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FLAGELLATES, BACTERIA, AND FUNGI ASSOCIATED WITH TERMITES: DIVERSITY AND FUNCTION IN NUTRITION – A REVIEW

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Abstract. Termite nutrition depends on a spectrum of symbiotic organisms. The members of the fungus-growing subfamily Macrotermitinae of the higher termites, i.e., the Termitidae, cultivate fungi of the genus *Termitomyces* in special gardens. These fungi are of a high nutritional value and their enzymes probably contribute to cellulose digestion, to varying degrees among different termite species. Lower termites digest cellulose with the help of symbiotic gut flagellates. After a partial breakdown by the termites' own enzymes, the ingested cellulose is engulfed by the flagellates and processed further. The flagellates liberate acetate as the major metabolic product for absorption by their termite hosts. Various bacteria also supply nutrients and warrant the maintenance of a special gut milieu. Most symbiotic flagellates and many of the bacteria cannot be cultivated, thus many aspects of their role in termite metabolism remain unknown. The phylogenetically ancient gut flagellates, i.e. trichomonads, hypermastigotes, and oxymonads, are characterized by typical cell organelles. They all lack mitochondria. Instead, trichomonads and hypermastigotes possess hydrogenosomes that deliver ATP and release acetate.
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TERMITES

Termites are classified in the order Isoptera of pterygote insects. Most of the approximately 2000 species are found in tropical and subtropical regions. There they are abundant, with local densities exceeding 6000 specimens per m^2 and biomasses (>50 g per m^2) not infrequently surpassing those of grazing mammalian herbivores (Lee & Wood 1971, Collins & Wood 1984). Termites are regarded as harmful insects because of their ability to destroy all materials containing cellulose. They have a great economic impact on wood used in and around buildings. Living plants, including agricultural products, are also destroyed by many species. In the United States, the annual costs for damage repair and control efforts probably exceed $750 million (Beal *et al.* 1989). In their natural habitats, however, termites are important decomposers. They reduce organic material to small pieces, thereby improving the nutrient contents of tropical soils, which are generally infertile due to nutrient poverty (Coventry *et al.* 1988, Park *et al.* 1994). They also improve soil aeration and drainage.

Termites are highly social insects. Hundreds to millions of individuals may coexist in one nest (Krishna & Weesner 1969/70, Grassé 1984). One colony consists of one or several pairs of reproductives and a large number of workers, soldiers, and larvae. The kings and queens of a termite colony reproduce, the workers forage and feed their nestmates, and the soldiers defend their home colony. Unlike the social Hymenoptera, termite societies contain individuals of both sexes, and they show a larval instead of an imaginal polymorphism (Noirot 1991). Moreover, lower termites can change their physical caste during development, i.e., a case of temporal polymorphism (Noirot 1991). Usually, termite nests contain countless subterranean ducts and chambers, and the spectacular hill-shaped colonies may rise to several meters. Wood-living termites inhabit living wood and rotten logs or stems.

Termites, cockroaches, and praying mantises represent a monophyletic group. Molecular phylogenetic data suggest a sister group relationship of the termites to a cockroach-mantid complex (order Dictyoptera), and the Blattaria is a sister group of the Mantoidea (Thorne & Carpenter 1992, Kambhampati 1995). Termites can be separated into seven families: the Mastotermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, Rhinotermitidae, Serritermitidae, and Termitidae. The first six families are collectively referred to as the "lower termites." Lower termites, however, do not form a monophyletic taxon but

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an ancestral grade composed of a series of families sharing several plesiomorphic characters (Noirot 1995). For cellulose digestion they all depend on intestinal flagellates. The Termitidae, referred to as the "higher termites," are the largest family comprising about three-quarters of termite species. Higher termites do not possess symbiotic protozoa in their gut, and display a more complex external and internal anatomy and social organization. Phylogenetic trees based on family-level relationships inferred from molecular data and from morphological characters differ in some aspects. Mastotermitidae seem to be the basal lineage. According to molecular data, a clade comprised of Termitidae (higher termites) and Rhinotermitidae is the sister group of the Kalotermitidae, and the Termopsidae are arranged at a more basal position than the Kalotermitidae (Kambhampati *et al.* 1996). These results were partly supported by Noirot's (1995) proposal, based on a comparative study of gut morphology, that the Termitidae, Rhinotermitidae, and Serritermitidae might be the sister group of Kalotermitidae. Morphological analyses by Krishna (1970), however, grouped the Kalotermitidae as a relatively basal group, implying a sister group relationship with the Mastotermitidae.

NUTRITION AND DIGESTION IN HIGHER TERMITES

Diets, and their digestion, differ among lower and higher termites. The Termitidae ingest a wide range of materials, for example leaves, roots, grass, dung, and soil (humus) (Wood & Johnson 1986). There are two groups within the Termitidae, fungus-cultivating and non-fungus-cultivating species. Non-fungus-cultivating higher termites probably digest their food, including cellulose, by enzymes that are produced by their own midgut and salivary glands (Slaytor 1992, Breznak & Brune 1994). Thus, intestinal bacteria do not seem to play a major role in their cellulose digestion (Breznak & Brune 1994). Cellulolytic bacteria could indeed be isolated from gut contents, but their cellulolytic activity and growth rate on cellulose was very low and insignificant (Breznak & Brune 1994). Cellulolytic genera are, e.g., *Cellulomonas* and *Bacillus* (König & Breunig 1997).

Termites of the fungus-cultivating subfamily Macrotermitinae create large fungal gardens in their nests. These gardens are constructed by assembling partially digested plant material that is permeated and further digested by the fungal mycelium (Wood & Thomas 1989). For example, plant polysaccharides and lignin are partially digested within the comb (Rohrmann & Rossmann 1980, Veivers *et al.* 1991). The garden fungi mostly belong to the genus *Termitomyces* and are exclusively found in termite nests. They are maintained and distributed by the termites, who for their part depend on the high nutritional value of the fungi. Termite workers eat the fungus comb, which includes *Termitomyces* mycelium and roundish asexual spores (mycotetes) (Crosland *et al.* 1996). In addition to the direct nutritional value, the ingested fungi may deliver 'missing enzymes' essential for the 'completion of cellulose digestion' (Martin & Martin 1978; Martin 1987, 1991, 1992). This opinion, however, has become controversial (Slaytor 1992, Bignell *et al.* 1994, Crosland *et al.* 1996). The amount of enzymes delivered by fungi seems to depend on the termite species (Rouland *et al.* 1991). In one group of species the fungus supplies a relatively high enzymatic activity, while in others its contribution is very low when compared with the enzyme secretion of the termites' midgut epithelium. In *Macrotermes barneyi*, for example, most cellulolytic activity (exoglucanase, endoglucanase, and ß-glucosidase) is located in the midgut and not in the mycotetes of the fungus (Crosland *et al.* 1996). In another species of the same termite genus, *M. natalensis*, fungal enzymes seem to be necessary at least for the digestion of crystalline cellulose (Martin & Martin 1978; Martin 1987, 1992). According to Slaytor (1992), however, the experimental results are not entirely convincing. Enzymes for hemicellulose digestion, i.e., xylanases, may also be supplied by the fungus (Rouland *et al.* 1988). In any case, the nature and relative importance of ingested fungal enzymes varies from species to species.

NUTRITION AND DIGESTION IN LOWER TERMITES

Many species of lower termites feed almost exclusively on wood, although this food is hard to digest and poor in nutrients, particularly nitrogen. Therefore many termites prefer wood that has been attacked by fungi, which is easier to utilize and richer in protein due to the presence of fungal mycelia. The following paragraphs describe in some detail the mechanisms by which lower termites manage to digest lignocellulose and extract their dietary requirements from such food. The following presentation focuses on the symbiotic relationships of termites with the intesti-

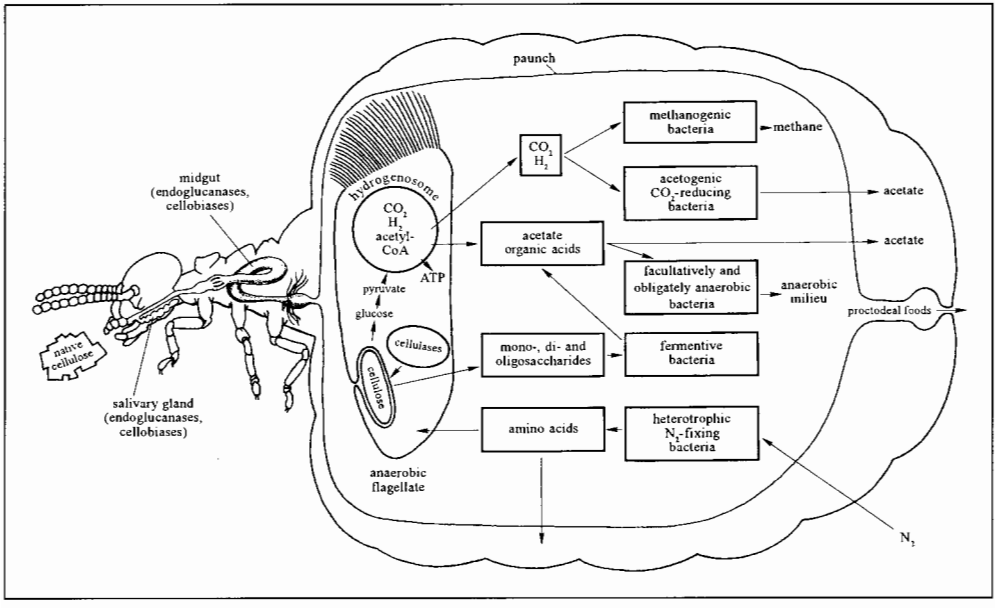


FIG. 1. Schematic view of the ttophic interactions in lower termites. Besides the digestion of cellulose by the termites' own enzymes and phagocytozing symbiotic flagellates, the metabolic roles of some groups of gut bacteria are sketched. The proportions of the termite and the sites of its organs are altered in favor of an enlarged presentation of the hindgut processes.

nal flagellates and bacteria contained in a large dilatation of their hindgut, the paunch (Fig. 1).

CONDITIONS OF LIFE IN THE TERMITE GUT ENVIRONMENT

The physico-chemical conditions in the rermite hindgut are influenced by the host as well as by the intestinal inhabitants. The pH-values in the mid- and hindgut of lower and higher termires range typically around neutral, i.e., at pH = 6 to 7.5 (O'Brien & Slaytor 1982). Soil-feeding termites (Termitidae) may have an extremely alkaline hindgut milieu (Bignell & Anderson 1980, Brune & Kühl 1996). While conditions in the midgut are aerobic, oxygen concentrations are low in the hindgut. Oxygen conditions were examined by feeding the termites redox dyes, and observing their color transformation in the gut (Veivers *et al.* 1980), or by physiological measurements on intestinal homogenares (Bignell & Anderson 1980). Redox potentials ranging from –230 to –270 mV were registered, i.e., practically anaerobic conditions were revealed by this method (Breznak 1983). Recently, more precise measurements were obtained by employing microelectrodes (Brune 1998). The rather simplistic concept of the termite hindgut as a purely anoxic fermentor therefore has to be revised. While the central portion was proven to be strictly anoxic, the outer zone of the hindgut was shown to contain oxygen due to diffusion from outside. A steep oxygen gradient is maintained by the respiratory activity of facultatively and obligately aerobic members of the gut microorganisms (Brune 1998). An anoxic milieu in the center of the paunch is essential for the obligately anaerobic symbiotic protozoa of the lower termites (Cleveland 1925a, Hungate 1939). Furthermore, there is a radial hydrogen gradient in the hindgut. The highest concentration is found in the center, and methanogens act as a major hydrogen sink in the gut periphery (Brune 1998). The spatial arrangement of metabolic groups of microorganisms and protozoa in the termite gut is thus governed by different gradients of substances which themselves depend on the activity of the symbionts.

THE ROLE OF BACTERIA

Although most gut bacteria are not directly involved in cellulose degradation in termites, they are essential for the survival of their hosts (Slaytor 1992). This is demonstrated, for example, by antibiotic defaunation which reduces the termite's life expectancy to a couple of days only, and has a similar devastating effect as starvation (Eutik *et al.* 1978). Some of the different roles of the gut bacteria have been identified (Fig. 1). Bacteria may, for example, protect the gut from invasion by foreign bacteria (Yoshimura 1995). Anaerobic and facultatively anaerobic bacteria consume a portion of the acetate, and of the other organic acids, that have been released by the gut flagellates. Thus they compete with the termite tissues that also use these substances. However, the anaerobic bacteria use the oxygen diffusing into the gut for oxidation processes maintaining the anoxic status of the internal gut regions (Veivers *et al.* 1982). A negative redox potential resulting from this activity is a prerequisite for the survival of the obligately anaerobic symbiotic protozoa. Fermenting bacteria, e.g., of the genera *Streptococcus*, *Bacteroides*, *Fusobacterium*, and *Lactobacillus*, profit by the low amount of soluble mono-, di-, and oligosaccharides liberated by the flagellates (Breznak 1983). Their metabolic end products, acetate and other organic acids, can be transported across the gut wall. Other microbes remove the H_2 and CO_2 that is released from the hydrogenosomes of the protozoa. So, acetogenic, CO_2-reducing bacteria produce acetate for use by the termites (Breznak *et al.* 1988). Species so far identified include *Acetonema longum* (Kane & Breznak 1991) and *Enterococcus* sp. (Tholen *et al.* 1997). Methanogenic bacteria (e.g., *Methanobrevibacter*-like species) primarily use CO_2 and the methyl group of acetate as electron acceptors (Odelson and Breznak 1985a, Breznak & Brune 1994). Acetogenesis outcompetes methanogenesis as the major electron (H_2) sink in the hindgut fermentation in almost all lower termites, and in wood-feeding higher termites (Breznak & Switzer 1986). Since nitrogen compounds are scarce in wood, the ability of nitrogen-fixing bacteria (e.g., *Enterobacter*, *Rhizobium*, and *Desulfovibrio*) is also important for the symbiotic community (Lovelock *et al.* 1985, König & Breunig 1997). These bacteria produce amino acids that are partly liberated and thus may be used by termites and flagellates. Other bacteria recycle nitrogen from uric acid. The metabolic role of the large spirochetes (Figs. 2, 3) is completely unknown. They belong to the family Pillotaceae (Balows *et al.* 1991) that is only found in termites and the cockroach *Cryptocercus*, and have a relatively complex ulttastructure (To *et al.* 1978) (Fig. 3). Some function as motility symbionts since they propel the flagellate cells to which they are attached, e.g., *Mixotricha paradoxa* (Cleveland & Grimstone 1964). The gut bacteria are phylogenetically diverse (Ohkuma & Kudo 1996). The taxonomic affiliation of many of them has yet to be determined neither are their functions for the gut ecosystem completely understood. This is also true for the bacteria which ate epi- or endobiotically associated with numerous species of gut flagellates (Ball 1969, Radek *et al.* 1992).

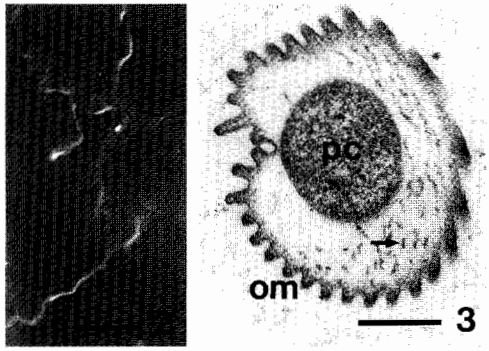
SYMBIOTIC RELATIONSHIPS OF TERMITES AND FLAGELLATES

The pseudergates, i.e. older latvae that are workers in the lower termites, feed on wood with their mandibles. The lignocellulose particles are then ground by the cuticular spines of the proventriculus. Some hydrolyzing enzymes, including cellulolytic ones (endoglucanases and cellobiases), from the salivary glands and the midgut begin to digest the food in fore- and midgut. The chief site of cellulose digestion, however, is the large dilatation of the hindgut, the paunch. Here, the extremely numerous symbiotic flagellates ingest and digest small wood particles, and excrete metabolites for use by the termites. Protozoan symbionts constitute one-third to one-seventh of the total body weight of, e.g., the nymphs of the termite *Zootermopsis* (Hungate 1939).

The association of termites and flagellates is not only advantageous for the termites, but also for the unicellular partners (Radek & Hausmann 1991). The flagellates are supplied with food by their host, protected from enemies, and receive shelter in a constant environment. They are protected from desiccation, and changes in temperature are avoided by their hosts moving actively to suitable sites. However, life in an intestine also holds dangers, for example being expelled from the host through the anus. When the paunch is filled to its capacity its contents are pushed into adjacent gut regions. An enteric valve prevents an overflow into the midgut. The termites may also actively empty part of their paunch content in order to supply, via proctodeal feeding, young larvae, soldiers, reproductives, or nest-mates that have molted (Honigberg 1970). Since the hindgut has an ecto-

dermal origin, the paunch is equipped with an intima. During molt the contents of the paunch are more or less wrapped by the old intima and get lost. The molted termites have to be refaunated by proctodeal feeding, or possibly by ingestion of the shed cuticula. Even though many of the ingested flagellates are damaged or digested during passage through the gut, sufficient numbers can reach the paunch to multiply again.

There is no convincing evidence for the formation of true cysts in termite flagellates (Imms 1919, Grassé & Noirot 1945, Honigberg 1963a). This does not seem to be necessary since they are transmitted to their hosts by proctodeal feeding. Morphological changes, however, occur during the molt of their hosts. The molting hormone, ecdysone, seems to be responsible for inducing the sexual cycles in some flagellates, e.g., in *Mixotricha paradoxa* (Cleveland 1966a), *Deltotrichonympha* (Cleveland 1966b), and *Koruga* (Cleveland 1966c), which are symbionts of the primitive termite *Mastotermes darwiniensis*. Hormone-induced sexuality was also reported from several other genera of protozoa living in termites (Cleveland 1965a, b), and in flagellates from the cockroach *Cryptocercus* (Cleveland 1953). Howevet, parts of the life-cycles described are uncertain, since individuals of different flagellate species seem to be put together in a common cycle. Honigberg (1970) suggested that the capacity for sexual reproduction was present in ancestral flagellates and may have been lost by many of the protozoa during the process of evolution. The method of transmission of the flagellates by termites would render sexuality rather superfluous.

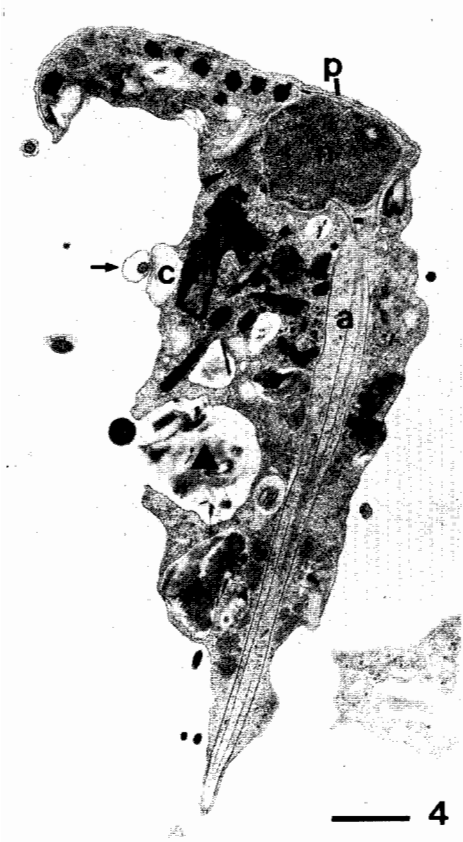


FIGS. 2, 3. Large spirochetes of the family Pillotaceae in the gut of the lower termite *Kalotermes flavicollis*. FIG. 2. Several spirochetes. Differential interfetence contrast. Bat = 10 mm. FIG. 3. Ultra-thin cross-section of *Pillotina* sp. depicting the protoplasmic cylinder (pc), axial fibrils corresponding to flagella (artow), and a wavy outer membrane (om). Bat = 0.5 µm.

THE ROLE OF FLAGELLATES IN THE DIGESTION OF WOOD

Lowet termites are incapable of digesting plant tissues containing lignocellulose without the help of symbiotic gut flagellates. They die within two weeks despite continued feeding if the protozoa are killed (Honigberg 1970). Defaunation can be achieved, fot example, by increasing temperature (Cleveland 1924) or oxygen pressure (Cleveland 1925a, b; 1928), by starvation of the termites (Cleveland 1925a, Yoshimura 1995), or ultraviolet irradiation (Inoue *et al.* 1997). It has been suggested that the associated epi- or endobiotic bacteria of the flagellates might be involved in enzyme production. However, experiments with bacteria-freed, axenically cultivated flagellates, e.g., *Trichonympha sphaerica* (Yamin 1981) and *Trichomitopsis termopsidis* (Yamin 1978, Yamin & Trager 1979, Odelson & Breznak 1985a), proved the capacity of these species at least to decompose cellulose without bactetial aid.

The hindgut flagellates ingest their food particles by phagocytosis (Figs. 1, 4). Thete are no special feeding apparatuses or cytostomes. Ingestion can take place throughout the plasma membrane except the flagellated body surfaces. When the body is covered with ectobiotic bacteria these are also engulfed and remain attached to the membrane of the digestion vacuole for some time (Radek *et al.* 1992). Bacteria living in the gut fluid and occasionally small flagellates are also phagocytosed. Dissolved substances may be incorporated by pinocytosis (Hollande & Valentin 1969). Each flagellate species of a certain termite host has its specified role in digestion (Yoshimura *et al.* 1993, 1994; Yoshimura 1995). For example, the largest protozoon in the termite *Coptotermes formosanus*, *Pseudotrichonympha grassi*, is involved in the decomposition of highly polymerized cellulose, while the other two species, *Holomastigotes hartmannii* and *Spirotrichonympha leidyi*, can only use low molecular weight cellulose (Yoshimura 1995). Many small flagellate species do not ingest particles and may play a role in later stages of cellulose metabolism such as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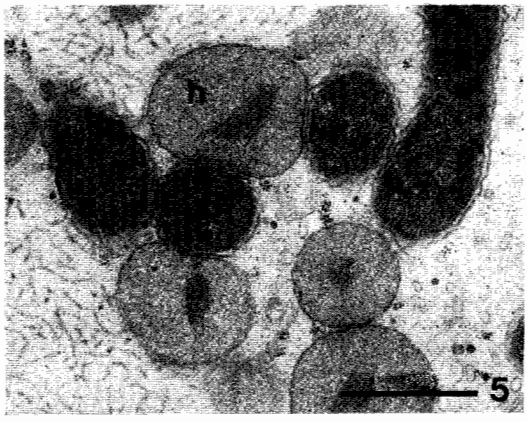
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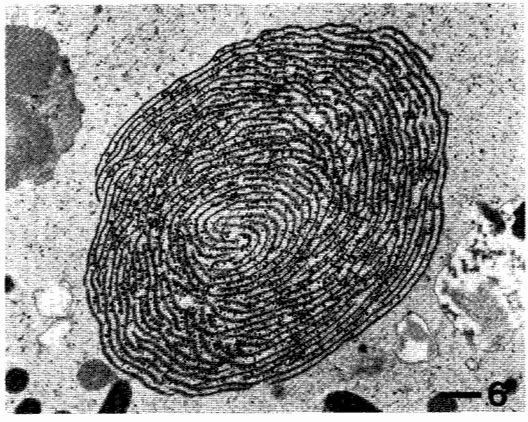
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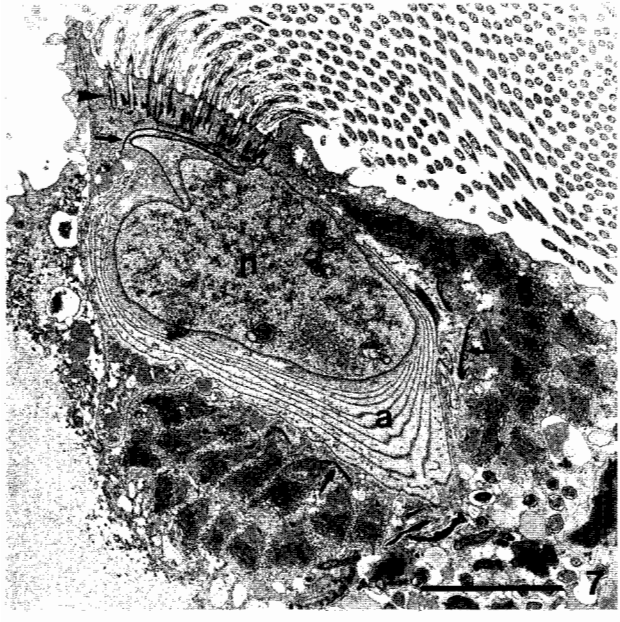
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methanogenesis (Yoshimura *et al.* 1996). The specific function of each protozoan species seems to be correlated with distribution in the gut, showing specific niches for the respective flagellates (Yoshimura 1995).

The lignocellulosic food of the termites consists of the major fractions cellulose (28–50%), hemicelluloses (20–30%), and lignin (18–30%) (Breznak & Brune 1994). Since covalent lignin-carbohydrate linkages impede the enzymatic degradation of lignocellulose, a high lignin content renders material hard to digest (Jeffries 1990). The published results on lignin degradation are somewhat ambiguous (see Breznak & Brune 1994, Varma *et al.* 1994). The limited information obtained so far indicates that lignin is not totally inert during its passage rhrough the termite gut. The breakdown of the aromatic ring system requires oxygen. Microorganisms near the aerated paunch epithelium may start the breakdown of lignin and continue digesrion outside the gut (Varma *et al.* 1994). Therefore repeated recycling of feces may increase the efficiency of lignin digestion.

Hemicellulose is digested to a considerable degree (Mishra 1979), but our understanding of hemicellulose digestion in termites is still meager (Breznak & Brune 1994). Hemicellulose is a heterogeneous group of polysaccharides composed chiefly of D-xylose, D-mannose, L-arabinose, or D-galactose, or of combinations of these. Xylanase activity by protozoa has been demonstrated in the hindgut of lower termites (Odelson & Breznak 1985b, Inoue *et al.* 1997).

Considerable research attention has been paid to the digestion of cellulose, the most important component for termite nutrition. In general, enzymatic hydrolysis of cellulose to glucose occurs through the action of cellulase, a general term referring to a mixture of enzymes consisting of three major classes: endoglucanases, exoglucanases, and cellobiases (Breznak & Brune 1994). Endoglucanases (endo-1,4-ß-glucanases) cleave internal glycosidic bonds along the polyglucan chain. They are active against amorphous cellulose and water-soluble derivatives (e.g., carboxymethylcellulose), and release glucose, cellobiose, cellotriose, and other higher oligomers. However, they are less active against crystalline cellulose. Exoglucanases (cellobiohydrolase and exoglucohydrolase) attack the terminus of a polyglucan chain including highly crystalline cellulose, liberating cellobiose or glucose from the non-reducing end. Cellobiases (ß-1,4-glucosidases) hydrolyze cellobiose and water-soluble cellodextrins ro glucose.

Lower termites are able to synthesize several cellulase components. Thus the cellulose and hemicellulose components of the wood particles ingested by the flagellates are already partially attacked (reviewed by Breznak & Brune 1994). The enzymes, for example endoglucanases, cellobiases and other carbohydrate hydrolyzing enzymes, are produced in the salivary glands and the midgut. However, the flagellates are the major agents of wood cellulose hydrolysis in lower termites (Honigberg 1970; Breznak 1982, 1983; O'Brien & Slaytor 1982; Odelson & Breznak 1985a). They play an important role in delivering further enzymes, including those which convert microcrystalline cellulose to glucose (Yamin & Trager 1979, Odelson & Breznak 1985b). With this supply, most of the cellulose can be hydrolyzed. Recently, Itakura *et al.* (1997) found all three types of cellulases that are essential for the hydrolyzation of natural cellulose in the salivary glands, foregut, and midgut of the lower termite *Coptotermes formosanus*. Thus part of the cellulose degradation into glucose can clearly be performed by the termites' own enzy-

FIGS. 4–8. Ultrastructural features of trichomonads and hypermastigotes. FIG. 4. Longitudinal section through the trichomonad *Foaina* sp. Besides the internal structures such as axostyle (a), cresta (c) with attached recurrent flagellum (arrow), nucleus (n), parabasal apparatus (arrowhead), and pelta (p), the ingestion of cellulose (large triangle) and already engulfed cellulose particles (small triangles) are depicted. Bar = 10 µm. FIG. 5. Hydrogenosomes (h) with crystalline structures, and cytoplasmic bacteria (b). Bar = 1 µm. FIG. 6. Cross-section of the axostyle of the hypermastigote *Joenia annectens*. The spirally wound microtubular plates enclose glycogen particles. Bar = 1 µm. FIG. 7. Oblique section through the anterior cell pole of *J. annectens*. The nucleus (n) lies just below the basal bodies (arrowhead) of the flagellar tuft and is enveloped by the microtubular plates of the axostyle (a). Parabasal filaments (arrows) run along the dictyosomes (d) and thus fix them in a particular position. Bar = 5 µm. FIG. 8. Dictyosomes of *J. annectens* with parabasal filaments in cross-section (arrows). Longitudinally sectioned filaments (arrowhead) reveal periodical striations. Bar = 1 µm.

mes. However, the highest level of activity, especially that of exo-cellobiohydrolase (87%), is found in the hindgut due to the protozoan fauna (Yoshimura 1995).

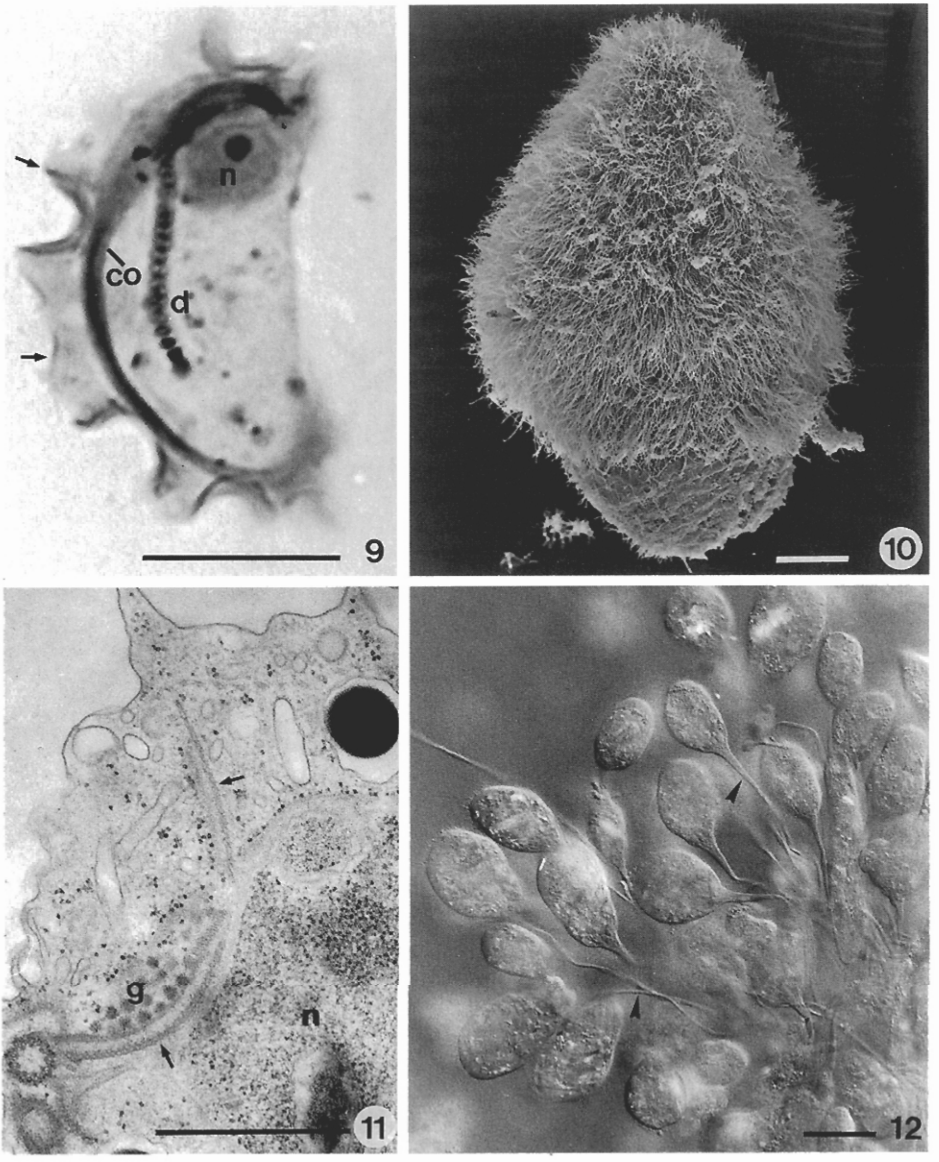
Microscopic investigations of symbiotic flagellates showed a gradual decomposition of ingested wood and cellulose within the food vacuoles, independent of bacterial activity (Yoshimura *et al.* 1994, 1996). Indigestible residues, probably lignin, are released from the cells as a loose fibrous material. The water-soluble nutrients, such as glucose and small sugar oligomers, are liberated into the cytoplasm. Glucose is stored in the protozoa as glycogen. Only a low percentage of the glucose reaches the fluid of the hindgut. Most of it is glycolytically transformed to pyruvate, and utilized for energy production by special organelles, the hydrogenosomes (Lindmark *et al.* 1989, Müller 1993). Hydrogenosomes often substitute for mitochondria in unicellular organisms that live in anoxic environments, such as rumen ciliates, chytridiomycete fungi, and termite flagellates (Müller 1993). In trichomonadid and hypermastigote termite flagellates these organelles are spherical bodies measuring 0.5–1 mm in diameter (Kulda *et al.* 1986). They are bounded by two closely adjacent membranes and are filled with a granular matrix and dense amorphous or paracrystalline inclusions (Fig. 5). The pyruvate:ferredoxin oxidoreductase and dehydrogenase are enzymes typical of hydrogenosomes (Müller 1993). Hydrogenosomes produce ATP by an anaerobic substrate-level phosphorylation reaction, releasing acetate, CO_2, and H_2 (Brul & Stumm 1994). Acetate is subsequently absorbed by the wall of the paunch and used as the major oxidizable energy source of the termites, as well as constituting an important biosynthetic precursor (Odelson & Breznak 1985b). Acetate is indeed the major volatile fatty acid in the hindgut fluid of lower termites, accounting for 94–99 mole% (Odelson & Breznak 1983). Small amounts of other volatile fatty acids, e.g., propionate and butyrate, are also present.

SYMBIOTIC FLAGELLATES: TAXONOMICAL AND MORPHOLOGICAL CHARACTERISTICS

The symbiotic flagellates of lower termites belong to several phylogenetically ancient protist taxa. Their classification, however, differs from author to author. According to Lee *et al.* (1985), termite flagellates are found in three orders: Trichomonadida, Hypermastigida, and Oxymonadida, within the class Zoomastigophorea. In more recent publications they receive a higher taxonomic rank. Hülsmann & Hausmann (1994) combined them in the phylum Axostylata, taking into account the universal presence of the axostyle, a microtubular rod. Trichomonadida and Hypermastigida were united in the phyla Parabasala and Trichozoa by Corliss (1994) and Cavalier-Smith (1996/97) respectively. The oxymonads are classified in the phylum Metamonada of the kingdom Archaezoa and receive the rank of a class, Oxymonadea, by Corliss (1994), and the rank of a subphylum, Axostylaria, with the single class Oxymonadea by Cavalier-Smith (1995).

The flagellate species are typical of the respective termite host species (Yamin 1979). Isolated populations of host termites may, however, contain slightly different flagellate communities, for example if the pair of reproductives that had founded the nest contained a restricted spectrum of symbionts. Occasionally, the same flagellate species are found in different hosts. A termite species may contain only a few different symbionts or more than 20 species of flagellates, e.g., *Coptotermes heimi* or *Reticulitermes lucifugus* (Yamin 1979). Approximately 80 different genera of gut flagellates comprising nearly 500 distinct species have been described from termites (Yamin 1979). Probably many further species exist, since only about 200 of the 400 known species of lower termites have been examined to date.

A specific organelle of termite flagellates is a rod composed of microtubules, the axostyle. It extends from the anterior cell pole adjacent to the basal bodies of the flagella back to the posterior cell pole. Its length varies according to the species. The microtubules are arranged in more or less spirally wound plates which form a rod (Fig. 6). The bridges that connect the microtubules within and between adjacent plates have motor functions in some oxymonads, e.g., *Notila*, *Oxymonas*, and *Saccinobaculus*, thus conferring motility to the respective axostyles (Grimstone & Cleveland 1965, McIntosh 1973, Mooseker & Tilney 1973). In the anterior body portion the axostyles often enclose the nuclei and form extensions (called pelta) that support anterior plasma membrane regions (Fig. 4). The Golgi apparatus (= parabasal apparatus) of trichomonads and hypermastigotes is a special, very complex organelle. The membranous stacks of the dictyosomes are composed of numerous



FIGS. 9–12. FIG. 9. Silver staining with protargol of the trichomonad *Trichomitopsis termopsidis* from *Zootermopsis angusticollis*. The recurrent flagellum forms an undulating membrane (arrows) with the body surface. A rib-like costa (co) subtends the undulating membrane. The nucleus (n) with one nucleolus and the sole dictyosome (d) are also stained. Bar = 10 µm. FIG. 10. The surface of the hypermastigote *Koruga bonita* from *Mastotermes darwiniensis* is densely covered with rows of flagella. Scanning electron micrograph. Bar = 20 µm. FIG. 11. The electron micrograph of the oxymonad *Monocercomonoides hausmanni* from *Kalotermes sinaicus* shows one pair of basal bodies connected to the paracrystalline preaxostyle (arrow). g = glycogen granules, n = nucleus. Bar = 1 µm. FIG. 12. Several individuals of the oxymonad *Microrhopalodina multinucleata* are attached to the gut wall of *Cryptotermes dudleyi* with a slender, anterior extension of the cell body, the rostellum (arrowheads). Bar = 20 µm.

cisternae, for example about 25 in *Joenia annectens*, and they are associated with striated fibers (Figs. 7, 8). These fibers take their name 'parabasal fibers' from their origin near the basal bodies. Oxymonads possess neither a Golgi apparatus nor parabasal fibers.

Most of the termite flagellates possess only one nucleus. In some groups of trichomonads (Calonymphidae) and oxymonads (e.g., *Microrhopalodina* and *Barroella*) however, parallel to a multiplication of flagella and axostyles, the nuclei are present in plurality. During mitosis the nuclear envelopes always remain intact (closed mitosis). In the oxymonads the division spindle lies intranuclearly, while the two half spindles make contact with the outside of the nuclear envelope in trichomonads and hypermastigotes. In the latter case the kinetochores of the chromosomes are integrated in the nuclear membranes. During cell division, axostyles, flagella, and parabasal apparatuses either disintegrate or distribute to the daughter individuals in a unique manner (Grassé 1952).

The typical mastigont systems of the symbionts contain the flagella with their basal bodies, and the associated organelles such as undulating membrane, costa or cresta, the parabasal apparatus, the axostyle, etc. (Brugerolle 1976/77, 1991; Lee *et al.* 1985). Trichomonads specifically possess four to six flagella per mastigont. Generally, one of them is directed towards the posterior cell pole. This recurrent flagellum may be attached to the cell body via an undulating membrane (Fam. Trichomonadidae; e.g., *Pseudotrichomonas*, *Trichomitopsis*, and *Tritrichomonas*) (Fig. 9). In this case, a rod with a periodical pattern, the costa, supports the cell body along the attachment site (Fig. 9). Otherwise, there is only a small, crescent cresta (Fam. Devescovinidae; e.g., *Devescovina* and *Foaina*). In the family Calonymphidae, numerous mastigont systems exist with or without associated nuclei (Kirby & Margulis 1994, Rösel *et al.* 1996). Hypermastigotes always possess abundant flagella but only one nucleus and one or no axostyle (Hollande & Valentin 1969, Lee *et al.* 1985). The numerous flagella may be arranged in different patterns, such as in tufts (e.g., *Joenia*), in vertical (e.g., *Trichonympha*) or horizontal rows (e.g., *Teratonympha*), or in spirals (e.g., *Spirotrichonympha*) (Fig. 10). Hypermastigotes and trichomonads share a common ancestor (Honigberg 1963b, Dacks & Redfield 1998, Ohkuma *et al.* 1998), so that the multiple flagella of the hypermastigotes probably arose by a multiplication of a trichomonad-like mastigont system.

Oxymonads have one or more mastigont systems and nuclei. Each mastigont system contains four flagella that are typically arranged in two pairs of two (Hollande & Carruette-Valentin 1970, Brugerolle 1981, Radek 1994). One of the four flagella is recurrent. The basal bodies of the two pairs are connected by a paracrystalline structure, the preaxostyle (Fig. 11). Many oxymonads may attach to the wall of the paunch via an apical cell protrusion, a rostellum, which is stiffened by microtubules of the axostyle(s) (Fig. 12) (Rother *et al.* 1999) . Oxymonads differ from the lineage of the parabasalids (trichomonads and hypermastigotes) in the flagellar apparatus ultrastructure and organization, mitotic apparatus, and attachment to the host. Furthermore, they have no mitochondria or hydrogenosomes, and a Golgi (parabasal) apparatus is missing.

FUTURE PERSPECTIVES

Many researchers have investigated the relationships of termites with their diverse pro- and eukaryotic intestinal and external symbiotic co-inhabitants. Nevertheless, various questions have been answered only partially, and various contradictory results await clarification. This situation is doubtless due to the multitude of termite species and the even higher species richness of the associated organisms. Hence, not only do a large number of organisms remain to be investigated, but divergent evolutionary pathways leading to different symbiotic systems and strategies can be expected. Most of the flagellates and many of the bacteria have never been grown in culture, thus severely restricting experimental investigations. Progress in molecular biology, notably the introduction of the polymerase chain reaction (PCR), now offers opportunities to characterize non-cultivatable species at the genetic level. The complex, still puzzling interactions of termites, flagellates, bacteria, and fungi will remain a fascinating field of research.

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