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Synaptonemal complexes as evidence for meiosis in the life cycle of the monomorphic diplokaryotic microsporidium *Paranosema grylli*

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Abstract

Characteristic configurations of the nuclei and synaptonemal complexes, indicative of the onset of meiosis, were observed in the meronts of the monomorphic diplokaryotic microsporidium, *Paranosema grylli*. This finding indicates that a process similar to the meiosis previously reported in polymorphic and some monomorphic monokaryotic microsporidia probably occurs in the development of *P. grylli*. It is the first evidence for the possible presence of a sexual phase in the life cycle of this microsporidium, which for a long time has been considered asexual.

Keywords: Diplokaryon; Meiosis; Microsporidia; *Paranosema grylli*; Synaptonemal complexes; Ultrastructure

Introduction

More than half of known microsporidian genera possess diplokaryotic nuclear apparatus, at least at some stages of their life cycle. The diplokaryon consists of two morphologically identical nuclei, closely adjacent to each other and dividing synchronously and equally. An alteration from diplokaryotic to monokaryotic nuclear arrangement takes place in the life cycle of many genera. It occurs either by the fusion of nuclei followed by meiosis (Hazard and Brookbank 1984) or by the dissociation of the diplokaryon (Becnel et al. 1987, 1989). The shift from one nuclear arrangement to another leads to ploidy reduction in the subsequent life cycle stages and results in the formation of monokaryotic spores. Evidence of the occurrence of meiosis in various microsporidian species was seen in the presence of synaptonemal complexes (Loubès et al. 1976; Vávra 1976a; reviewed by Larsson 1986), in the discrete change of DNA content indicated by microphotometry (Hazard and Brookbank 1984) or in the characteristic meiotic configuration of chromosomes (Hazard et al. 1979; Chen and Barr 1995).

There are about 20 phylogenetically non-related genera of monomorphic diplokaryotic microsporidia which are believed to maintain the diplokaryotic nuclear arrangement during the entire life cycle. The life cycle of these genera involves mitotic proliferation of diplokaryotic meronts and sporonts (usually by binary fission), and a single sporogony sequence which results in the production of diplokaryotic spores. Such a cycle is referred to as a "*Nosema*-like cycle" for disporoblastic forms developing in direct contact with the host cell cytoplasm. This cycle is thought to involve neither nuclear alteration nor ploidy reduction. It is supposed to

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be an asexual fragment of an ancestral more complex life cycle with a sexual phase characteristic of the polymorphic species (Sprague 1978; Loubès 1979; Baker et al. 1997; Sokolova and Issi 1997). However some authors doubt the asexuality of the monomorphic diplokaryotic microsporidia and suggest the existence of hidden sexual stages in their life cycle (Sprague et al. 1992). The question whether this group of microsporidia is truly asexual remains open.

Paranosema grylli (formerly known as *Nosema grylli*), a fat body parasite of the cricket *Gryllus bimaculatus*, was originally described as a monomorphic diplokaryotic microsporidium (Sokolova et al. 1994). Further studies of this species revealed some deviations from a typical *Nosema*-like cycle in its development, suggesting the existence of a brief unikaryotic stage at the end of merogony, but no evidence for meiosis has been observed (Sokolova et al. 2003). Recently we have found synaptonemal complexes in the diplokarya of *P. grylli* meronts. In the present paper, we describe this phenomenon and discuss its possible biological significance.

Material and methods

Paranosema grylli was maintained in a laboratory culture of the cricket *G. bimaculatus* as described by Sokolova et al. (2003). Insects were infected with diluted water suspension of spores (approximately 10^5 spores per ml) used instead of water supply for second–third instar nymphs. Fixation and embedding for transmission electron microscopy (TEM) was carried out on the fat body of a male late-instar nymph, 6 weeks after infection. The proliferative stages of *P. grylli* were abundant in fresh smears from the fat body of this specimen, but no immature or mature spores were seen. Portions of infected fat body were fixed in 2% OsO_4 for 20 min at room temperature, washed in 0.1 M phosphate buffer (pH 7.6), dehydrated in an ethanol series and propylene oxide and embedded in Epon resin. Sections were stained with uranyl acetate in 70% ethanol and Reynolds' lead citrate.

Results

The interphase diplokaryon in meronts and sporonts of *P. grylli* consisted of two closely apposed nuclei (Fig. 1). In the area of contact between the two nuclei the nuclear envelopes were closely adjacent to each other. No nuclear pores were found in this area. The space between the outer membranes of the two nuclei was narrower than the space between the inner and outer membranes of the nuclear envelope, but no specific structures connecting nuclei were seen. During mitosis the diplokaryon did not change in general configuration and both nuclei remained in tight contact (Fig. 2). The mitotic chromosomes appeared as electron-dense patches of chromatin connected to the microtubules of each mitotic half-spindle. The centriolar plaques were embedded in the nuclear envelope (Fig. 2).

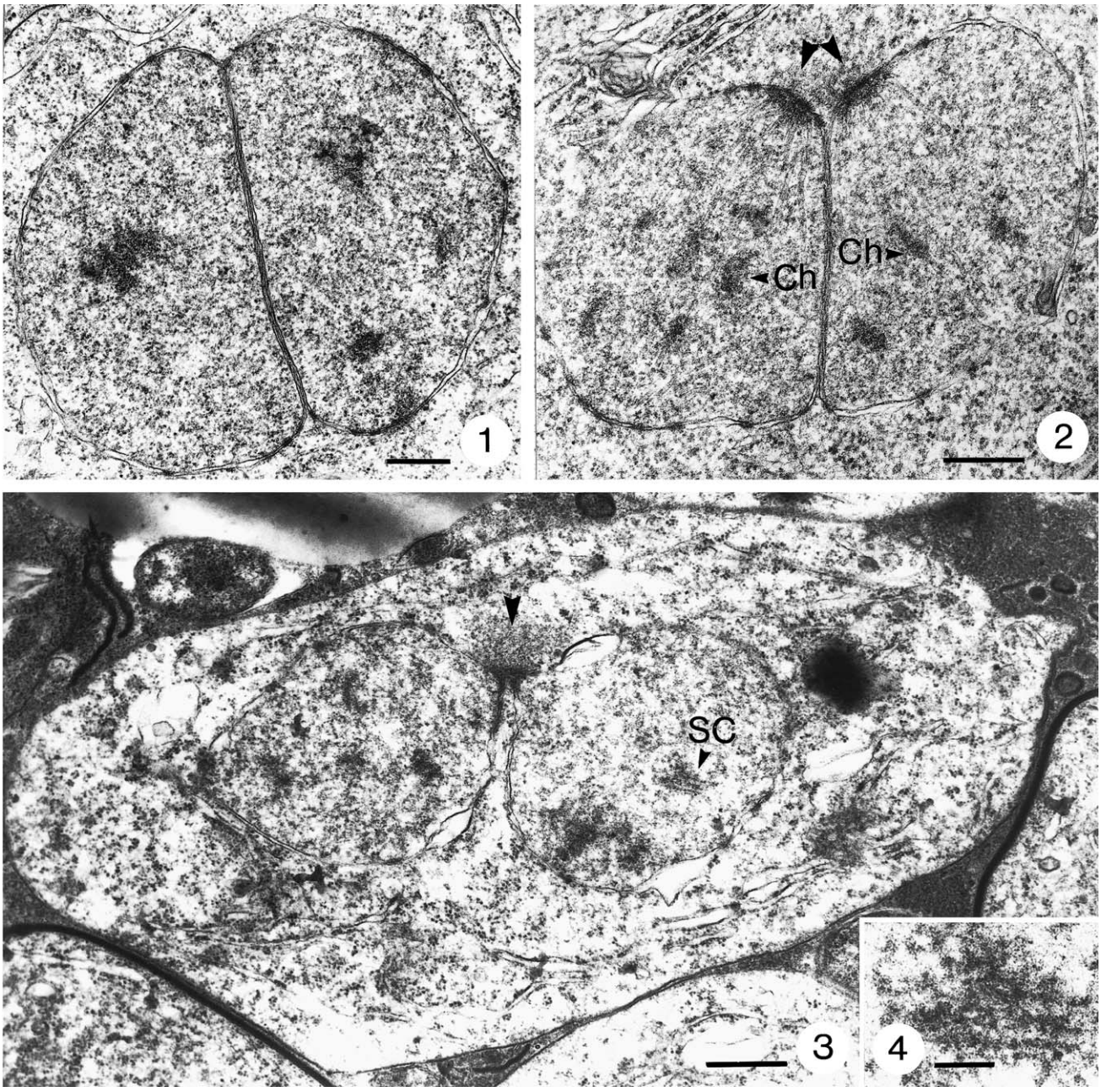
Elongate diplokaryotic meronts with peculiar nuclear configurations, never seen during the previous 10 years of investigations of *P. grylli*, were present in this material. In these meronts, the nuclei of diplokarya were loosely associated and connected to each other by electron-dense amorphous material in plaque-like structures (Fig. 3). In some sections two such structures were seen (Fig. 5). Some of this material was deposited on the inner surface of the nuclear envelope, while most of it formed a kind of cone, connecting the nuclei outside the nuclear envelope. The tip of the cone was oriented towards the site of closest contact between the two nuclei; fine filamentous material radiated from the base of this cone into the cytoplasm (Fig. 6). In contrast with the interphase and mitotic nuclei (Figs. 1, 2, 7, 10), the nuclear pores were distributed over the whole surface of these loosely associated nuclei (Fig. 8). In some sections the nuclear pores were symmetrically arranged in the zone of internuclear contact, giving the appearance of connections between the nuclei (Fig. 11).

In both nuclei of such diplokarya electron-dense filamentous structures, bar-like in longitudinal sections and as three dots in cross-section, were observed (Figs. 3–5, 8–9). Sometimes they appeared to be concentrated toward the plane of contact between the two nuclei (Fig. 9). These structures had the appearance typical of synaptonemal complexes. In the best photographs both central and lateral elements were clearly seen (Fig. 4). The total width of the synaptonemal complex was 95–100 nm.

Discussion

Synaptonemal complexes and meiotic configuration of the diplokaryon

The appearance and width of synaptonemal complexes in *P. grylli* are typical for microsporidia (Larsson 1986). Grassé (1970) suggested that detection of synaptonemal complexes could not on its own be an ultimate proof of meiosis. We must reserve the possibility that synaptonemal complexes may occur in cases of mitotic recombination. This phenomenon is known for a number of protists (reviewed by Seravin and Goodkov 1999). However, given that somatic pairing of the lengthy regions of chromosomes is a relatively rare event, while meiosis is known in many



Figs. 1–4. Fine structure of nuclei of *Paranosema grylli*. **1.** Interphase diplokaryon. **2.** Mitosis, centriolar plaques are arrowed. Ch, chromosome. **3.** Meiotic features in *P. grylli.* In this meront, whose slightly separated nuclei are connected by a plaque-like structure (arrowed), there are clearly visible synaptonemal complexes (SC). **4.** The synaptonemal complex shown in Fig. 3 at higher magnification. Scale bar is 500 nm in Figs. 1–3 and 100 nm in Fig. 4.

microsporidian species, we believe that the finding of synaptonemal complexes in *P. grylli* is evidence of meiosis in this organism. This is the first indication for the existence of a sexual phase in the life cycle of this monomorphic diplokaryotic microsporidium, which for a long time has been considered asexual.

A further indication in favour of meiosis in the nuclei of *P. grylli* is the characteristic arrangement of slightly separated but yet paired nuclei. This kind of nuclear separation is known to be indicative of the beginning of meiosis in various microsporidian species (Andreadis and Hall 1979; Hazard et al. 1979; Loubès 1979; Vivarès and Sprague 1979; Andreadis 1983; Hazard and Brookbank 1984). Such a morphology of the diplokaryon usually precedes the breaking down of the nuclear envelope and "arranging of chromosomes from the two nuclei so as to form a single nucleus" (Andreadis 1983) prior to the first meiotic division.

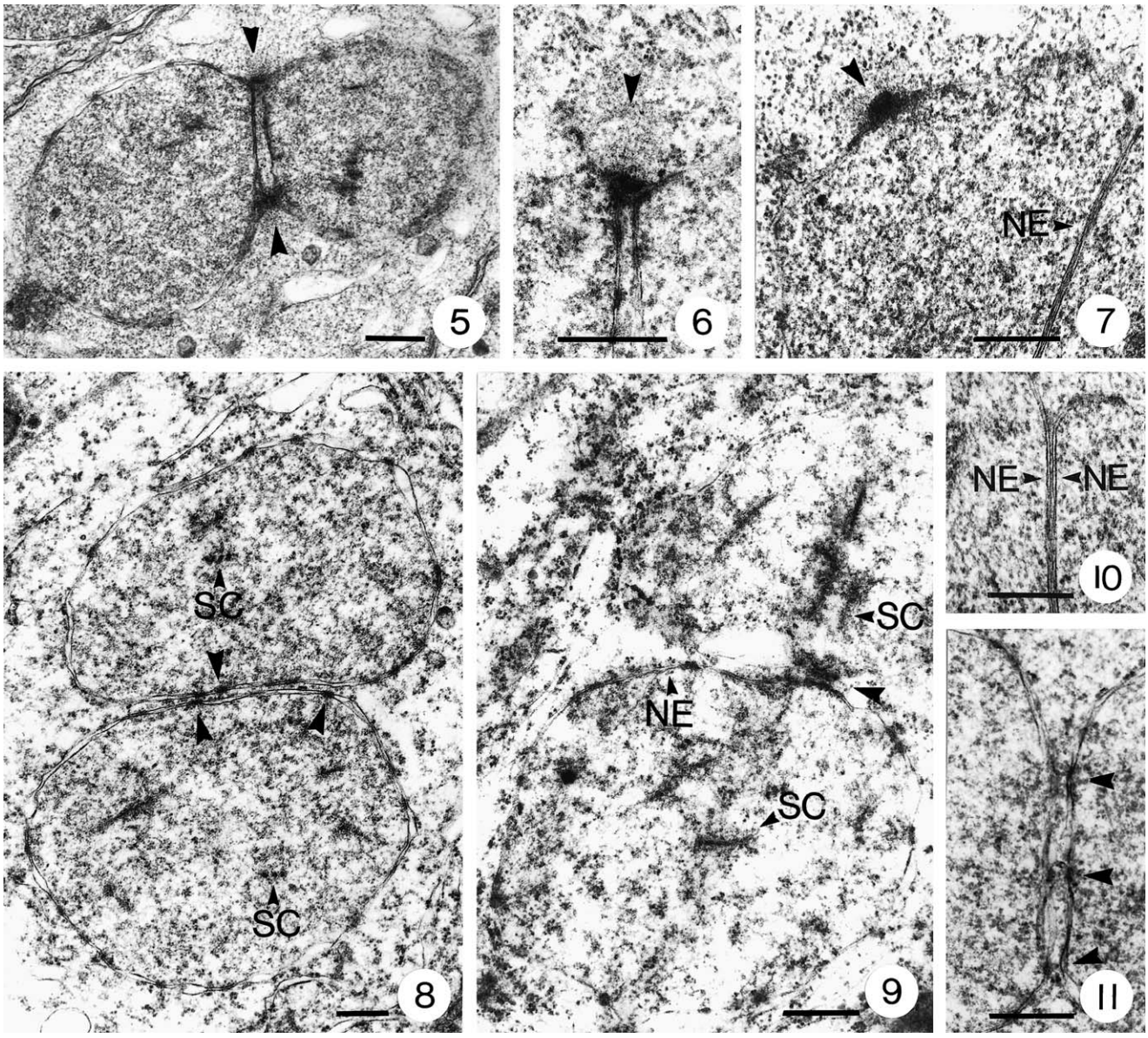


Fig. 5–11. Fine structure of nuclei of *P. grylli*. **5.** Meiosis in *P. grylli.* Two plaque-like structures connecting nuclei are visible. **6.** A plaque-like structure with radiating filamentous material (arrowed). **7.** Persisting centriolar plaque in the interphase nucleus; filaments radiating in the cytoplasm are arrowed. NE, nuclear envelopes at the site of contact between the nuclei of the diplokaryon. **8.** The meiotic diplokaryon. SC, synaptonemal complexes appearing as rows of three dots; nuclear pores at the region of contact between two nuclei are located asymmetrically (arrowed). **9.** Sagittal section of meiotic diplokaryon, synaptonemal complexes (SC) are concentrated close to the nuclear envelope at the region of contact (arrowed) between the nuclei. **10.** The interface of two nuclei of an interphase diplokaryon showing the absence of nuclear pores, NE—nuclear envelope. **11.** Zone of interface between two nuclei of meiotic diplokaryon with symmetrically arranged nuclear pores (arrowed). Scale bar is 500 nm in Figs. 5–9 and 250 nm in Figs. 10 and 11.

Taken together, the above considerations warrant the suggestion that we have observed meiosis in *P. grylli* similar to that reported in various polymorphic and monomorphic monokaryotic microsporidia.

The plaque-like structures connecting partially separated nuclei in *P. grylli* look identical to similar structures found by other authors (Loubès 1979; Sweeney et al. 1985; Toguebaye and Marchand 1985; Becnel et al. 1989; Andreadis and Vossbrinck 2002). Their origin and functions are unclear. Toguebaye and Marchand (1985) suggested that these are the special ''nuclear separation organelles''. Loubès (1979) considered them to derive from the centiolar plaques. Becnel et al. (1989) supposed that these are the shared centriolar plaques, which ''probably prevent complete separation of the nuclei'' at the early stage of meiosis preceding the

fusion of the diplokaryon. These authors demonstrated the "spindle fibers" (microtubules) radiating from such a plaque across each nucleus to the chromosomes at later stages of meiosis.

In *P. grylli*, the plaque-like structures connecting the nuclei are smaller than completely formed mitotic centriolar plaques and do not have as much granular material adjacent to the inner nuclear membrane (cf. Figs. 2 and 6). They somehow resemble the interphase centriolar plaques in size and appearance, as if to suggest that these structures are each composed of two centriolar plaques (Fig. 7). A characteristic halo of filamentous material radiating from these structures into the cytoplasm (Fig. 6) is further evidence that the connecting structures may be derived from centriolar plaques. The formation of the intranuclear spindle normally occurs at the end of meiotic prophase. Thus, it is reasonable to suggest that, at the early steps of prophase when the formation of synaptonemal complexes takes place and the microtubular spindle is yet to be formed, the centriolar plaques are not fully developed. The remarkable localization of these structures at the interface between the paired nuclei probably indicates that they participate in the positioning of paired nuclei at the onset of meiosis. However the question about the assembly of these structures and their evolution during meiosis remains to be solved.

Possible position of meiosis in the life cycle of *P. grylli*

Meiosis in microsporidia usually occurs at the time of conversion from merogony to sporogony, generally in the early sporont (Loubès, 1979; Hazard and Brookbank, 1984). However, the synaptonemal complexes in *P. grylli* were observed in cells which had no patches of electron-dense amorphous material outside the plasmalemma. Deposition of the electron-dense material (interpreted as the primordium of the exospore) on the cell surface is the first sign of transition to sporogony in *P. grylli*. Thus, the stages with synaptonemal complexes probably correspond to meronts and therefore the position of meiosis in the developmental sequence of *P. grylli* is rather unusual for microsporidia.

Examples which could be interpreted as later stages in meiosis in *P. grylli* have not been seen. In the same embedded material we have not observed any stages that could reliably be interpreted as uninucleate or having unpaired nuclei. However, Sokolova et al. (2003) found stages with two and four unpaired nuclei, which were attributed to a "second merogony", a transitional phase to sporogony. These stages could be intermediates in a meiotic sequence.

There are two possible explanations for the fact that, up to now, meiosis has not been found in this species. Only disporoblastic sporogony and diplokaryotic spores are known in *P. grylli*. Therefore, a post-meiotic stage of monokaryosis in the life cycle of this microsporidium can occur only before the beginning of sporogenesis and may be rather ephemeral. Early phases of development of *P. grylli* and its propagation in the host are still rather poorly studied, and the meiosis might have been overlooked. Another possible explanation may be the facultative occurrence of meiosis in *P. grylli*. Asexual reproduction with infrequent rounds of sex is characteristic for many protists (Dacks and Roger 1999), but has never been considered for microsporidia.

There are several examples of abortive meiotic sequences in polymorphous microsporidia, like *Culicosporella lunata* (Hazard et al. 1984) or *Edhazardia aedis* (Becnel et al. 1989). In discussing the consequences of meiosis in *P. grylli* we should also keep in mind the question whether this meiotic sequence is functional.

A specific diplokaryon arrangement accompanied by the formation of synaptonemal complexes has been described in only one other monomorphic diplokaryotic microsporidium—*Nosema rivulogammari* (Larsson 1983). In contrast to *P. grylli*, synaptonemal complexes in *N. rivulogammari* were found in diplokaryotic sporonts. Numerous uninucleate stages of sporogony were observed, but the further fate of these stages and the way they transformed to diplokaryotic sporoblasts remains obscure. It was unclear if "the nucleus of the uninucleate cell duplicated and the cell matured into a sporoblast" or if there was an intermediate quadrinucleate stage which gave rise to two diplokaryotic sporoblasts. Quadrinucleate sporogonial plasmodia were not seen in TEM sections, but there were stages which appeared to be quadrinucleate under the light microscope (Larsson 1983).

Biological significance of recombination for monomorphic diplokaryotic microsporidia

Despite the widespread opinion that diplokaryosis in monomorphic diplokaryotic microsporidia is permanent, there are several light-microscopic observations on the occurrence of uninucleate stages in the life cycle of such species. They were found in *Nosema bombycis* (Stempell 1909; Ohshima 1973), in *N. locustae* (Canning 1953), *N. acridophagus* (Henry 1967), *N. cuneatum* (Henry 1971) and *N. bombi* (McIvor and Malone 1995). Also, uninucleate stages and binucleate stages with unpaired nuclei were reported from TEM sections of *N. apis* (Youssef and Hammond 1971) and *N. rivulogammari* (Larsson 1983).

In early studies of *N. bombycis*, the occurrence of uninucleate forms was explained by autogamy (Stempell 1909; Ohshima 1973). Sprague et al. (1992) speculated that the diplokaryotic sporoplasms of *N. bombycis*

"undergo haplosis by nuclear dissociation" after the invasion into the host cell. The uninucleate products initiate a proliferative sequence that includes gametogony and ends with diplosis by union of gametes to form the first diplokaryotic cell in this round of the life cycle. This suggestion was based on the interpretation of data on uninucleate forms of *N. bombycis* (Stempell 1909; Ohshima 1973) and on analogy with *Nosema*-like development in some heterosporous microsporidia, e.g. *Culicospora magna* (Becnel et al. 1987). Larsson (1983) and the present study suggest the occurrence of meiosis in the development of at least two species of monomorphic diplokaryotic microsporidia. Further study will probably reveal evidence of meiosis in other microsporidia with *Nosema*-like cycles and warrant revision of views about their asexuality.

The biological significance of meiotic recombination for monomorphic diplokaryotic microsporidia is probably very high. The diplokaryon divides equally, so that each nucleus of a pair contributes one, and only one, daughter nucleus to each daughter diplokaryon (Vávra 1976b; Toguebaye and Marchand 1984). In the absence of recombination, mutations will accumulate independently in each nucleus of the diplokaryon. Thus a "diplokaryotic cell will evolve to comprise genetically divergent nuclei, which would be in effect two different clonal lineages within the same cell" (Cavalier-Smith 1995). Long-term diplokaryosis would thus lead to profound divergence. Kondrashov (1994) discussed the genetic consequences of asexuality in multigenomic unicellular eukaryotes and conceived that in such organisms, living apparently without a regular sexual process, selection against harmful mutations would be less effective than if there were a regular ploidy cycle with periodic reduction to a single genome per cell. Questions about possible mechanisms of recombination and maintenance of genetic stability in monomorphic diplokaryotic microsporidia still await answers.

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