Microsporidian Pathogens in European Gypsy Moth Populations

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Abstract

The significance of microsporidian pathogens as mortality agents of gypsy moth (Lymantria dispar L.) in Europe frequently is overlooked. Collections of isolates from 10 different countries suggest that three genera and several biotypes are extant. It is important that the taxonomic placement and phylogeny of currently described genera and species be clarified and that regulatory issues be addressed before exotic entomopathogens are introduced into North America to regulate L. dispar populations.

Introduction

Microsporidia are a diverse group of obligate intracellular parasites that use most animals and humans as hosts. They are among the oldest eukaryotes and have many primitive yet uncommon and complex characteristics. At the molecular level, they possess a ribosomal RNA structure that is thought to be more prokaryotic than eukaryotic. Microsporidia have no mitochondria and undergo a primitive nuclear division (Vossbrinck et al. 1987), though sequence analysis of several microsporidian genes suggest that they are fungi (Weiss et al. 1999; Van de Peer et al. 2000).

Microsporidia were elevated to the phylum Microspora by Sprague (1977) and amended to the Phylum Microsporidia by Sprague and Becnel (1999). According to Larsson (1999), about 1,300 species have been described but a substantial number of identified genera and species have not been studied or named. The genus Microsporidium was introduced by Sprague (1977) as a temporary designation for species that have been excluded from other genera and are awaiting proper taxonomic classification based on new information.

The taxonomy of microsporidia—specifically, their identification in field collections—has been hampered by their size (typically 2-10m) and because few species have been described adequately and placed in appropriate genera (Larsson 1999). It is estimated that about half of microsporidian genera contain only a single species. The advent of electron microscopy and recent advances in molecular technology have improved the systematics of microsporidia but also raised questions about the taxonomic placement and phylogeny of currently described genera and species.

According to Maddox et al. (1999), six species of microsporidia have been described from gypsy moth populations in Europe, and several isolates that have not been described or identified are recorded in the literature. Despite a rich microsporidian complex in European gypsy moth populations, microsporidia have not been reported from gypsy moth populations in North America (McManus et al. 1989). Table 1 is an updated version of the information provided initially by Maddox et al. (1999).

Of the listed species, probably only N. lymantriae (Weiser 1957) and N. serbica (Weiser 1964, Pilarska and Vavra 1991) satisfy the diagnostic criteria defined by the International Code of Zoological Nomenclature (1985) for describing a new taxon. With respect to the identification of the other species, it should be noted that ultrastructural and molecular information was not available to the authors at the time these microsporidia were described as new species.
Importance of Microsporidia in Gypsy Moth Population Dynamics

In Central Europe, the gypsy moth is considered a periodic pest, with outbreaks occurring every 7-10 years. Further south in the Mediterranean and Balkan regions, where the climate is more conducive to gypsy moth development and survival, outbreaks are reported every 3-4 years (Weiser 1987).

In Europe, several entomopathogens have been reported from \textit{L. dispar} populations particularly during outbreak years (Weiser 1987). These include the baculoviruses (nuclearpolyhedrosis virus (NPV) and granulosis virus (GV), cytoplasmic polyhedrosis virus (CPV), microsporidia, fungi, bacteria, and nematodes. In North America, where the gypsy moth was introduced in 1869, NPV is the dominant pathogen causing outbreak populations to collapse. Bacteria, nematodes, and CPV are recovered only occasionally; microsporidia have not been recovered. In 1989, the fungus \textit{Entomophaga maimaiga}, which was introduced in 1910-11 from Japan but never reported as established, was found simultaneously in several locations in the northeastern United States and subsequently caused a regional epizootic in U.S. gypsy moth populations (Hajek et al. 1995). Since then, \textit{E. maimaiga} has been the most dominant mortality factor in preoutbreak populations and acts in a density-independent manner, which is atypical of most entomopathogens.

Among the pathogens in European gypsy moth populations, NPV is primarily responsible for the termination of \textit{L. dispar} outbreaks whereas microsporidia are prevalent during the gradation period prior to outbreaks and then persist at low levels among gypsy moth populations in the years between outbreaks. Whereas the NPV causes acute disease and results in dramatic epizootics among mid- and late-instar larvae, microsporidia usually produce a chronic disease state that results in the death of some larvae and pupae and prolongs the developmental period of larvae, possibly lengthening the window of opportunity for early-instar parasitoids such as \textit{Cotesia melanoscela} and \textit{Glyptapanteles sp}. Weiser and Novotny (1987) reported that hymenopteran parasitization of the gypsy moth was 58% higher in plots treated with \textit{N. lymantriae} than in control plots.

Different microsporidian species that infect the gypsy moth target different tissues within their host, including the silk glands, midgut epithelium and associated muscle tissue, fat body, Malpighian tubules, nerve tissue, and reproductive organs. Species in the genus \textit{Nosema} infect the gonads and ovarioles, reducing fecundity and killing overwintering embryos. Zelinskaya (1980) reported that the

<table>
<thead>
<tr>
<th>Microsporidian species</th>
<th>Location</th>
<th>Reference</th>
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<tbody>
<tr>
<td>\textit{Nosema lymantriae}</td>
<td>Czechoslovakia</td>
<td>Weiser (1957)²</td>
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<tr>
<td>\textit{Nosema muscularis}</td>
<td>Czechoslovakia</td>
<td>Weiser (1957)²</td>
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<tr>
<td>\textit{Nosema muscularis}</td>
<td>Spain</td>
<td>Romanyk (1966)</td>
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<tr>
<td>\textit{Nosema muscularis}</td>
<td>USSR, Ukraine</td>
<td>Zelinskaya (1980)</td>
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<tr>
<td>\textit{Nosema serbica}</td>
<td>Yugoslavia</td>
<td>Weiser (1964)</td>
</tr>
<tr>
<td>\textit{Nosema serbica}</td>
<td>USSR, Ukraine</td>
<td>Zelinskaya (1981)</td>
</tr>
<tr>
<td>\textit{Nosema serbica}</td>
<td>Bulgaria</td>
<td>Pilarska and Vavra (1991)</td>
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<tr>
<td>\textit{Thelohania disparis}</td>
<td>Russia</td>
<td>Timofejeva (1956)</td>
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<tr>
<td>\textit{Thelohania similis}</td>
<td>Czechoslovakia</td>
<td>Weiser (1957)²</td>
</tr>
<tr>
<td>\textit{Nosema portugal}</td>
<td>Portugal</td>
<td>Maddox et al. (1999)²</td>
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<tr>
<td>\textit{Nosema sp.}</td>
<td>Portugal</td>
<td>Cabral (1977)</td>
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<tr>
<td>\textit{Nosema sp.}</td>
<td>Yugoslavia</td>
<td>Sidor (1979)</td>
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<tr>
<td>\textit{Nosema sp.}</td>
<td>Romania</td>
<td>Safroiu et al. (1978)</td>
</tr>
<tr>
<td>\textit{Thelohania portugal}</td>
<td>Portugal, Bulgaria, Hungary</td>
<td>Solter et al. 1997</td>
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*Modified from Maddox et al. (1999).
  ²Species descriptions.
mortality of overwintering eggs laid by severely infected females was 58.5% compared to 34.2% in healthy females. Survival rates for neonates that emerged from infected egg masses also were reduced significantly.

According to Weiser (1987), microsporidian infections become more prevalent in the progradation period prior to a gypsy moth outbreak and may infect 15-30% of the population during an outbreak’s peak period. As soon as the NPV appears in the population, microsporidian prevalences decline. However, many researchers suggest that microsporidia might have a more significant role in the population dynamics of $L.\text{dispar}$. Purrini and Skatulla (1978) reported that the prevalence of $N.\text{lymantriae}$ in Sardinia reached 60% in 1977. Sidor and Jodal (1983) studied the prevalence of a microsporidia identified as $N.\text{serbica}$ and NPV in the Acacia Forest “Bagremara” during a gypsy moth outbreak in 1978-81. The average larval mortality attributed to $N.\text{serbica}$ exceeded that caused by NPV in each of the 4 years of the investigation and reached a maximum of 72% in 1978 when the estimated density of $L.\text{dispar}$ was 16,000 egg masses/ha. Zelinskaya (1980) concluded that a complex of four microsporidian species was the major cause of a decrease in the population of I-III instar gypsy moth larvae in forest plantings along the lower Dnepr River in the Ukraine during 1976-77, and that 26-60% of egg-laying moths were infected during the outbreak phase. Sierpinska (2000) reported a prevalence of infection of 91% among late-stage $L.\text{dispar}$ larvae that were sampled from the Biebrza National Park in Poland. Larvae were infected by a $Nosema$-like microsporidium.

Strong evidence that microsporidia are significant mortality factors in the dynamics of gypsy moth populations in Central Europe was the motivating factor behind a foreign exploration program that was initiated in 1985. The long range objective was to explore the feasibility of introducing candidate microsporidia from Europe as classical biocontrol agents to enhance the natural control of gypsy moth in North America.

**Published Description of Species**

In 1986, the USDA Forest Service supported a foreign exploration trip to Europe by J. Maddox and M. Jeffords (Illinois Natural History Survey) to search for microsporidian isolates from gypsy moth populations (McManus et al. 1989). Isolates were recovered from Portugal and the Czech Republic; a description of the microsporidium recovered from Portugal was published (Maddox et al. 1999). Included was a tabular listing for six species of microsporidia that were described or reported from European gypsy moth population at that time (Table 1).

The original description of these microsporidia contained few ultrastructural details and little data on life cycles. This lack of information led to confusion in identifying microsporidia isolated from Lepidoptera because some species are dimorphic (Maddox and Sprenkel 1978), i.e., they produce two different types of spores. Unfortunately, the microsporidia from the gypsy moth in Europe were described before dimorphism was recognized. Consequently, species reported in the literature as mixed infections of $Thelohania$ sp. and $Nosema$ sp. probably represent a single dimorphic species in the genus $Vairimorpha$. According to Sprague et al. (1992), the formation of uninucleate spores in an octosporous sporulation cycle is characteristic of $Vairimorpha$. Figure 1 shows the probable relationships among the described species of gypsy moth microsporidia.

*Nosema lymantriae*: Probably a valid species as described by Weiser (1957). Mention in the literature of mixed infections of $Nosema$ and octosporous $Thelohania$, though probably refers to a different species in genus $Vairimorpha$.

*Thelohania similis*: Probably not a valid species and always was reported as mixed with diplokaryotic spores ($Nosema$ type) spores, suggesting a $Vairimorpha$ sp. No $Thelohania$ sp. has been recovered in our surveys of microsporidia in European gypsy moth populations.

*Thelohania disparis*: Close examination of the description in Timofejeva (1956) leads us to believe that this is a $Vairimorpha$ sp.
Nosema muscularis: Its description matches the characteristics of “primary spores,” an autoinfective stage that apparently occurs in all lepidopteran Nosema and Vairimorpha species as well as in other microsporidia genera (Solter and Maddox 1998a).

Endoreticulatus sp: The status of this species and its synonomy with Pleistophora schubergi, which was described from Euproctis chrysorrhoea (L.), is being investigated. The isolate from Portugal was first referred to as Vavraia sp. (McManus et al. 1989), but later was called Endoreticulatus sp. (Solter et al. 1997). It has since been recovered from L. dispar populations in Bulgaria and Hungary.

Nosema serbica: It is not known whether this species is similar to or conspecific with N. Lymantriae; unfortunately, neither isolates nor stained slides are available for analysis. Pilarska and Vavra (1991) discussed the similarities and differences between N. serbica and N. lymantriae. Attempts are being made to isolate this microsporidia from gypsy moth populations in the Balkans from where it was described originally.

Nosema portugali: First reported by Cabral (1977) and later isolated and described in detail by Maddox et. al. (1999) this species is closely related to other Nosemas isolated from gypsy moth populations. However, on the basis of molecular data, it may stand as a distinct species.

Ultra structural and molecular studies have been completed on isolates of Vairimorpha sp. recovered from gypsy moth populations in Bulgaria. A report is being prepared that describe this species and clarifies the taxonomy of the species listed in Table 1.

Foreign Exploration in Search of Microsporidian Isolates
As mentioned earlier, a foreign exploration program was initiated by the Forest Service in 1993 with support provided by the USDA Foreign Agricultural Service, International Cooperation and Development Research and Scientific Exchange Division, to search for microsporidia among gypsy moth populations in several countries in Europe, to compare these isolates with previously described species, and to evaluate isolates that might be candidates for introduction as classical biological control agents against L. dispar populations in North America. Although gypsy moth populations were at low densities throughout most of Europe during the period of exploration, we recovered microsporidian isolates representing three genera from more than 20 locations in seven countries. All of the isolates are stored in liquid nitrogen at the Illinois Natural History Survey laboratory of the junior author.

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Figure 1.—Relationships of gypsy moth microsporidia.
Since 1994, an ad hoc team of 10 scientists from six countries has conducted research on various aspects of several of the isolates that we recovered: ultrastructure and life cycle, modes of transmission, effects of infection on the gypsy moth host, impacts on nontarget Lepidoptera, and molecular sequencing of small subunit rDNA. A synthesis workshop was held in Prague in October 1997 and attended by 12 scientists from the United States and Europe to report results of individual studies, exchange relevant data, and identify laboratory and field research that is needed to realize accomplishments in a reasonable period of time. Much of the progress that is documented here resulted from the combined efforts of workshop participants.

Related Research

Since the stated goal of the research program was to evaluate the feasibility of introducing microsporidia into U.S. gypsy moth populations as a classical biological control agent, a basic knowledge about their host specificity is fundamental in resolving safety and regulatory issues (Maddox et al. 1992). A series of laboratory bioassays was initiated in 1994 that elucidated the host range of five of the biotypes listed in Table 1 by testing larvae of 49 lepidopteran species that are indigenous to North America and sympatric with gypsy moth larval populations (Solter et al. 1997). The microsporidia produced a variety of responses in the nontarget hosts such that the latter were placed in one of three categories: (1) no infection (refractory); (2) a typical infection in which few or no typical environmental spores were produced and horizontal transmission is unlikely; (3) heavy “host-like” infections in which mature environmental spores are formed that may be infective to conspecific individuals. On the basis of the response of nontarget species to the five microsporidian biotypes, Solter et al. (1997) concluded that N. portugal was the least infectious to nontarget Lepidoptera, and that Endoreticulatus sp. was most infectious, producing a “host-like” infection in 15 different species. The other isolates, Nosema-like isolates from Slovakia and Romania and N. lymantriae, were intermediate in infectivity to lepidopteran larvae, but host ranges and responses of hosts to infection were closer to those of N. portugal than to Endoreticulatus sp. The infectivity of Endoreticulatus sp. is not surprising. Although it has been recovered from gypsy moth populations in Portugal, Hungary, and Bulgaria, Endoreticulatus sp. is considered a generalist; its taxonomic placement is under study.

Solter and Maddox (1998b) conducted studies to determine the usefulness of laboratory studies in predicting ecological host specificity by feeding microsporidia isolated from other Lepidoptera to L. dispar larvae and then comparing the horizontal transmission between infected and uninfected L. dispar larvae to that which occurs among individuals of the natural hosts. Although nine microsporidian species infected L. dispar larvae, only one was transmitted to uninfected L. dispar larvae. This supports the concept that there is a much higher level of ecological host specificity among Lepidoptera microsporidia than laboratory host-range studies might predict.

Although laboratory bioassays provided a method to assess the host range of microsporidia in sympatric populations of Lepidoptera, there was a need to accumulate supporting data on the presence and prevalence of L. dispar and other microsporidia from natural populations of Lepidoptera in the field. Long-term monitoring of L. dispar populations in several sites in central and western regions of Bulgaria revealed that three species were present (Endoreticulatus sp., Nosema sp., Vairimorpha sp.) and that the same species were recovered repeatedly from the same site (Solter et al. 2000). Eleven microsporidia isolates from the same three genera were recovered from 1494 insect larvae representing 12 families of Lepidoptera. However with one possible exception, none of these isolates produced a typical host-like infection when fed to L. dispar larvae. The infective isolate was genetically different from the local endemic microsporidium. That no L. dispar microsporidia were found in Lepidopteran larvae feeding sympatrically with gypsy moth larvae on the same host-tree species supports predictions from laboratory studies that the ecological host specificity of L. dispar microsporidia is much narrower than physiological (laboratory) host specificity.

More definitive studies being conducted in Slovakia to evaluate the infectivity of N. portugal and Vairimorpha sp. against nontarget Lepidoptera when the isolates are applied to control gypsy moth
larval populations. In this multiyear study, suspensions of each isolate are applied via ULV spray in replicated 500-m² plots to which gypsy moth larvae had been introduced previously. There were intensive collections of all larval Lepidoptera 7 and 14 days after spraying. Preliminary results suggest that the Vairimorpha isolate is more infectious to nontarget Lepidoptera, particularly certain genera of noctuids, than N. portugal. These results corroborate the results of laboratory studies of Solter et al. (1997), though additional field studies will be conducted in subsequent years to better clarify the impacts of these two isolates.

As mentioned earlier, a recurring dilemma concerning microsporidia, including the gypsy moth isolates, is related to their taxonomic placement. Many of the morphological and life-cycle characters that traditionally were used in microsporidian systematics are not holding up to molecular scrutiny, and other new techniques are elucidating characters such as multiple spore types in some species that understandably confused early taxonomists. Nevertheless, pathogens identification is a necessary component of a classical biological control program where exotic organisms are being considered for release into the environment. From analyses of ribosomal DNA sequences we know that the gypsy moth Vairimorpha and Nosema species are taxonomically distinct from the type species (Nosema bombycis from silk moth and Vairimorpha necatrix from the true army worm), and that gypsy moth Nosema and Vairimorpha isolates are more closely related to each other than to other members of the genera to which they are assigned. While there are sufficient differences between the gypsy moth Nosema and Vairimorpha isolates to designate them as good species groups, the Nosema group continues to be difficult to analyze. The rDNA sequences (both large and small) are nearly identical between isolates but most differ by one base pair. The rDNA sequence may be too conservative to analyze differences in closely related species or distinguish differences between populations. The problem is twofold: to which of the previously described species are they most closely related (or are they synonymous), and do they represent one variable species or several species?

We will need to rely on the extant records (the literature and Giemsa-stained specimen slides) to determine whether our isolates are synonymous with previously described species. However, to evaluate relative differences between our viable isolates, we are experimenting with PCR-RAPDs. In preliminary studies we have been able to distinguish definitive differences between Vairimorpha sp. and Nosema spp. as well as certain differences between the Nosema isolates. We are not yet certain that this method will yield the information we seek and will consider using AFLP or protein analysis in future studies. Overcoming the taxonomic difficulties within these groups will allow us to take more positive steps toward satisfying the regulatory issues that must be addressed before an exotic entomopathogen is introduced into North America to control gypsy moth populations.

References Cited


