result of reduced fecundity as well as their longevity. This was implied as the long-term control measure. However, the short-term measure to restrict pest population growth, that attained critical peaks, emerged to be the mortality of larvae that were to be employed to rescue and ensure relief measures against adult mosquitoes.

The efforts have also been made to reveal mystery of short-term and long-term population control of pests that comprised proven record of *N. pyrausta* (Paillot) (Tables 8.3, 8.4) and *V. necatrix* (Kramer) (Tables 8.3, 8.4) as the most suited candidates for being agents of biocontrol.

8.3.1 *N. Pyrausta* (Paillot) (Fig. 8.5)

The European corn borers, *Ostrinia nubilalis*, introduced into the United States of America in 1917, along with *N. pyrausta* (Paillot) (Fig. 8.5), parasitizing their Malpighian tubules, comprised one of the most extensively studied moth host-microsporidian systems. The European microsporidian introduced sporadically in 1951 into the USA (Steinhaus 1951) was the subject of investigation (Zimmack et al. 1954) conducted on its distribution in 1954 in seven of the North Central states. The studies dealing with relative fecundity status of female moths revealed striking differences between moths that showed marked differences in their infectivity status. Fewer eggs were laid by the infected moths, whose longevity was short and the non-infected moths, who lived longer, laid numerous eggs (Zimmack and Brindley 1957). Simultaneously, faster-paced growth was hampered in the former, coupled with dim survival prospects.

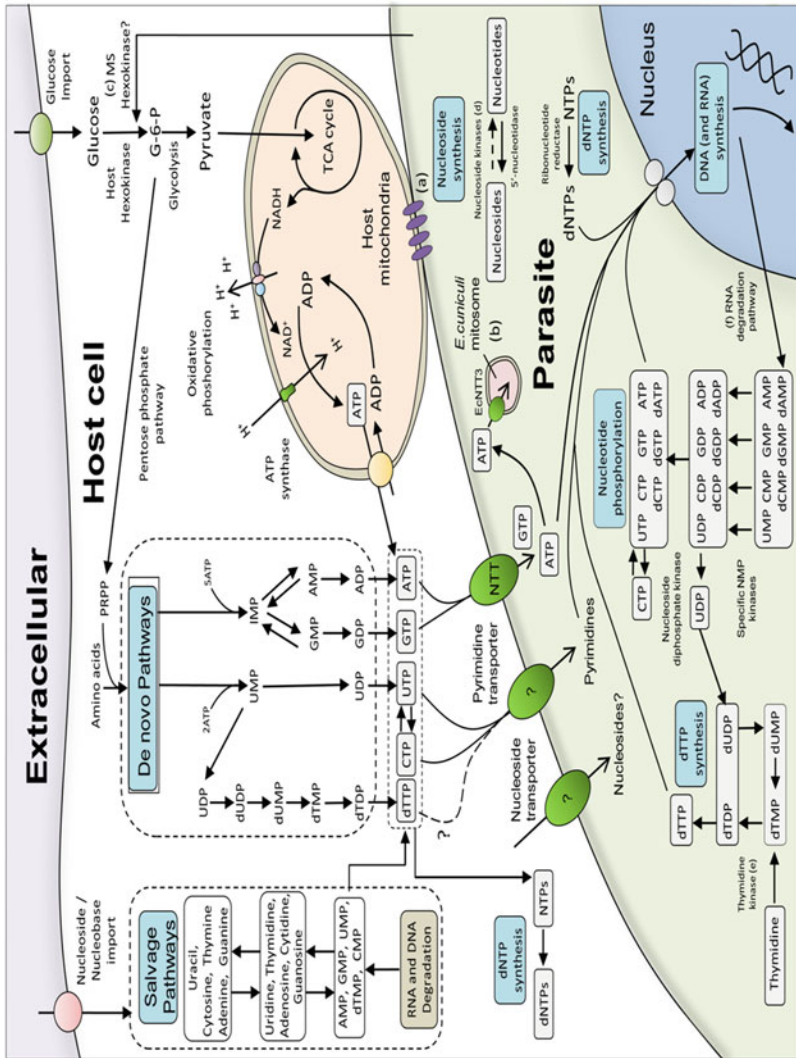


Fig. 8.4 Nucleotide acquisition and metabolism in host cells and microsporidia. Schematic illustration showing nucleotide metabolism in a typical microsporidian parasite within an infected host cell. Host cells can make nucleotides via de novo biosynthesis and regenerate ATP by oxidative phosphorylation – these pathways are absent in microsporidia. Host purine nucleotides can be stolen using

Fig. 8.4 (continued) microsporidia NTT transporters and then efficiently used and recycled by the parasites. Key: (a) *E. cucullii* physically tethers mitochondria using an unidentified protein. (b) Only EcNTT3 has been found in the mitosome. (c) Nematocida may secrete a hexokinase into the host cell to stimulate host nucleotide production. (d) Nucleoside kinases are apparently absent from some microsporidian genomes but are present in *Trachypleistophora hominis*. (e) Thymidine kinase is present in some microsporidia but not all. (f) The microsporidian RNA degradation pathway is shown in the figure below. (Reprinted from: Archibald, J.M., Simpson, A.G.B. & Slamovits, C.H. 2020. Handbook of the Protists. Springer. second Ed., p.1657)

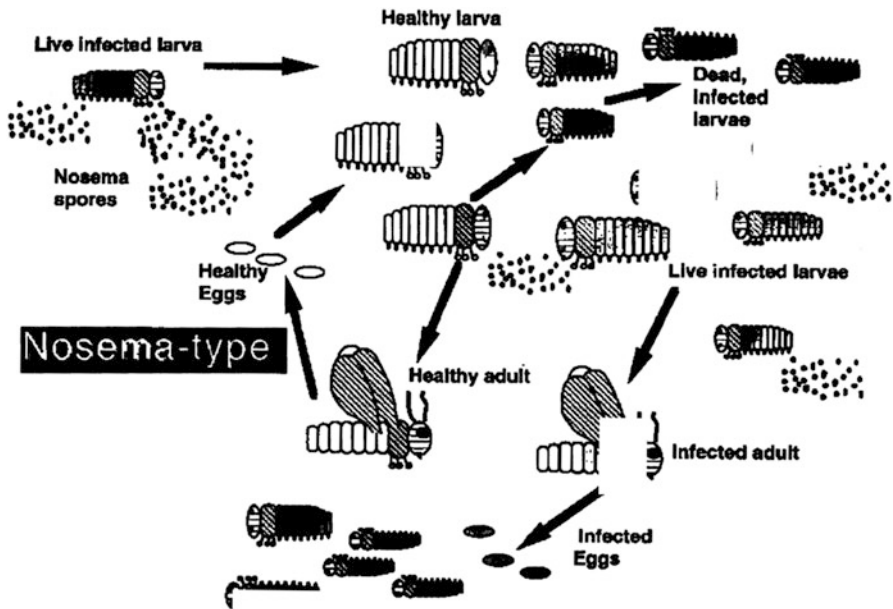


Fig. 8.5 Diagrammatic representation of the interactions between *Nosema*-type microsporidia and their hosts. Healthy larvae become infected by ingesting microsporidian spores which are present in the faeces and/or silk of infected individuals. Larvae infected by ingesting spores may die from the infection if they consume many spores at an early larval stage, but many infected larvae can develop into infected adults. Mortality may occur during pupation and emergence as adults. Much of the mortality caused by *Nosema*-type microsporidia (Tables 8.3, 8.4) occurs in the transovarially infected offspring in infected females. Transovarially infected larvae may be heavily infected and die in early larval stadia

(Reprinted from Maddox et al. 1998. Microsporidia affecting forest Lepidoptera. (In: (Eds.) Maddox, J.V., McManus, M.L. & Solter, L.F. Proceedings: Population Dynamics, Impacts, & Integrated Management of Forest Defoliating Insects.). USDA Forest Service General Technical Report NE-247.).

The evidence of invasive spores of microsporidian organisms was traced by Zimmack and Brindley (1957) in the yolk accompanying embryonated oocytes in the ovary. This was compiled in histological analysis. The infected female moths laid embryonated eggs with spores and the shallow foveae on the egg shell were found embedded with spores by Kramer (1959a). Several other reports have illustrated noticeable prevalence of infection in larvae, pupae as well as adult insects.

The concept of stress-induced effect of fatality to the insect pests infected with *N. pyrausta* was formalized by Kramer (1959b). The stress of winter was under investigation. The natural populations of *O. nubilalis* with reduced longevity and fecundity, under influence of the infection by *N. pyrausta*, were thus regulated, as reported by Van Denburgh and Burbulis (1962). The natural populations of *O. nubilalis* infected by the larvae of *N. pyrausta* in two counties of Nebraska between 1957 and 1972 were correlated with the density of parasites by Hill and

Gary (1979). The two epizootic waves of *Nosema* (Tables 8.4, 8.5, 8.6) occurred at the onset of crash in borer populations that co-occurred with peak in parasite prevalence. The event could thus be correlated with the introduction of mortality on augmentation of prevalence by *N. pyrausta* in larvae of *O. nubilalis*.

Hill and Gary (1979) provided evidence of the eventuality that the parasitic forms did not act directly to the detriment of pest populations, as those due to a combination of environmental factors that regulated fecundity. The intricacies of regulatory dynamics put forth by Anderson and May (1981) could explain that most of the features of the population cycles concluded could actually conform to the host/parasite association model, derived earlier by Anderson and May (1980).

On the basis of the review of long-term observations, the effect of environmental factors emerged to be the significant operator that induced a supportive role to the dynamics of infections by *N. pyrausta*, which on its own was unable to cause significant damage to the pest, but, in combination with environmental attributes, was much more destructive.

8.3.2 *V. Necatrix* (Kramer) (Fig. 8.6)

The utility of *V. necatrix* (Kramer) as a stronger pathogen than *N. pyrausta* and *V. necatrix* (Kramer) is established, and its application as a microbial insecticide has been illustrated effectively (Tanada and Chang 1962). Its initial recovery from the army worms, *Mythimna (Pseudaletia) unipuncta* (Haworth) in Hawaii from more than half of the dead larvae, was ensured from a decimated pest population. The alarming signals emanated from it due to its uninhibited substantial prevalence the following year, at the same site again, while the population density of the army worms declined (Tanada 1964). Though its prevalence in the routine field crops is minimal, its outbreak in certain forest insects has been substantial (Maddox et al. 1981). A noticeable occurrence of *V. necatrix* has been encountered in several major agricultural pests, particularly a variety of phytophagous Lepidoptera (Maddox et al. 1981).

As the ruptured gut due to polar filament extrusion to reach at haemocoel triggered bacterial septicaemia drawing source bacteria from the midgut lumen, cent percent mortality level was attained following infection. The domain of this disease is with a larval base, in which the sites of infections are primarily the fat bodies. Adult insects evade infections because of the non-survival of larvae under the influence of disease as well as non-occurrence of transovarial transmission. This disease primarily being fatal to larvae than adult insects, it has been recognized as a larvicide for quickened depletion of the pest population.

The entry of protozoan organisms into the body of their hosts is most of the time through ingestion of their cysts. It is very rare that any other route is employed by protozoans to attack the susceptible pest hosts; for instance, the penetrative cysts of the ciliate *Lambornella* invade the mosquito's cuticle. It is though intriguing that the life cycle of the flagellate, *Trypanosoma* sp., commonly parasitizing insects (Wallace 1979), does not comprise true cysts, but the transmittance of infection

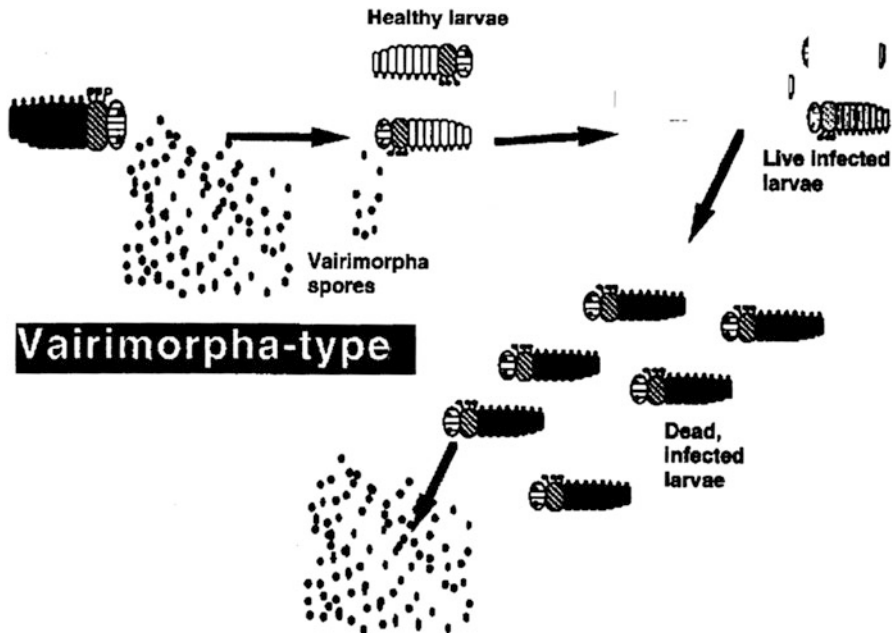


Fig. 8.6 Diagrammatic representation of the interactions between Vairimorpha-type microsporidia and their hosts. Healthy larvae become infected by ingesting microsporidian spores which are released when infected larvae die and disintegrate. Spores are seldom present in the faeces or silk of infected larvae. Most Vairimorpha-type microsporidia are very pathogenic; individuals infected during the larval stage seldom develop into infected adults. These infected individuals usually die as larvae

(Reprinted from Maddox et al. 1998. Microsporidia affecting forest Lepidoptera. (In: (Eds.) Maddox, J.V., McManus, M.L. & Solter, L.F. Proceedings: Population Dynamics, Impacts, & Integrated Management of Forest Defoliating Insects.). USDA Forest Service General Technical Report NE-247.).

was suggested to have occurred by ingestion of contaminated faecal material. This infection is never passed on from mother to offspring, but it is transmitted from individual to individual.

8.3.3 *Endoreticulatus Schubergi* (Zwölfer) (Fig. 8.7)

The protists (microsporidia) in *Endoreticulatus* group (Table 8.2) were entirely distinguished from the other two, namely *N. pyrausta* and *V. necatrix*, described as above. Restricted to midgut epithelial sites, their phylogenetic interrelationships segregated these from others in the other two groups, while simultaneously their life cycles and decimated pathogenicity were also the differentiating features. They were capable of producing only a uninucleate environmental spore, enveloped in a cover, that encompassed 16, 32 or more spores. The most commonly encountered

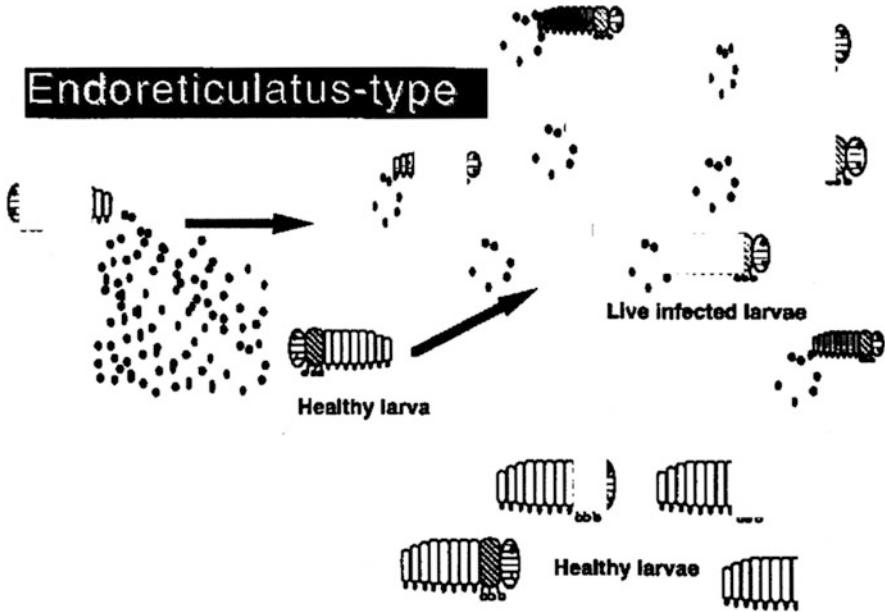


Fig. 8.7 Diagrammatic representation of the interactions between *Endoreticulatus* – type microsporidia and their hosts. Healthy larvae became infected by ingesting microsporidian spores which are present in the faeces of infected individuals. The mortality rate is very low in individuals infected with *Endoreticulatus*-type microsporidia. Infected larvae develop more slowly than healthy larvae and produce faeces contaminated with spores throughout their larval development (Reprinted from Maddox et al. 1998. In: (Eds.) McManus, M.L. & Liebhold, A.M. Proceedings: Population Dynamics, Impacts, & Integrated Management of Forest Defoliating Insects. USDA Forest Service General Technical Report NE-247, p.187–197.)

Endoreticulatus schubergi (Fig. 8.7) never adopted the transovarial pathway to reach at the site of infection in larval populations of forest Lepidoptera. It, however, had the potential to produce chronic infections. Though their vertical transmission on the surface of eggs existed (transovum transmission) to a minimal extent, this pathway was even a subdued one when compared to the actual vertical transmission within the environment of the egg (transovarial transmission). The mechanism of transmission of *Endoreticulatus schubergi* and associated protists are illustrated in Fig. 8.7, identifying the stages at which mortality occurred. A large variety of Lepidopteran pest hosts harboured *Endoreticulatus* spp. quite frequently, but the impact was felt to the maximum extent of a 'sublethal' type only, because of the chronic nature of infection by this species (Maddox et al. 1998).

8.4 Transmission

Protozoa normally enter their hosts when encysted stages are ingested and rarely by any other route (e.g. the use of invasion cysts of the ciliate *Lambornella* (Table 8.3) to penetrate the cuticle of mosquitoes). True cysts are unknown in some trypanosomatid flagellates of insects (Wallace 1979) but even in these cases the mode of infection is probably by ingestion of contaminated faecal material. Transmission is defined as horizontal when the pathogen is passed from individual to individual within and between generations but not directly from parent to offspring, whatever the mode of entry. When a parasite infects the gonads of its host, vertical transmission from parent to progeny is possible. This may be transovum, when the infective stages contaminate the external surface of the egg and are consumed by the larvae at eclosion, or transovarial, when the infection is located within the eggshell. Occasionally the infected parent is the male. The different explored routes summarizing transmission pathways of a large variety of protozoans in the insects were reviewed earlier by Fine (1975). Two specific modes of transmission, (i) Horizontal alone and the other (ii) Horizontal as well as Vertical, were concluded. Though the horizontal transfer of cysts has been a natural component of the life cycle's pathway, the transfer of protozoan organism or their cysts from parents to the offspring is likely facilitated only by the invasion by a parasite or its developing entities into the reproductive bodies, like gonads, or eggs. This could be termed transovarial transmission.

The uniformly adopted strategy by a majority of protozoans to effectively propagate individual to individual transmission essentially required maintenance of parasites throughout the year, including the potential of their reproductive entities, particularly cystic bodies to survive during the unfavourable environment. In such a manner, these bodies enabled overwintering of the developing stages, for example, cysts, diapausing larvae, pupae or non-feeding adults, so as to ensure continuation of the progeny.

8.4.1 Horizontal Alone

Most Protozoa are maintained this way in invertebrate host populations. There is no strong evidence that any amoebae, flagellates, ciliates or Apicomplexa are transmitted by the vertical route. Restriction of transmission to the horizontal route requires that the parasites are maintained in the living hosts throughout the year or that they can survive in the environment through periods when the host is quiescent and non-feeding, that is, as eggs, diapausing larvae, pupae or non-feeding adults. In seasonal pests, it is likely that the initial generation of the new season will have a low prevalence of disease because infected hosts may have a poor over-wintering survival rate, and the number of available infective stages may be low. The latter could have resulted due to loss of viability with time and their ultimate removal from the environment. Infections will multiply in young larvae, releasing infective stages at death to spread through the new generation. Spread will be enhanced if spores are

released continuously from live hosts, as would occur with infections of the gut, Malpighian tubules and salivary or silk glands. In multivoltine host, prevalences would increase with each generation. The characteristics of *V. necatrix* are noticeably remarkable to have a rapid spread within a generation. But its virulence (effecting rapid removal of the parasitized sector from the population), coupled with the absence of a vertical route of transmission and low survival rate of spores in the environment, is sufficient to account for the low prevalences and rare sightings of this parasite in nature.

8.4.2 Horizontal Alone As Well as Vertical & Horizontal Routes

The other alternative mode of transmission could be that of vertical transmission in a way that the invasion of microsporidians into the contents of the eggs could result into hatching of larvae from the contaminated egg material (Kramer 1959a; Brooks 1968). The winter moth, *Operophtera brumata* (L.) (Canning and Lom 1986), population in an oakwood habitat recorded augmented prevalences of microsporidia. The spread of as many as three species of microsporidia was noticed (Canning 1982). The possibility of occupation of microsporidia on the surface of egg shells as well as inside the contents of the eggs (Kramer 1959b; Brooks 1968) opened up interesting avenues. The rising proportion of incidence of spores and developmental stages of the three species of microsporidia harboured by the winter moth, *O. brumata* (L.), were encountered in the newly laid, non-embryonated eggs examined by Canning and Lom (1986).

Prior to eclosion, the non-occurrence of parasites either in the larval tissues contained in eggs, or in larvae post 1 day of hatching necessitated the location of site of survival of spores during embryogenesis. The spores were shelved in polar plug, meconium, that represented remnants of yolk entering into the lumen of the gut, during late embryogenesis that facilitated merger of stomodaeum with proctodaeum to form midgut. This not only extended disinfecting protection to larvae but also ensured transovarial transfer for vertical transmission. Sections of larvae within the eggs showed that spores were present only in a plug of material, occupying the lumen of the gut. This plug, the meconium, represents the remnants of the yolk taken into the gut lumen at the time when the anterior and posterior parts of the gut, stomodaeum and proctodaeum, join up via the midgut late in embryogenesis. This suggested itself as a remarkable adaptation to ensure that the larvae did not succumb to overwhelming infections while still within the egg. If the larvae were to die, the purpose for which transovarial transmissions has evolved would be defeated. Therefore, it remained to be ascertained whether the infections in newly hatched larvae are initiated in the epithelial cells of gut due to sporoplasm inoculation after hatching of spores, or whether these spores are passed in the meconium and are eaten by, or accidentally contaminate, the larvae. Thomson (1958) confirmed the occurrence of a similar phenomenon for *N. fumiferanae* (Thomson)-spruce budworm, *Choristoneura fumiferana* (Table 8.3) system. *N. fumiferanae* were fatal only in the actively feeding larvae post-hatching.

Gaugler and Brooks (1975) emphasized that the failure of heavier egg infections of *N. heliothidis* (Lutz & Splendore) to reduce egg hatch in corn earworms, *Heliothis zea*, and account for the normal, embryonic development and lack of disease symptoms in newly hatched larvae apparently occurred due to the failure of the egg stages to penetrate larval tissues during embryogenesis. Therefore, the mystery of the mechanism of attained infections by eggs in larvae in the microsporidian—host systems remained to be cracked. The sex-wise differentiation in the larval infestation of the dimorphic diptera, namely *Amblyospora* (Table 8.2) and *Thelohania*, revealed heavier infections in males than females (Hazard and Oldacre 1976).

8.5 Protozoans as Biocontrol Agents to Restrict Grasshoppers and Locusts

The density of the pests, attributes of the environment, as well as the conditions of plants are, in fact, instrumental to attract the wrath of the grasshoppers that might be recognized as chronic pests (Watts et al. 1982). Though due to behavioural advantage to the grassland songbirds, utilizing feeding on grasshoppers resulted into extensive breeding of the former (Martin et al. 1997), yet colossal loss was caused to the crops and vegetation, due to chewing and clipping by even lower densities of pests that consumed the precious crops of farmers and poor people. Although the protozoans were considered to be the slow-acting control agents, which affected rapid breeding and feeding speed of acridid organisms, yet their potential for action fell short of comparably rapidly acting fungi and certain chemical insecticides. Bidochka and Khachatourians (1991), Brooks (1988), McLaughlin (1971), Henry (1990), Maddox (1987), and Raina et al. (1987) put forth extensive reviews to succinctly account for the protozoan potential as biocontrol agents of insect pests. A number of earlier workers (King and Taylor 1936) have been assertive about the enfeebling effect of a variety of protozoa on grasshoppers. Microsporidia have spearheaded the targeted detriment of acridid grasshoppers, but besides Phylum *Microspora*, a great variety of Amoebida, and Eugregarinida, Phylum *Apicomplexa* of Neogregarinida as well as Phylum Ciliophora have succeeded as a biocontrol agent. The adverse influence of *Malameha locustae* (King and Taylor 1936) harboured by *Locusta pardalina* (Walker) resulted into effect on fecundity of these locusts. The proof of their ferocious attacks on the colonies too, of several susceptible species (Pickford and Randell 1969), maintained in the laboratory was obtained by Jackson et al. (1968). The common occurrence of the pathogen on the colonies of *Schistocerca gregaria* (Forskil) including the nondiapause strain of *Melanoplus sanguinipes* was noticed. Interestingly, simultaneous enhanced resistance against insecticides in the cyst-harboured Malpighian tubules (Hinks and Ewen 1986) co-occurred in certain extreme cases, whose account of life history and pathogenesis has been recorded by Braun et al. (1988). Several Orthoptera commonly harboured gregarines in nature as well as in certain poorly maintained colonies of *S. gregaria* (Harry 1970). Certain well-known protozoans that could succeed in making field

crickets physiologically dysfunctional as well as brought disturbances in the ecology of field crickets (Zuk 1987) were brought on record. The taxonomic status of Gregarinida, placed under Apicomplexa, was reviewed by Levine et al. (1980). These were reportedly encountered in the gastro-intestinal tract of more than 30 species of grasshoppers. Though not thoroughly analyzed, gregarines existed in the relationship of symbionts with grasshoppers but their role as potential pathogens is being actively investigated world over (Johnson et al. 1997). However, serious efforts are being made to employ microsporidia as candidates for the potential control of grasshoppers and locusts (Henry and Oma 1981). Based on the characteristics of rapid transmission, fecundity and ease of formulation, *N. locustae* are the favoured candidates for use in the biocontrol of locusts and grasshoppers. A wide spectrum of *Nosema* spp. were encountered in the field populations of *Locusta migratoria migratorioides* (Reiche and Fairnaire) at the Imperial College, London, by Canning (1953, 1962b). Northern Great Plains and Prairies of North America were the native sites of *N. locustae*. Its spores were also frequently harboured by *M. bivittatus*, *M. mericanzs Saus.* (*M. sanguinipes*) and *M. dawsoni* (Scudder). This has commonly been recognized as a common inhabitant of Acrididae with more than 80 species having frequently harboured spores of *N. locustae* (Henry 1969; Henry 1985; van der Paauw et al. 1990; Bomar et al. 1993). The life cycle of *N. locustae* illustrated that unless its infective spores have arrived in the lumen of the gut through ingestion of *N. locustae*, they shall not germinate to release binucleate sporoplasm into the live cells of host pests by rapid extrusion of polar tubes. Therefore, through this mechanism the contact of sporoplasms with the epithelial cells of the midgut is successfully established or the sporoplasms reach directly into the gut cells (Raina and Ewen 1979). The invasion of the yellow organ, the principal fat body organ performing the function of storage of energy, by the sporoplasm enabled generation of developmental entities, like meronts, sporonts, sporoblasts and spores in succession (Canning 1962a). A starved energy reserve of the pest due to microsporidial infection and the ensuing hypertrophy, thereafter, disrupted the dynamicity of intermediate metabolism in the fat cells of the principal fat body, and weakened the body of the pest to induce debilitating conditions. In certain other cases, the virulent pathogeny of microsporidians, greater than *N. locustae*, induced mortality occurred in acridids. As for example, the pathogenicity by *N. cuneatum* Henry enhanced susceptibility to protozoan infections with effective hindrance to development resulting into frequent mortality at an even greater rate. The experiment enumerated by Mussgnug (1980) that the effective outcome on control of pathogenicity by application of *N. locustae* alone was at a relatively lower level than when pathogens combined in the formulations of Malathion- *N. locustae* were applied.

8.6 Forest and Tree Crop Pests

The typical example of vertical transmission of microsporidia of *N. fumiferanae* brought to the fore the primary pathway of disease transmission through consumption of its spores by the spruce bud worm, *Choristoneura fumiferana* (Table 8.3), in the forest and tree crops. Virtually, the fourth and fifth instars received a spore inoculum from the frass and cadavers that were generated after the death of larvae, which were naturally infected from parenteral infections, but the latter could not attain maturity. On the other hand, the larvae that did not receive infections from their parents, and instead their fourth and fifth instar stages received the spore inoculum from the frass and cadavers, attained maturity as well as harboured infections. Thus microsporidia are maintained in the pest hosts by vertical transmission, once the consumption of contaminated spores triggers the augmented incidence of infection.

The spores of *Nosema* were illustrated to be germinated in gut and live cells of gut, fat body and other tissues of Lepidoptera, beetles, locusts and a variety of Orthoptera. They multiply to trigger sporulation that culminated with destruction of organs resulting into being chronic and debilitating. The protozoans were transported by rotten food stuff, cannibalistic behaviour of the organisms through the transovarial pathway (Capinera and Hibbard 1987). The living insects happen to be the fertile ground for the spores of protozoans that sustain with appreciably reduced virulence. It was demonstrated that specifically *Nosema locustae* has had a restricted role in the biocontrol of grasshoppers. Certain formulations of other protozoans, like *Nosema* combined with *Bacillus thuringiensis*, were utilized as dry baits sprinkled over the surface of the soil against other pests, but their availability was restricted commercially.

8.7 Observations on the Sporoplasm

The transport of sporoplasm into the cells of the host pests preceded by the microsporidia infection of host cells involved expedited ejection of the polar tube (Fig. 8.2) (Weidner 1970; Frixione et al. 1992; Takvorian et al. 2005; Han et al. 2017). Weidner (1972) and Han et al. (2017) elucidated that the event of the onset of the infection triggered sticking of microsporidian spores to the cells of the host pests or the surrounding tissues. The indomitable association of the polar tube during infection of the host cells by microsporidia to target spore germination was propagated by Cali et al. (2017). It was also asserted by Takvorian et al. (2013) and Vavra and Larsson (2014) that the transfer of infection is possible by the outflow of infectious sporoplasm, to reach polar tube after its passage from spores. The mature spore formation occurred after initiation of a reproduction cycle, that terminates into meronts' emergence, once the sporoplasm invaded into the host cell (Cali et al. 2017; Han and Weiss 2017), and the formation of proliferative forms, meronts was preceded by the production of constituents of the cycle, like sporonts and sporoblasts. The microsporidia not only were devoid of functional

mitochondria, but also did not comprise the full complement of genes capable of ATP generation, yet could contribute to the completion of glycolysis. The protein coding gene complement of the microsporidial genome was restricted to ~3000.

Three of the total four nucleotide transport proteins (NTT1–4), identified to date from certain microsporidia namely, *Encephalitozoon cuniculi* and *Trachipleistophora hominis*, have been known to be the constituents of the membrane of sporoplasm. It has been illustrated in the literature dealing with microsporidia evolution, enumerating the findings of Tsaousis et al. (2008), Heinz et al. (2014) and Dean et al. (2018) that the horizontal gene transfer was instrumental in the displacement of these nucleotide transport proteins from bacteria to microsporidia. Resultantly, these nucleotide transport proteins illustrated their potential to transport ATP, GTP, NAD⁺ and purine nucleotides from the cytoplasm of the host. The mechanism to transport energy as well as nucleotides from the cells of the hosts is also undertaken by microsporidia major facilitator superfamily (MFS), which is an alternative to the nucleotide transport proteins (NTT1–4), being a sporoplasm surface located protein family. These were ThMFS1–4, of which the location of ThMFS1 and ThMFS3 was traced to be in the sporoplasm plasma membrane during infection. These were also reported (Major et al. 2019) to be performing the similar function of transportation of ATP, GTP and purine, identical to those by NTTs. But it is remarkable that as yet the pyrimidine nucleotide import system of NTTs nor ThMFS remained undeciphered (Heinz et al. 2014; Dean et al. 2018; Major et al. 2019).

Microsporidia are supposed to possess appreciably reduced mitochondria, called mitosome, *sans* mitochondrial genome as well as ATP generation capacity (Williams et al. 2002; Goldberg et al. 2008). Typically, mitosomes constitute an essential constituent of Microsporidia, Diplomonads, Amoebozoa and Apicomplexa (Tovar et al. 1999, 2003; Williams et al. 2002; Keithly et al. 2005) that are a double membrane bound entity. The role of nucleotide transport proteins to ensure the import of nuclear encoded proteins for the functional activity of mitosomes, as well as the maintenance of organelles, to compensate the absence of cristae as well as their own DNA in the morphologically reduced sized mitosomes than mitochondria is well recognized (Burri et al. 2006; Hans-Peter Braun 2009; Tachezy 2019). The energy produced through glycolytic pathway becomes available for use by microsporidian mitosomes because of the process of oxidative phosphorylation to produce ATP being dysfunctional. However, the former process of energy generation operated only in spores, but became inactivated within the cytosol of pests, while intracellular growth and replication were initiated (Dolgikh et al. 2011; Heinz et al. 2012; and Williams et al. 2014). Han et al. (2019) demonstrated aggregation of mitochondria around microsporidia of the cells of their host pests, for energy supply, as confirmed in their experimentation on Encephalitozoonidae harboured by a parasitophorous vacuole of the cells of host pests.

8.8 Molecular Characterization of the Organisms of Biocontrol

The validity of the names of species of microsporidians was conducted in recent years by using chromosomal DNA comparisons (Munderloh et al. 1990). The control basis for vectors of *Nosema* spp. has been analyzed to highlight their molecular characteristics (Strett & Henry 1985). The *N. Locustae* spore structure profiling for polypeptide configuration was done to record pre-eminent polypeptides of exospores and spores post-buffer treatment comprising 4% sodium dodecyl sulphate and 0.02 M dithiothreitol (Heckmann 2020). An electron optically oriented recent approach to characterize a variety of biological control agents was introduced by Avery and Anthony (1983) to crack the mysteries of the molecular basis for biocontrol. In addition, the cell culture revelation could be helpful to elucidate the elements of biology of the candidates for microbial biocontrol. The biology of microsporidians in the in vitro cultures comprising continuous cell lines was demonstrated by Raina et al. (1987). The successful elements required to conduct such studies were aseptic spores in adequate number; applicable stimulus for germination; convenient accessibility to cell lines, along with a sensitive collaborators to conduct research (Kurti et al. 1990; Heckmann 2020). The apparent proximity between the two microsporidians, *N. payrausta* and *N. furnacalis*, naturally parasitizing the European corn borer, *Ostrinia nubilalis*, and the Asian corn borer, *O. furnacalis*, respectively, was test cultured on experimentally reared caterpillars of *O. nubilalis* and centrifugally purified. This experimental illustration reassuringly proved the closeness of the two strains of *N. payrausta* and *N. furnacalis*, with even greater strength than it was previously demonstrated (Munderloh et al. 1990). Since the intricacies of distribution and interactions of microsporidia in the intracellular environment have long remained an enigma, certain advanced methods of detection, for example, ELISA (Enzyme-linked Immunosorbent Assay) of these spores, have been applied. These have helped to assess potential length of survival of spores in the habitat, in addition to their detection in the microhabitat. The facts that have emerged from the interactions of microsporidia of *N. fumiferana* infesting *Choristoneura fumiferana* (Table 8.3) revealed influence on the nutritional physiology of the hosts, which rendered them nutritionally deficient. This provided comparative data on nutritional efficiencies that revealed noticeable decline in Consumptive Index (CI), Nitrogen Consumptive Index (NCI), Relative Growth Rate (RGR) and gross (ECI) and net (ECD) production efficiencies in the infected than non-infected larvae. The approximate digestibility (AD), N utilization efficiency (NUE) and larval moisture content are taken into account to assess that of the healthy and diseased insects, reared on 2.5%N and 4.5% N diets, the enhanced CI in the former with deficient NCI than those reared on the latter diet, were the significant findings. However, no effect of dietary N was seen on the mortality of healthy insects. The outcome thus indicated sharing of products of host cell metabolism for parasitic metabolism and productivity requirements. A presumptive improvement in the growth and survival of host as well as biocontrol agent at the augmented levels of nitrogen was concluded (Heckmann 2020).

8.9 Molecular Mechanism in Pathogeny

The aspects of physiology, as well as biochemistry of microsporidians, have been scarcely worked upon, and information on the operative Krebs's cycle in these has been wanting. Isotopic labelling and analysis of intensities of radiolabelled bands have come in handy to crack the mysteries of metabolism in the microsporidia. The cell fractionation achieved by segregating molecular entities based on differentiating isoelectric points, electrophoretic analysis of the enzymes encased within microsporidia, HPLC of the routine precursors or labelled metabolites of biologically significant molecules, functional localization of the specific enzymes in the cellular environment by chemical applications could reveal successfully the details of metabolic pathways within microsporidia (Heckmann 2020). It was presumptively postulated that the pre-emptive regulation of cyclic events within the host cell provided alternative energy pathway for microsporidia, as mitochondria and endoplasmic reticulum aggregated intracellularly, in the vicinity of the former. The greater variety of species diversity and their origin could be deciphered by the knowledge on aspects of evolution of form within the built environment of microsporidian organism, but the information on these was conclusively unavailable (Heckmann 2020). The microsporidia has essentially to survive within the host cell itself after invasion into it, especially following the principle of mutualism by triggering critical inputs during host–cell protein synthesis, growth and endomitosis during merogony. Soon thereafter, at the onset of sporogony, the microsporidia attained the characteristics of a pathogen, after this organism enveloped as a separate entity within the environment of host environment, and finally lysed the cell (Bulla Jr. and Cheng 1976).

The microsporidians predominantly being intracellular parasitic organisms, it's only at the cellular level that their response could be manifested. But a variety in these manifestations was obvious; for instance, in the genus *Pleistophora*-type of event the interiors of the host cell are systematically engulfed completely. Once the invader started division to ensure growth, the manifestation into destruction of the cell was evident. Once the microsporidia occupied the interior of a cell, the lytic action to convert the cytoplasm into an array of vesicles, endoplasmic reticulum, cisternae and ribosomes jumbled into an unorganized mass (Bulla Jr. and Cheng 1976). No pathogenesis was encountered beyond the infected region in this event. However, in other genera, exorbitant hypertrophy in the infected cell under influence from the microsporidian resulted. The connective tissue cells and those of mesenchyme origin, reaching up to 14 mm size comprised the hypertrophic cells (Weiser 1969). These are termed xenoma. The physiological and morphological integration of the cell of the host whose internal environment and composition are totally altered, mainly because of the enhanced sized microsporidian, contributed significantly to the formation of xenoma, as a separate entity that was nurtured depleting natural constituents of the host cell. With the gradual growth of xenoma noticeable alterations in its wall triggered inflammation as well as proliferation. This resulted into replacement of xenoma by the granuloma, whose tissues invaded into the former, but with a diminished centrally placed spore mass. The molecular

mechanism operating to express mitochondrial association with microsporidia has been a mystery even today (Lom and Dykova 1992). Though the secondary xenomas emanated from the reminiscent spores within, yet these never protruded or were liberated to the exterior. It is obvious that the granulation tissue invasion brought about changes into the wall of xenoma, and thus isolated the microsporidian with restricted auto-infection probability. The viable spores thus could only dehisce out if the host perished or else these were engulfed by the fibroblasts. Thus the mechanism of defence at the cellular level is strengthened with the central role of phagocytes that diminish the macrophage-engulfed spores, if their initial disintegration failed within granuloma. The gradual disintegration of the contents of the spore in the interior of phagocytes left an emptied folded membrane, that itself disintegrated ultimately. The evidence of chitinase enzyme activity was thus concluded by Heckmann (2020) confirming complete disintegration of the phagocytised spores that are redundant not to trigger any fresh infection activity episode further. The molecular level implications of the entry of microsporidians into the live cells were dealt with by Heckmann (2020) to emphasize that to analyze response of the host to the invader, such interactions would have to be understood at the level of cell.

The indications of interactions involving a surface protein 1 (EhSSP1) on the surface of the microsporidial sporoplasm of *E. hellem* with mitochondria from the cell of the host pest have been expressed (Han et al. 2019). All the three forms of voltage-dependent anion selective channels (VDAC1–3) being expressed in the cytoplasm of the outer membrane of mitochondria interact with EhSSP1. Han et al. (2019) asserted that the strength of the association of the mitochondria with microsporidian parasitophorous vacuole would weaken on account of this interaction being disrupted. Han et al. (2019) demonstrated that the energy retention encountered by the microsporidia in the cell of its host was presumably facilitated by the interaction of EhSSP1 with VDAC. It was peculiar to note that an unidentified cell protein within the host pest incorporated with the constituents of an invasion synapse (sporoplasm and polar tube in the cellular environment, form a synapse) also succeeded to interact with EhSSP1. This could trigger a novel response while sporoplasm is transported into the host cell cytoplasm. Further additional investigations could be conducted to explain this phenomenon. The flagellates, *Trypanosoma* sp., followed distinctively segregated life cycle pathway *sans* cysts, and instead, transfer of developmental stages occurred through direct ingestion of excretory matter. Naturally occurring DNA catenanes (Waraich et al. 2020) encountered primarily in the mitochondrial DNA isolated from HeLa cell lines (Hudson and Vinograd 1967) were assigned the task of the predominant method of organizing DNA in the mitochondria of trypanosomes. The network of the latter mitochondrial DNA, also called kinetoplast DNA (kDNA) (Englund et al. 1982), comprised two types of DNA circles that are interlinked to form an intricate network of maxicircles (20–40 kbp) and mini circles (2.5 kbp) (Lukes et al. 2002).

The soil-dwelling amoeba of genus *Dictyostelium* was the first free-living protozoan genome to be fully sequenced. It provided opportunity to examine activity of mutants of pathogens, as attenuation of their virulence resulted into depletion of their

virulence in *Drosophila* as well (Han et al. 2020). This meant that *Dictyostelium* provided a valid tool to examine the virulence mechanism of pathogens of insect pests. Han et al. (2020) carried out genetic manipulation in *Dictyostelium* that could illustrate the application of autophagy at relative ease during infections by pathogens. The cysteine protease, Paracaspase, was found in *Dictyostelium*. But sufficient data is not available to examine molecular function of these caspase-like proteins or their role in programmed cell death. Such detailed investigations could also lead to development of treatment therapies against parasitic protozoans (Han et al. 2020).

8.10 Microsporidia Invasion

The transport of sporoplasm into the cells of the host pests preceded by the Microsporidia infection of host cells involved expedited ejection of the polar tube (Fig. 8.2) (Weidner 1970; Frixione et al. 1992; Takvorian et al. 2005; Han et al. 2017). Weidner (1972) and Han et al. (2017) elucidated that the event of the onset of the infection triggered sticking of microsporidian spores to the cells of the host pests or the surrounding tissues. The significant contribution to these initial steps during infection is those of spore wall proteins, that is, SWPs, as illustrated by Southern et al. (2007). The investigations revealed a number of SWPs interacting with host cells due to stronger affinity to the heparin-binding motif (HBM) and sulphated glycosaminoglycans (GAGs) associated with the surface of cells of the host pests on *Nosema bombycis*, (Table 8.6) *Encephalitozoon* spp. and *Antonosporea locustae* (Table 8.6) (Hayman et al. 2001; Hayman et al. 2005; Southern et al. 2007; Li et al. 2009; Wu et al. 2009; Chen et al. 2017). A simultaneous report of the critical involvement of the integrin constituent of the host cell into the infection process by the microsporidium, *Encephalitozoon intestinalis*. *E. intestinalis* has also been on record in recent years (Leonard and Hayman 2017).

The canonical integrin-binding motif, arginine-glycine-aspartic acid (RGD), that regulated the binding of extracellular matrix (ECM) proteins with host cell integrins, were supposed to be present within a large number of hypothetical proteins, as revealed by the *E. intestinalis* genome analysis. A variety of pathogenic microbes, viz., several parasites, bacteria and viruses, that are reported to have stuck to the host cells were attributed to comprise proteins conducting interactions with host cell integrins (Patti et al. 1994; Bartlett and Park 2010). It was further demonstrated that the microsporidial spore attachment and host cell infection were barred by the incubation of host cells with RGD-peptides or recombinant alpha3 beta1 and alpha 5 beta 1 human integrin proteins (Leonard and Hayman 2017). It, therefore, conclusively suggested that, for the germination for production of spores and subsequent host cell invasion to succeed, the attachment of spores was an important part of the whole mechanism (Leonard and Hayman 2017).

8.11 Environmental Interactions

The body constituents of microsporidia are usually very soft and fragile that are usually susceptible to a variety of environmental attributes, but its spores can withstand adversities, externally to the cellular environment. The protozoans, with bacteria (decomposers), and ciliates (bacterivores) entered into effective interspecific and intraspecific interactions, in association with mosquito larvae (primary consumers) succeeded to influence characteristics, like cell size, cyst production and growth rate (TerHorst 2010, 2011). According to Maddox and Solter (1996), the survival period of life cycle stages of microsporidia, that belonged to the terrestrial category, distinct from the aquatic ones, was normally over 30 years, in liquid nitrogen storage conditions. But the usual survival time was from 1 month to a year, under natural environmental conditions, when these were under protection from UV radiation and other co-occurring microbes or degradation factors (Maddox 1973; Maddox et al. 1981; Brooks 1980, 1988; Goertz and Hoch 2008). However, microsporidia from aquatic environment were not that sturdy, and could easily be under the influence of environmental degradation (Becnel and Johnson 2000). It was quite an unusual characteristic of microsporidia that it could coordinate its presence or absence while their hosts are unavailable under variable conditions of seasonality or wide fluctuations in their population density. Subsequently, their unique potential to develop in a suitable host species, which was related to its original host species (Lange and Azzaro 2008) or in an alternate or intermediate host, when their routine hosts were not available, was an advantageous adaptation (Micieli et al. 2009).

The vertical transmission in certain types of infections by *Edharzardia aedis* in the host, *Aedis aegypti*, (Table 8.1) might not be so ferocious to the extent as to facilitate its inoculation to the local environment with spores for horizontal transmission among larvae of the mosquitoes (Koella et al. 1998). The most common instance of adoption of survival strategy by *N. pyrausta* microsporidia in diapause stages of the host, as well as to pass over the overwintering period through its fifth instar stage in *O. nubilalis* (Andreadis 1986; Siegel et al. 1988), and the relatively more common transovarial transmission through infected eggs of *N. Portugal* (Maddox et al. 1999) are available. This, of course, is quite imperative that the infection stress employed under additional physiological alterations during diapause could as well be fatal to the host; yet the protection of the infecting agent in the surviving host's environment is facilitated to enable these to remain available, till the breeding of the forthcoming next generation of mosquito pest (Andreadis 1986).

8.12 Advantages & Disadvantages

8.12.1 Advantages

The management of biodiversity in a variety of ecosystems could only be sustained when the insect pest hosts remained under the stress of natural enemies (protists, i.e. microsporidia) to achieve biocontrol. It was, therefore, obvious that the

protozoan pathogens that acted to the detriment of such pests be conserved for the safety of human beings, and in this way, the nontarget organisms would also succeed to contribute for the benefit of a balanced ecosystem. The formal recognition to the only species, namely, *P. locustae*, the pathogen of a grasshopper species, registered by the US Protection Agency (USEPA), as a microbial insecticide, is a distinctive step towards the advantage to human beings under the biocontrol programme, after a passage of half a century of expeditious researches world over in this field.

8.12.2 Disadvantages

The typically recognized slow-acting potential of microsporidia to control insect pests quite often leads to the prolonged wait to record detectable impacts on the pest host populations. Under such an eventuality, newer methods of appropriate formulations, keeping in view the ecological fate of protozoan spores, would be required to be developed. An eye would have to be kept on the cost-effective aspects of economy for the management of protozoan organisms, particularly microsporidia to care for their potential of biocontrol in the context of IPM.

8.13 Production and Storage

8.13.1 Production

Culture media are generally available for ciliates and trypanosomatid flagellates, and there is reason to be optimistic that only minor modifications would be needed to support the growth of species, which are endoparasitic. Since the freshwater environment of the host mosquitoes would be normal habitats for the infective stages of *Lambornella* and *Tetrahymena*, the cultured ciliates could be introduced directly. The problems of preventing the ciliates being consumed by the mosquitoes, as observed by Clark and Brandl (1976), would have to be overcome. Cultured flagellates cannot be introduced in this way as they neither form cysts nor have free-swimming stages, which can survive the physical and physiological changes as do ciliates. So far no species of flagellate has been deemed pathogenic enough to warrant efforts being addressed to the problem of introduction. Other Protozoa harboured by invertebrates, suitable for development as biocontrol agents, are all obligate intracellular parasites with resistant spores or cysts for transmission. None can be cultured free of living cells, and methods for mass production are limited at present to growth in natural or experimental hosts. In vitro culture in cell lines has been achieved for a few species, but spore production is well below that which is obtained in living hosts.

In Vivo Production

The episodes of production and efficacy of the insect infested microsporidia and neogregarines were reviewed by Brooks (1980). It was hypothesized that the

optimum replication rates to produce hosts and to minimize their losses should be ensured by keeping an eye on the dose applied, age of the host and the time of incubation from the standpoint of economic production. Brooks (1980) summarily described a large variety of protozoans that were produced in live hosts, but strikingly in pretty lower numbers. The protozoan infective agents, namely, *Malpighamoeba locustae*, were encountered in day-to-day faecal matter collections from the host pests. The turnover was substantial in case of *V. necatrix* from *Helicoverpa zea*. The cost analysis report by McLaughlin and Bell (1970) presented a comparable account of protozoan organisms, viz., *Mattesia grandis* (McLaughlin) and *N. gasti* (McLaughlin), from an experimental report involving pest hosts, that is, cotton boll weevils, *A. grandis*. Schwalbe et al. (1974) contemplated production of spores of *Melanoplus troglodermae* to assert biocontrol of *T. glabrum* in the live environment to store the stored grains. To envisage the production of supernumerary instars of the host pest, the use of an insect growth regulator was suggested by Brooks (1980) to trigger production of spores/larvae en masse. The efforts to produce the protozoan, *N. locustae* Canning, from the commercial standpoint in grasshoppers, *Melanoplus bivittatus*, were recognized by the US Environment Protection Agency (EPA) (Henry and Oma 1981).

In Vitro Production

The procedures have been developed to date to grow suspension cultures or monolayers of cells for the enhanced output of a variety of microsporidia as well as to ensure simultaneously the availability of the spores of these microsporidia at the required period of intervals from the culture medium. But their status of product output as on to the level of cost-effectiveness could not be ensured. Several earlier investigators (Gupta 1964; Kurtii and Brooks 1971; Bayne et al. 1975; Sohi and Wilson 1976) utilized an already infected host resource to establish primary cell cultures of these microsporidia.

The uninfected cultures were maintained to introduce sterile harvested spores. It was also not necessary to grow the microsporidia or other cells that could only exhibit closer affiliation to their host cells, whether those of invertebrate or vertebrate origin. Primary cultures of silkworms, *Bombyx mori*, were used by Ishihara and Sohi (1966) and Ishihara (1969) for growth of *N. bombycis* Nageli (Table 8.6) and cell lines were used by Undeen (1975) for *N. algerae* in pig kidney cells by Kurtii and Brooks (1977) for *N. disstriae* and by Atwell et al. (1985) for *H. zea* cells. The findings of several earlier investigators did not find consistent growth and replication rates in cultures of different kinds, though they did not report comparisons with in vivo systems to record the rates of replication beyond a few days. An improvement in the technique for infection of cells in culture was introduced by Barker et al. (1980), who centrifuged the cultures when the spores were introduced. The close contact between spores and cells, during the process of polar filament eversion, gave a higher level of infection from which the parasites could spread to other cells. Sohi and Wilson (1976) found that spore production of *N. disstriae* was depressed in culture, although the numbers of parasites remained high. This would be a serious

impediment to mass production, and a similar effect has been reported for cultures of *N. algerae*, in which spore production stopped after six passages (Streett et al. 1980).

8.13.2 Storage

The resistivity of the entities that participate in transmission of certain Protozoa has a greater role to play but the most convenient and safest form of these could be to keep the cysts or spores of these in dry state. Storage presents no insurmountable problem for Protozoa with resistant stages of transmission, but the simplest method of keeping the cysts or spores in the dry state can only be used for certain categories. Dry storage is essential to prevent extrusion of the polar filaments, which occurs automatically in water. The protrusion of polar filaments can be prevented by maintaining these in dry form, which otherwise is attained automatically in the moist state in *N. whitei* Weiser, a microsporidium of flour beetles *Tribolium* spp. No apparent loss of viability was observed by Milner (1972) in the spores of *Tribolium* spp., if stored in a dry flour–yeast mixture at 4 °C to the extent of 15 months. On the other hand, the exposure to even shorter periods of desiccation was fatal to some of the microsporidia. The dried spores of *N. algerae* lost their potential to infect the larvae of *Anopheles stephensi* Liston in a study by Alger and Undeen (1970). However, an optimal survival of most of the microsporidia and a fewer variety of neogregarines were attained when these were stored in a sterile water suspension kept at a temperature above freezing point. The spores of *N. bombycis* Nageli could survive viable for as long as up to a decade, as recorded by Oshima (1964). The treatment by antibiotics was applied to maintain viability of these microsporidia as it deteriorated rapidly under the influence of these contaminants. Contrary to this, the treatment by the required concentrations of antifungal mixtures, needed to deactivate yeasts or growth of hyphae, proved damaging to a larger chunk of the microsporidia in a manner that a span of storage of *N. algerae* for 5 weeks in natamycin, fungizone and nystatin resulted in a loss of their viability (Lai 1980). Simultaneously, appreciable issues of rearing to maintain insect colonies during conduct of experiments were faced when the antibiotics, like benomyl and fumagillin, were applied to keep check on the growth and spread of microsporidia (Vavra and Maddox 1976). The noticeable reduction in viability of microsporidian spores ensued at the higher temperatures that could incapacitate these in as much as that it was reduced in most species, to weeks, hours or minutes by temperatures that have risen to 40 °C (Maddox 1977). Considerable loss in viability of microsporidian spores of a large number of protozoan species, like *N. locustae*, resulted at lowered thermal regime (3 years in distilled water at –10 °C), as well as in case these were stored dry (Henry and Oma 1981). The imminent requirement to resolve the problem of storage of such magnitude was appreciated because these protozoans have been recognized as a workable biopesticide over the years by now. But the issues like availability of these biocontrol liquids in huge quantities for applications in the fields kept the farmers baffled, because of large areas under attack by grasshoppers and locusts, and simultaneously methods to be

adopted for the delivery of these biopesticides over large farm areas were cumbersome. Thus, the riddles of cost-effectiveness kept the farmers away from immediate adoption of such measures, and instead these were adopted only in cases of extreme emergencies. However, the outcome of a large number of investigations (Kramer 1970a, b; Brooks 1980) illustrated a storage regime at the freezing temperature (i.e. 4 °C) was appropriately helpful for the growth of several species. The need has been felt to enhance adequate quantities of storage of *N. locustae* at the commercial scale. The methods of lyophilization and cryopreservation in liquid nitrogen, employed to achieve long-term viability (Vavra and Maddox 1976), were not found to be cost-effective because of the high cost of liquid nitrogen.

Later, however, some apprehensions were expressed about the loss of viability of spores to a small extent immediately followed by lyophilization, but the spores that survived the onslaught retained infectivity for longer periods. The loss of viability of a negligible magnitude of *Nosema apis* Zander post-lyophilization was concluded by Bailey (1972), and, therefore, its prolonged use for storage of viable microsporidia was recommended.

8.14 Future Prospects of Protozoans Biocontrol of Insect Pests

The pests, to the extent of 98%, are known to have been controlled by biocontrol agents. Certain ecological invaders comprising newer predators and parasitoids could conform to the significant constituents of biocontrol that regulate a variety of pests. According to the modern trend, the utilization of emerging strains, biotypes, parasitic hybrids, protozoan and fungal biopesticides required utmost attention for various applications in the management of pests. The principal beneficiaries of the application of protozoan control methods to regulate populations of pests have been the aquaculturists and farmers, particularly those dedicated to modernized aquacultural techniques, like cage culture etc., organic farming and other modern agricultural methods. Newer substitutes would be added day by day by the application of modernized researches to develop formulations of newer substances. Further advancements would target commercialization and adaptability of biopesticides. These efforts would also restrict cost escalation as more and more natural means of biocontrol become acceptable to the public at large. Once more and more advancements in the development of promising candidates among natural products emanated from the farmed products, the test of their efficacy and reliability in application could be ensured by extensive checks on the target pests in a variety of cropping systems.

It is a challenging task to conduct detailed investigations on biochemical pathways in the intracellular organisms that lack mitochondria but are the potential candidates to be employed in biocontrol of insect pests.

8.15 Conclusions

Microsporidia (Protists) belong to the significant group of biocontrol agents that had the potential to keep noticeable benefits of human populations conserved as against the potential of insect pest hosts that could keep the former divested of noticeable economic gains. The unique mechanism of invasion adopted by these pathogens incorporated the use of a specialized apparatus whose functional details are yet not completely available. They are important pathogens of economically important insects and animals. It is noticeable that considerable information is available to assign (i) vertical, (ii) horizontal, as well as (iii) transovarial transmission pathways in the dynamics of the life cycle of microsporidia. But additional information on their mode of entry into the cells of insect pests, along with the mechanism of its emergence post-replicative cycle, would provide solution to the riddle of incomprehensible process of impact on the pest host. The limitations of the excessive applicability of chemicals to control or kill the insect pests have indeed not led us too far in as much as to pave safer pathways down the lane. This is, in particular, due to the well-known adverse ill-effects on the environment, nontarget species as well as public health. This does not necessarily mean that only a fast action strategy could serve meaningful purpose, particularly in the wake of a virtual slow-action reactivity exhibited by most of the known protists (microsporidia). The elements of reliable non-chemical control method could safely have the potential to long-term suppression and maintenance of low densities of insect pests, like grasshoppers and locusts, and these could well be the critical elements of control by natural enemies in terms of application of protists as tools of biocontrol. The judicious mix of ecologically safe biocides utilizing microsporidian protozoans as its constituents, to replace hazardous uneconomical chemicals for control of insect pests, is propagated.

8.16 Points to Remember

- Biocontrol elements in pathogenic interactions, other than non-chemical and other methods of control, encompassing the tiny protista (Microsporidia) and other protozoans comprise unique entomopathogenic organisms.
- The invasion of these minute pathogens primarily in the form of cyst into the cells of insect pest hosts is a commonly known event. These infective cysts have the potential to survive over unfavourable conditions as well as overwinter to survive withering conditions, during seasonal change.
- Pathogens have, of late, been recognized as suppressor of rapid breeding and feeding speed of acridid and a variety of other insect pest hosts, although their action was labelled initially as slow-paced. This could well be a successful biocontrol strategy.
- The microsporidian invaders made inroads into the intracellular environment of insect pests through cysts. Amongst *Nosema*-type group of microsporidia, the transmittance of the flagellate, *Trypanosoma* sp., did not occur by direct ingestion of true cysts but through the ingestion of faecal material. *Endoreticulatus* group

(Table 8.2) was differentiated from the other two, that is, *Nosema* type and *Vairimorpha* type, phylogenetically as well as in the status of pathogenicity.

- The horizontal transmission of microsporidians is a unique pathway in which entry of cysts through eggs, diapausing larvae, pupae or non-feeding adults is attained. The alternative vertical mode facilitated invasion of microsporidians into the contents of the eggs that terminated the process at the hatching of larvae from the contaminated egg contents.

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