14 Termitomyces/Termite Interactions

Corinne Rouland-Lefèvre, Tetsushi Inoue, Toru Johjima

14.1 Introduction

The first observation of brain like formations inside termite nests were made by the German naturalist, J. D. König (1799), in the East Indies. These formations were several centimeters in diameter and were later given the name of fungus combs. A fungus was developing on these plant structures in the form of mycelium with small white nodules (mycotetes) which are characteristic of the group. More than half a century later, the English cryptogamist, G. Gardner (cited by Petch 1913), collected a large carpophore-bearing fungus of the Agaricales order from a termite nest in Ceylon, However, it was only in 1906 that Petch (1906) pointed out the relationship between the mycotetes and carpophore fungal formations and showed that these large mushrooms were cultivated by the termites inside their nests. Heim (1941) created a new genus for these fungi, *Termitomyces,* which contains all the "termitophilic Agaricales".

Termitomyces have since been studied by entomologists seeking to clarify the relationship between termites and fungi. Grassé (1959) was the first to show that *Termitomyces* were associated with only a single subfamily of termites, the Macrotermitinae, and that this is a real symbiosis as neither of the two symbionts can exist without the other. Other more recent works have shown the details of this symbiosis: on the one hand, because termite nests are both temperature and humidity regulated (Matsumoto 1977; Johnson 1981; Thomas 1987a), they provide ideal conditions for the growth of these *Termitomyces* and, on the other hand, the fungus helps the termites to degrade the plant matter (e.g. wood, dry grass, leaf litter) on which they live. It grows on a special structure in the nest, the fungus comb, which is actively maintained by the termites, by continuously adding plant matter and consuming the comb.

Corinne Rouland-Lefèvre, Tetsushi Inoue: Université Paris 12-Val de Marne, UFR des Sciences et Technologie, 61, avenue du Général de Gaulle, 94010 Creteil Cedex, France, E-mail: corinne.rouland@bondy.ird.fr

Toru Johjima: International Cooperative Research Project, Japan Science and Technology Agency, Kasetsart University Research and Development Institute, 10900 Bangkok, Thailand

Soil Biology, Volume 6
Intestinal Microorganisms of Soil Invertebrates
H. König, A. Varma (Eds.)

The precise nature of the relationship between termites and the associated fungus, as well as its specificity, have provoked considerable controversy which has been largely resolved by new biochemical and molecular biology techniques. In this chapter, we shall present the results obtained using molecular biology to study termite/*Termitomyces* co-evolution. We shall then look at recent scientific advances regarding the contribution of the termite and its symbiotic fungus to the degradation of major plant components (cellulose, hemicellulose, lignin).

14.2 Phylogeny and Co-Evolution of Fungus-Growing Termites and *Termitomyces*

Symbiotic relationships have played an essential role in termite evolution, involving a range of symbionts including protists, methanogenic archaea and bacteria (Bignell 2000). Only one Termitidae subfamily, the Macrotermitinae, has evolved an external mutualistic symbiosis with fungi of the *Termitomyces* genus.

14.2.1 The Symbionts

Macrotermitinae or "Fungus-Growing" Termites. Macrotermitinae belong to the family of higher termites (Termitidae), which accounts for 70% of known species of termite. They are organized in complex societies with a number of well-defined casts and do not have symbiotic protists in their digestive tract. These characteristics are specific to the Termitidae family which encompasses 235 of the 281 currently recognized genera in the order Isoptera. Termitidae are divided into four subfamilies, one of which is the Macrotermitinae.

Macrotermitinae are only found on the African and Asian continents where they are the dominant group in tropical areas (Abe and Watanabe 1983; Wood and Thomas 1989). The Macrotermitinae have been divided into 11 taxonomically distinct genera and approximately 330 species (Khambhampati and Eggleton 2000). Most of the diversity occurs in Africa, where 10 of the 11 genera are found. Genera are also found in Asia and Madagascar, and one genus is exclusively Asian (Khambhampati and Eggleton 2000).

Termitomyces Fungi or "Termitophilic" Fungi. *Termitomyces* are Basidiomycotina fungi from the order Agaricales, the Tricholomatacea Roze family and

the Termitomyceteae (Jülich) Singer tribe. Approximately 40 *Termitomyces* species have been described. However, their taxonomy was based on the fruit structures (Heim1977) and, as some *Termitomyces* never fruit, their taxonomy is thus difficult to determine. However, since Heim, no other mycologist has been interested in the composition of the *Termitomyces* genus, even though it contains fungi of extremely diverse form, origin and biology. The common denominator of the *Termitomyces* fungi is that all species in the genus are cultivated by termites in their nests. Furthermore, all the fungi cultivated by Macrotermitinae termites belong to this genus.

Although additional varieties have been identified using molecular biology (Rouland et al. 2002; Aanen et al. 2002), the small number of fungal species compared with the number of Macrotermitinae species (40/330) suggests that many of these fungi are shared by different termite species.

14.2.2 Evolution of Fungus-Growing Termites and *Termitomyces*

There has recently been a resurgence in interest in phylogeny of *Termitomyces,* phylogeny of Macrotermitinae and co-evolutionary dynamics. The first study by Rouland et al. (2002) on 15 African *Termitomyces* species sequenced their internal transcriber spacer region (ITS1–5.8S-ITS2) and showed that *Termitomyces* was clearly a monophyletic group belonging to the family Tricholomataceae. The hypothesis that "each species of termite is associated with a separate species of fungal symbiont" (Heim 1942; Grassé 1982) seems unlikely since identical ITS sequences were obtained from fungi cultivated by three species of termites in different genera. On the other hand, Katoh et al. (2002) demonstrated, by sequencing ITS and 18S rDNA, that the symbiotic fungi cultivated by several colonies of the same termite species, *Odontotermes formosanus,* belonged to two different *Termitomyces* species. The work of Taprab et al. (2002) on the molecular phylogeny of symbiotic *Termitomyces* of several species of Asian Macrotermitinae also indicates that the relationships of symbiotic fungi with host termites and their locality were apparently complex.

The most comprehensive study on termite/*Termitomyces* evolution was conducted by Aanen et al. (2002) who created phylogenetic trees of the two symbionts in this mutualism across several genera and reconstructed both the origin and a number of key aspects of the co-evolutionary dynamics of the symbiosis. They studied 38 colonies of 32 species, covering 9 of the 11 genera of fungus-growing termites from three locations in Africa (Cameroon, Senegal and Gabon) and 3 in Asia (Sumatra, Kalimantan (Indonesia) and Sri Lanka), spanning most of the distribution of the Macrotermitinae. Maximum likelihood and Bayesian analyses of DNA

sequences were used to reconstruct the phylogenic trees of both symbionts. This study confirmed that the fungus-growing termites are monophyletic within the Isoptera order and the fungi cultivated by these fungus-growing termites are monophyletic within the Basidiomycotina order, as has been described in other papers (Rouland et al. 2002; Monçalvo et al. 2002). Moreover, these authors demonstrated that the symbiosis has a single African origin and that the secondary domestication of other fungi or reversion of mutualistic fungi to a free-living state has not occurred. According to the papers mentioned above (Taprab et al. 2002; Katoh et al. 2002), host switching has been frequent especially at lower taxonomic levels, and different nests of a particular termite species can have different symbionts. These observations are consistent with horizontal transmission of fungal symbionts. But, in spite of frequent host switching, there is a very high correlation between the termite and fungal phylogenic trees because the mutualistic interactions at higher taxonomic levels are highly specific. If horizontal transmission is the main transmission mode, the symbiont specificity at higher taxonomic levels is surprising. The question arises how this specificity has evolved. One possibility is that termites select their fungal symbionts indirectly (i.e. by supplying substrates suitable only for particular groups of fungi). If this is the case, we must consider that not all *Termitomyces* have the same capacity to degrade plant matter and therefore their importance in the digestive symbiosis with termites varies. This will be discussed in the following section.

14.3 The Role of Termitomyces in Mutualistic Symbiosis

14.3.1 Nature, Structure and Dynamics of the Fungus Comb

Fungus combs are sponge-like structures enclosed in specially constructed chambers inside a termite mound and the soil. The structure provides a large surface area, allowing air circulation and access by the termites. The fungus comb is the culture medium for *Termitomyces* as well as food for the termites and it is constructed with the undigested feces of termite workers (Josens 1991; Grassé 1978; Sieber and Leuthold 1981; Johjima et al. 2003). In most *Macrotermes*, the fecal material is deposited on the top rim of the fungus comb. There is an age gradient within the fungus comb, such that the upper part of the fungus comb is newer than the lower part. The termites eat the fungus comb from the lower rim (Josens 1971; Sieber and Leuthold 1981). The termites, therefore, eat the mature part of the fungus comb which creates a highly efficient method of digesting plant matter. The fungus

comb has a cycle time of approximately 40 days (Traniello and Leuthold 2000). An exception has been reported for *Macrotermes carbonarius* where gradient exists among individual combs (Hyodo et al. 1999). Other fungus comb structural forms have been observed in Macrotermitinae other than *Macrotermes*, and were described in detail in the review by Rouland (2000). Some species of fungus-growing termites have a food store in addition to the fungus comb. This comprises small particles cut from plant litter and looks like moist sawdust (Wood 1978; Darlington 1994). This food store is also ingested by the termites and then excreted on the fungus comb without obvious digestion. The function of this food store is not well understood. Lower concentrations of plant secondary metabolites have been detected in *Macrotermes gilvus* food stores than in the leaf litter around the mound (Johjima et al. 2003). The food store may well help in detoxification or improve the palatability of the litter.

Termitomyces have only been found in association with fungus-growing termites in the form of mycelium with aggregations of asexual spores which have been called various names such as mycotetes, synnemata, conidia, nodules. Mycotetes are also consumed by the termites. The sexual stage of the fungus, the basidiocarps with a long pseudorhiza extended from fungus comb (Singer 1986), is developed at specific times in the rainy seasons. However, as they last no more than few days, they are easily overlooked. *Termitomyces* is absent everywhere else in the nest structure including the food store and the internal walls of the nest. Outside the fungus comb it only occurs in the guts of workers (Thomas 1987a,b). Although the fungus comb seems to have a pure culture of *Termitomyces* when it is in the termite nest, other fungi such as *Xylaria* proliferate on the fungus comb within a few days if the comb is removed from the nest. It is not fully understood how the growth of contaminants is controlled in the termite nest. If there are still live termites on the comb after it has been removed from the nest, the growth of non-symbiotic fungi is suppressed for a few days (Grassé and Noirot 1958; Thomas 1987b). In a study of *Macrotermes bellicosus*, very little evidence of germination of fungal spores in the food store was observed despite the presence of various fungal spores (Thomas 1987b). The food store consists of comminuted food mixed with saliva. Furthermore, extracts of the food store, whole termites and the termite gut showed fungistatic activity, suggesting that termite secretions inhibit fungal spore germination and growth. Recently, antimicrobial peptides were identified in the fungus-growing termite *Pseudacanthotermes spiniger* (Lamberty et al. 2001). Termicin is present in hemocyte granules and salivary glands of the termite which has an activity against several fungi. However, the antimicrobial activity against *Termitomyces* and the typical contaminant fungus *Xylaria* has not been studied. The fungistatic activity of termite secretions, especially saliva, appears to be a major part of fungus comb

maintenance. Other possible factors that control fungal activities have also been discussed by Thomas (1987b).

14.3.2 Role of *Termitomyces* in the Digestive Metabolism of Termites

Sands (1956) showed that *Odontotermes badius* workers supplied with fresh fungus comb survived much longer than workers supplied with wood chips and filter paper containing starch or sugars. Workers supplied with fungus comb from which the mycotetes had been removed survived only slightly longer than starved workers. These observations indicate that fungus comb, especially mycotetes, is important for termite nutrition. Four roles in the symbiotic relationship with host termites have been proposed for *Temitomyces*: (1) enrichment in nitrogen (Matsumoto 1976; Collins 1983); (2) a source of metabolic water and heat (Lüscher 1951); (3) provision of glycosyl hydrolases which work synergistically with enzymes from the host termites (Martin and Martin 1978; Rouland et al. 1988b,c); (4) the degradation of lignin to improve cellulose digestibility (Grassé and Noirot 1958). Termites feed mainly on plant debris that is poor in nitrogen. The nitrogen content of the mycotetes was reported to be much higher (6–8%) than in the fungus comb (0.8–2.0%) and, therefore, the mycotetes are a nitrogen-rich food for the termites (Matsumoto 1976; Rohrmann 1978, Wood and Thomas 1989). The roles suggested in (1) and (2) will be more or less significant for all fungus-growing termites, while the roles suggested in (3) and (4) are controversial and would depend on the host as discussed below.

Provision of Polysaccharide-Degrading Enzymes. The role of *Termitomyces* in plant polysaccharide degradation was first clarified by determining the enzyme activities of the various parts of termite workers digestive tract as well as of the fungal nodules (Abo-Khatwa 1978; Martin and Martin 1979; Rouland et al. 1988a–d, 1991; Sengupta and Sengupta 1990). To define the respective role of each partner of the symbiosis, a multi-variance analysis of 19 enzymatic activities detected in 5 termites and fungal species was made. The first two axes of the PCA (Fig. 14.1a) accounted for 74% of the total variance (41% on axis 1, 33% on axis 2). Discriminant analysis carried out on 10,000 permutations showed that the PCA was highly significant ($P < 0.00001$). On axis 1, the correlation circle showed the separation of samples having polysaccharidase activity and those with oligosaccharidase activity. On axis 2, samples with β-xylosidase activity were separated from those with heterosidase activity. The projection of sample classes (termites and associated fungal species) on the first two PCA axes (Fig. 14.1b), on the one hand, clearly differentiate the low level of oligosaccharidasic and heterosidasic activity of the fungi from the higher level of activity of the

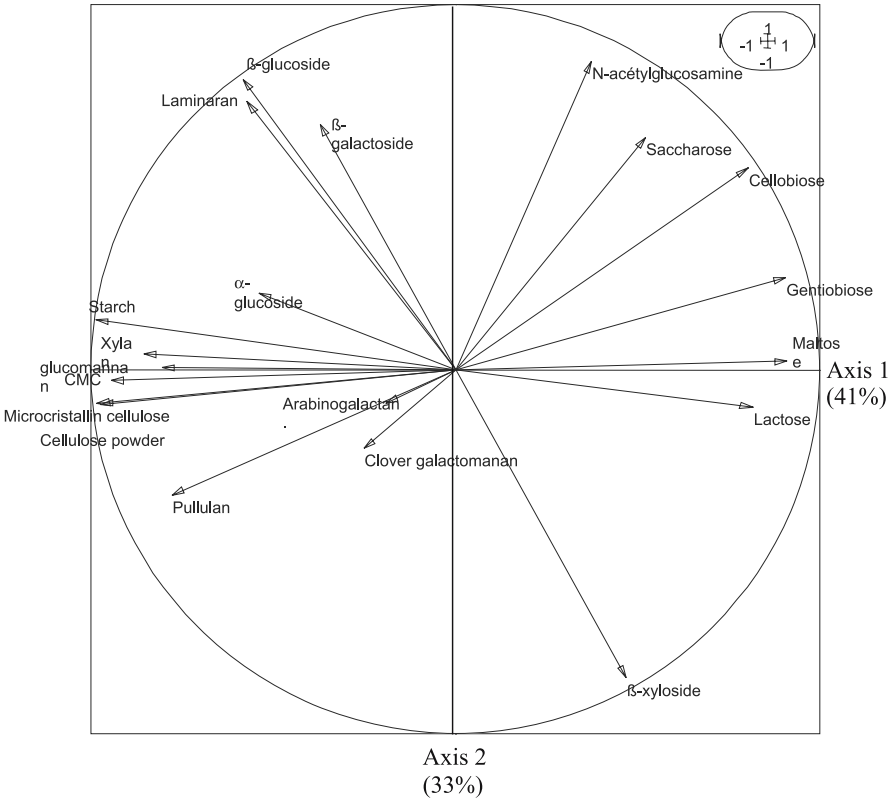

Fig. 14.1. Results of principal components analysis (PCA). **a** Correlation circle; Results of principal components analysis (PCA).

termites and, on the other hand, clearly differentiate the fungi/termite associations of the species *A. cavithorax* and *M. toumodiensis* from the other species of termites. The differentiation between these termite/fungus associations is principally due to a greater degradation of polysaccharides by *A. cavithorax* and *M. toumodiensis* workers than by their associated fungi whereas, in the other species studied, the two symbionts (termite/fungus) degrade polysaccharides to the same extent. Furthermore, for some associations, the substrate most degraded by the termites' digestive tract is also the substrate most degraded by the symbiotic fungus. In this case, the enzymes produced by the symbionts are not complementary unlike the enzymes produced by fungus-cultivating ants (D'Ettore et al. 2002). To explain this anomaly, several authors have proposed an acquired enzyme hypothesis (Martin 1991; Martin and Martin 1978, 1979; Kukor and Martin 1983). This hypothesis suggests that some fungi can produce polysaccharidases which act synergistically with the enzymes produced by the termites.

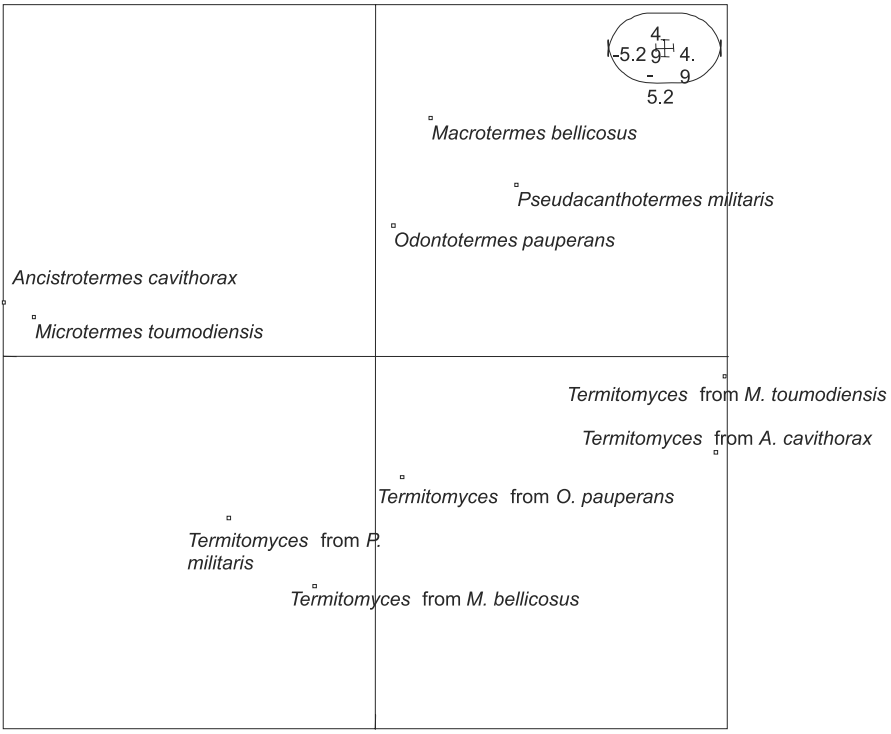

Fig. 14.1. (continued) **b** ordination of the samples in the plane defined by axes 1 (41%) and 2 (33%) of the PCA

Degradation of polysaccharides could, therefore, be achieved in the worker termite guts by the combined action of fungal and termite enzymes. Fungal cellulases and xylanases have been found in the digestive tract of two species of *Macrotermes* (Rouland et al. 1988a–c; Matoub and Rouland 1995), but without any equivalent recent work on other species, this hypothesis is still contested by some authors (see Rouland 2000).

For polysaccharide degradation, symbiotic fungi exhibit different enzyme activities. For example, *T. eurhizus*, associated with the termite *P. spiniger*, is highly amylolytic, whereas *Termitomyces*, associated with *Macrotermes bellicosus*, is both xylanolytic and cellulolytic (Rouland et al. 1991; Ikhouane 1995). The differences in enzyme activities could be due to different genetic patterns in the fungi or to different environmental conditions. In order to distinguish between these factors, the enzyme production of several species of *Termitomyces* cultivated under the same conditions has been examined (Table 14.1). It is, therefore, possible to distinguish two groups of *Termitomyces*: in the first group (*Termitomyces*. sp., *T. eurhizus* and *T. medius*), the fungi can produce enzymes which vary ac-

Table 14.1. Production of several osidasic enzymes on fungus comb extracts from *Ancistrotermes guineensis*, *Macrotermes bellicosus*, *Microtermes subhyalinus* and *Pseudacanthotermes spiniger*

	Cellobiase	CMCase	Maltase	Amylase	β−Xylosidase	Xylanase
T. eurhizus from *P. spiniger*						
A. guineensis comb	12 ± 3	84 ± 8	12 ± 2.5	18 ± 4	24 ± 3	112 ± 5
M. bellicosus comb	0	270 ± 36	11 ± 1.3	0	48 ± 5	1385 ± 59
P. spiniger comb	14.6 ± 1.3	5.2 ± 0.3	2.3 ± 0.1	10.6 ± 0.5	12.8 ± 1.2	18.2 ± 2.4
T. medius from *A. guineensis*						
A. guineensis comb	15.4 ± 1.1	76.05 ± 8.6	12.4 ± 2	278 ± 25	25.3 ± 1.6	824 ± 56
M. bellicosus comb	0	0	0	17.9 ± 2.4	22.7 ± 3.6	821 ± 47
P. spiniger comb	12.3 ± 0.6	76 ± 5.2	74.8 ± 8.3	75.2 ± 6	18.4 ± 2	992 ± 36
T. sp bellicosus from *M. bellicosus*						
A. guineensis comb	0	0	0	0	14.5 ± 2.3	672 ± 42
M. bellicosus comb	0	198 ± 36	28 ± 6.4	0	48 ± 9	986 ± 51
P. spiniger comb	0	0	0	0	10.9 ± 3	550 ± 37
T. sp subhyalinus from *M. subhyalinus*						
M. subhyalinus comb	4.5 ± 0.3	2.05 ± 0.2	5.3 ± 0.8	3.4 ± 0.6	14.6 ± 1.2	14.6 ± 1.6
P. spiniger comb	4.1 ± 0.5	2.0 ± 0.6	3.8 ± 0.8	2.5 ± 0.4	3.7 ± 0.9	12.3 ± 1

Activities are expressed in µmol permn per mg protein (Mora and Rouland 1994; Ikhouane 1995)

cording to the substrate and thus enzymatic production is correlated with environmental factors. These fungi are generalists and can be cultivated by several species of termite. In the second group, however, the variety of substrates that induce enzyme production is more limited. The enzymes produced in culture media are constitutive. This type of fungus appears to be more specialized in that it is found only in association with a single termite species. The group includes the symbiotic fungus associated with *M. bellicosus* (Ikhouane 1995) and *T. clypeatus* which produces several constitutive enzymes in vitro (Ghosh and Sengupta 1987; Sengupta and Sengupta 1990; Roy et al. 1994; Sinha and Sengupta 1995; Mukherjee et al.

1995). This classification of *Termitomyces* according to their metabolism seems to be correlate strongly with the co-evolution conclusions discussed above. *T. medius* has been found as a cultivated fungus associated with three species of *Microtermes*, two species of *Ancistrotermes* and with *Synacanthotermes heterodon*, whereas the symbiotic fungi associated with *M. bellicosus* have only been cultivated in the nests of this species (Aanen et al. 2002; Rouland et al. 2002).

Lignin Degradation in the Fungus Comb. Lignin is a heterogeneous random phenylpropanoid polymer that constitutes 15–30% of woody plant cell walls. Free-radical condensation of lignin precursors (coniferyl, sinapyl and *p*-coumaryl alcohols) results in the formation of an amorphous and highly branched polymer with at least 12 different types of linkage (Monties and Fukushima 2001). Since lignin forms a matrix surrounding the cellulose microfibrils, cellulose-depolymerizing enzymes are locked out and the decomposition of the cellulose will be retarded. Experimental evidence of lignin degradation in fungus combs was reported by Rohrmann (1978), and later by Hyodo et al. (2000). They determined proximate fractions for parts of the fungus comb differing in age, showing that lignin concentrations in the older parts of the combs were lower than those in the newer parts (Table 14.2). The structural changes of the lignin as the comb aged were analyzed using solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy of the molecules (Hyodo et al. 2000). Compared with the NMR spectrum of newer comb, the relative peak intensities at 56 and 154 ppm were clearly lower in the older comb. The peaks at 56 and 154 ppm were assigned to methoxyl and aromatic carbon in lignin, respectively. Further

Table 14.2. Chemical composition of various fungus combs (as dry weight %)

	Part of comb	Cellulose	Lignin	Ash
M. ukuxii[a]	Newer	20.82[c]	14.30	8.00
	Older	27.18[c]	2.43	7.45
M. natalensis[a]	Newer	17.78[c]	9.80	16.22
	Older	21.82[c]	5.67	15.47
M. gilvus[b]	Newer	17.1[d]	32.6	14.0
colony 1	Older	26.3[d]	15.0	15.5
M. gilvus[b]	Newer	21.5[d]	34.6	5.7
colony 2	Older	33.8[d]	53.4	11.2

[a] Data from Rohrmann (1987)
[b] Data from Hyodo (2000)
[c] Cellulose + chitin
[d] Calculated from glucose content

studies for precise peak assignment will be required because fungus comb consists of a variety of plant material so that the spectrum must be very complex.

There have been extensive biochemical studies of lignin degradation by basidiomycetes which have revealed that extracellular peroxidases are responsible for the depolymerization of lignin (see reviews by Kirk and Farrell 1987; Gold and Alic 1993; Hatakka 2001). Laccase is one of main components of ligninolytic enzymes in some basidiomycete (e.g. *Pycnoporus cinnabarinus*, Eggert et al. 1997). However, many fungi that are not able to degrade lignin also produce laccase (e.g. *Neurospora crassa*). Lignin peroxidase (EC 1.11.1.14) catalyzes the oxidation of various aromatic compounds to form aryl cation radicals that are unstable and undergo a variety of nonenzymatic (chemical) reactions such as ring-opening and side-chain cleavages (Kirk and Farrell 1987). The substrate of manganese peroxidase (EC 1.11.1.13) is Mn^{II}. The enzyme oxidizes Mn^{II} to Mn^{III}, which diffuses from the enzyme surface and oxidizes a variety of phenolic compounds (Gold and Alic 1993). Thus Mn^{III} acts as a diffusible oxidant and attacks macromolecular lignin. In the case of lignin peroxidase, substrate oxidation site is located on the surface of the enzyme (Doyle et al. 1998). Direct interaction and electron transfer between lignin peroxidase and lignin were observed (Johjima et al. 1999). Both enzymes show broad substrate specificities and are suitable for degradation of lignin because lignin structure is a random, highly branched polymer with various linkages.

The understanding of the lignin degradation mechanism in fungus comb is limited. Rohrmann and Rossman (1980) detected pigmentation in *Macrotermes ukuzii* and *Termitomyces* combs grown on comb agar in the presence of syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehyde). This suggests that *Termitomyces* produces extracellular phenol oxidase(s) such as peroxidase and laccase in the fungus comb. However, this does not show that *Termitomyces* can degrade lignin because laccase and peroxidase are secreted by non-ligninolytic fungi.

The work discussed above was based on *Macrotermes*. Hyodo et al. (2003) analyzed chemical composition of the fungus combs from *Odontotermes*, *Hypotermes makhamensis*, *Ancistrotermes pakistanicus* and *Pseudacanthotermes militaris* in addition to four species of *Macrotermes*. In all *Macrotermes* species, the carbohydrate:lignin ratio increased progressively with comb age, whereas it decreased or remained constant in three species of *Odontotermes*, *H. makhamensis*, *A. pakistanicus* and *P. militaris*. This indicates that, in *Macrotermes*, lignin is preferentially degraded in the combs, whereas in the other four genera carbohydrate is preferentially degraded. The authors also conducted a stable carbon isotope analysis and suggested that *Macrotermes* depend more on plant matter for nutrition than the other four genera. It is uncertain whether the differences are accounted for by the

different sources of the fungus combs or by the phylogenetic differences between the symbiotic fungi. The authors mentioned that the *Macrotermes* species examined in the study, where lignin content in fungus combs decreased with age, seem to tend to feed on leaf litter, while the other genera appear to feed predominantly on wood with the exception of *P. militaris*. *M. gilvus*, in which the lignin degradation was analyzed by solid-state NMR as described above, also mainly uses leaf, grass and stalks as a source of the fungus comb (Roonwall 1970). It has been observed that grass lignin is more easily degraded by fungi than wood lignin (Rodriguez et al. 1996). It is possible that *Termitomyces* can degrade grass and leaf lignin but not wood lignin. Molecular biological studies covering the cloning of ligninolytic enzymes and the distribution of the genes within the symbiotic fungi from various host termites will resolve the question.

The existence of plant secondary metabolites as well as lignin will interfere with carbohydrate utilization by termites because these compounds are apparently key components of plant defence against pests and pathogens and are, therefore, considered to be an important controlling factor for litter decomposition in soils (Swift et al. 1979; Bennett and Wallsgrove 1994). Phenolics such as tannins and flavonoids are one of major classes of plant secondary metabolites. The concentration of phenolics in the fungus comb of *M. gilvus* is much lower than in the fallen leaves around the termite mound (Johjima 2003). The concentration of water-soluble phenolics in extractable compounds from older comb is lower (40%) than that in newer comb, suggesting that phenolics are degraded in the fungus comb. Phenol oxidase activity has been detected in the fungus comb as mentioned above. This enzyme might be responsible for the degradation of phenolics in the fungus comb.

Chemical analyses of fungus comb at molecular level will provide further insights into the function of fungus comb. However, it is nearly impossible to determine the fungus comb compositions in molecular level using degradative chemical methods since the fungus comb consists of a variety of plant debris and microorganisms. For example, the Klason lignin method has been shown to overestimate lignin content in litter, where the residue contains cutin, suberin and tannins as well as lignin (Kögel 2003 and references therein). The results obtained by Rohrmann (1978) and Hyodo et al. (2000) may be due to degradation of cutin, suberin and tannins rather than lignin being degraded in the fungus comb. New analytical approaches such as solid-state NMR spectroscopy and analytical pyrolysis/gas chromatography/mass spectrometry combined with conventional methods will allow a detailed analysis of fungus comb components and help the understanding of the detailed mechanism of lignin degradation and the tracing the fate of lignin and plant secondary metabolites such as tannin in Macrotermitinae colonies.

14.4 Conclusions

The efficiency of degradation of lignocellulose by fungus-growing termites is largely attributed to their symbiotic *Termitomyces*. These fungi provide a variety of functions that termites do not possess. Recently, the application of new technologies and molecular methods has greatly improved our knowledge of lignin degradation by both Macrotermitinae termites and *Termitomyces* fungi and on the co-evolution of the symbionts. However, there are still gaps in our knowledge on the adoption of a particular species of fungus by termites. As described above, the same fungus can be cultivated by several different termite species. A crucial and as-yet-unanswered question is: to what extent do the Macrotermitinae manage to reduce the genetic diversity of horizontally acquired symbionts to a single strain to prevent the evolution and appearance of non-cooperative symbiont traits?

The other important unsolved questions concern the acquired enzymes: do these enzymes exist in genera of Macrotermitinae other than *Macrotermes*? Can lignin-degrading enzymes also be acquired by termites? New biochemical and molecular studies are required to answer these questions.

References

Aanen DK, Eggleton P, Rouland-Lefevre C, Guldberg-Froslev T, Rosendahl S and Boosma JJ (2002) The evolution of fungus-growing termites and their mutualistic symbionts. PNAS 99:14887–14892

Abe T and Watanabe H (1983) Soil macrofauna in a tropical rainforest and its adjacent cassava plantation in Okinawa- with special reference to the activity of termits. Physiol Ecol Japan 20: 101–104

Abo-Khatwa N (1978) Cellulase of fungus-growing termites : A new hypothesis on its origin. Experientia 34:559–560

Bennett RN, Wallsgrove, RM (1994) Secondary metabolites in plant defence mechanisms. New Phytol 127:617–633

Bignell DE (2000) Introduction to symbiosis. In Abe T, Bignell DE and Higashi M(eds) Termites: Evolution, Sociality, Symbioses, Ecology. Kluwer Academic, Dordrecht, The Netherlands, pp 189–208

Collins NM (1983) The utilization of nitrogen resources by termites (Isoptera). In: Lee JA, McNeill S, Rorison IH (eds) Nitrogen as an ecological factor, Blackwell Scientific, Oxford, pp 381–412

Darlington, JPEC (1994) Nutrition and Evolution in Fungus-growing Termites. In: Hunt JH, Nalepa CA (eds) Nourishment and Evolution in Insect Societies, Westview Press, Boulder, pp 105–130

D'Ettorre P, Mora, P, Dibangou V, Rouland C, Errard C (2002) The role of symbiotic fungus in the digestive metabolism of two species of fungus-growing ants. J Comp Physiol B 172:169–176

Doyle WA, Blodig W, Veitch NC, Piontek K, Smith AT (1998) Two substrate interaction sites in lignin peroxidase revealed by site-directed mutagenesis. Biochem 37:15097–15105

Eggert C, Temp U, Eriksson KE (1997) Laccase is essential for lignin degradation by the white-rot fungus *Pycnoporus cinnabarinus*. FEBS Lett 407:89–92

Gold MH, Alic M (1993) Molecular biology of the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Microbiol Rev 57:605–622

Gosh AK and Sengupta S (1987) Multisubstrate specific amylase from mushroom *Termitomyces clypeatus*. J Biosc 11:175–285

Grassé PP (1959) Un nouveau type de symbiose: la meule alimentaire des termites champignonnistes. Nature 3293:385–389

Grassé PP (1978) Physiologie des insectes. -Sur la véritable nature et le rôle des meules à champignons construites par les termites Macrotermitinae (Isoptera : Termitidae). CRAS Paris 287:1223–1226

Grassé PP (1982) Termitologia, Vol. I, Masson, Paris

Grassé P-P, Noirot C (1958) Le meule des termites champignonnistes et sa signification symbiotique. Annales des Science Naturelles, Zoologie et Biologie Animalle 20:113–128

Hatakka A (2001) Biodegradation of lignin. In: Hofrichter M, Steinbüchel A (eds) Biopolymers, Vol. 1, Wiley-VCH, Weinheim, pp 129–180

Heim R (1941) Etudes descriptives et expérimentales sur les agarics termitophiles d'Afrique tropicale. Mem de l'Acad Sc 64:25–29

Heim R (1977) Termites et Champignons. Boubée Ed., Paris, 207 pp

Hyodo F, Azuna J, Abe T (1999) A new pattern of lignin degradation in the fungus comb of *Macrotermes carbonarius* (Isoptera, Termidae Macrotermitinae). Sociobiology 34: 591–596

Hyodo F, Inoue T, Azuma J-I, Tayasu I, Abe T (2000) Role of the mutualistic fungus in lignin degradation in the fungus-growing termite *Macrotermes gilvus* (Isoptera; Macrotermitinae). Soil Biol Biochem 32:653–658

Hyodo F, Tayasu I, Inoue T, Azuma J-I, Kudo T, Abe T (2003) Differential role of symbiotic fungi in lignin degradation and food provision for fungus-growing termites (Macrotermitinae: Isoptera). Functional Ecology 17: 186–193

Ikhouane A (1995) Etude de la biodégradation de polymères végétaux par des champignons du genre *Termitomyces* symbiontes de termites. Thèse d'Université, Université Paris XII Val de Marne, Paris, France

Johnson RA (1981) Colony development and estabilishment of fungus comb in *Microtermes. umbaricus* Sjöstedt (Isoptera, Macrotermitinae) from Nigeria. J Nat Hist 32:3–12

Josens G (1971) Le renouvellement des meules construites par quatre Macrotermitinae (Isoptères) des savanes de Lamto-Pakobo (Côte d'Ivoire). CRAS, Paris 272:3329–3332

Johjima T, Itoh N, Kabuto M, Tokimura F, Nakagawa T, Wariishi H, Tanaka H (1999) Direct interaction of lignin and lignin peroxidase from *Phanerochaete chrysosporium*. Proc Natl Acad Sci USA 96:1989–1994

Johjima T, Inoue T, Ohkuma M, Kudo T (2003) Chemical analysis of food processing by the fungus-growing termite Macrotermes gilvus. Sociobiology 42: 815–824

Kambhampati S, Eggleton P (2000) Taxonomy and phylogeny of termites. In Abe T, Bignell DE and Higashi M (eds) Termites: Evolution, Sociality, Symbioses, Ecology. Kluwer Academic, Dordrecht, The Netherlands, pp 1–24

Katoh H, Miura T, Maekawa K, Shinzato N, Matsumoto T (2002) Genetic variation of symbiotic fungi cultivated by the macrotermitinae termite *Odontotermes formosanus* (Isoptera:Termitidae) in the Ryuku Archipelago. Molecular Ecology 11:1565–72.

Kirk TK, Farrell RL (1987) Enzymatic "combustion": The microbial degradation of lignin. Annu Rev Microbiol 41:465–505

Kögel I (2003) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol Biochem 34:139–162

König JG (1779) Naturgeschite der sog. Weissen Ameisen. Beschr der Berliner Gesellschaft naturforsch Freunde 4:21–23

Kukor JJ, Martin MM (1983) Acquisition of digestive enzymes by siricid woodwasps from their fungal symbiont. Science 220:1161–1163

Lamberty M, Zachary D, Lanot R, Bordereau C, Robert A, Hoffmann JA, Bulet P (2001) Insect immunity. Constitutive expression of a cysteine-rich antifungal and a linear antibacterial peptide in a termite insect. J Biol Chem 276:4085–4092

Lüscher M (1951) Significance of fungus garden in termite nests. Nature 167: 34–35

Martin MM (1991) The evolution of cellulose digestion in insects. Philosophical Trans Royal Soc London 333:281–288

Martin MM and Martin JS (1978) Cellulose digestion in the midgut of the fungus-growing termite *Macrotermes natalensi* : the role of acquired digestve enzymes. Science 199: 1453–1455

Martin MM and Martin JS (1979) Distribution and origins of the cellulolytic enzymes of the higher termite, *Macrotermes natalensis*. Physiol Zool 52:11–21

Matoub M and Rouland C (1995) Purification and properties of the xylanases from the termite *Macrotermes bellicosus* and its symbiont fungus *Termitomyces* sp. Comp Biochem Physiol 112:629–635

Matsumoto T (1976) The role of termites in an equatorial rain forest ecosystem of west Malaysia. I. Population density, biomass, carbon, nitrogen and calorific content and respiration rate. Oecologia 22:153–178

Matsumoto T (1977) Respiration of fungus combs and carbon dioxyde concentration in the center of mounds of some termites. 8 th Proc Int Congr I.U.S.S.I., 104–106

Moncalvo JM, Vilgabys R, Redhaed SA, Johnson JE, James TY, Aime M C, Hofstetter V, Verduin SJ, Larsson E, Baroni TJ (2002) One hundred and seventeen clades of euagarics Mol Phylogenet Evol 23:357–400

Monties B, Fukushima K (2001) Occurence, function and biosynthesis of lignins. In: Hofrichter M, Steinbüchel A (eds) Biopolymers, Vol. 1, Wiley-VCH, Weinheim, pp 1–64

Mora P., Rouland C. (1994) Comparison of hydrolytic enzymes produced during growth on carbohydrate substrates by *Termitomyces* associated of *Pseudacanthotermes spiniger* and *Microtermes subhyalinus*. Sociobiology, 26, 39–53

Mukherjee M, Khowzla S, Ghosh AK and Sengupta S (1995) Regulation of endo-1,4β-glucanase secretion from *Termitomyces clypeatus* by carbon catabolic product(s). Folia Microbiol 40:75–480

Petch T (1906) The fungi of certain termites nests. Ann of the Royal Bot Gardens Peradeniya, 3:4–67

Petch T (1913) Termite fungi, a resume. Ann Roy Bot Gardens Peradeniya, 5 : 303–341

Rodriguez A, Perestelo F, Carnicero A, Regalado V, Perez R, De la Fuente G, Falcon MA (1996) Degradation of natural lignins and lignocellulosic substrates by soil-inhabiting fungi imperfecti. FEMS Microbiol Ecol 21:213–219

Rohrmann GF (1978) The origin, structure, and nutritional importance of the comb in two species of Macrotermitinae. Pedobiologia 18:89–98

Rohrmann GF, Rossman AY (1980) Nutrient strategies of *Macrotermes ukuzii*. Pedobiologia 20:61–73

Roonwall ML (1970) Termites of the Oriental reagion. In: Krishna K, Weesner FM (eds) Biology of termites, Academic Press, London

Rouland C (2000) Symbiosis with fungi. In Abe T, Bignell DE and Higashi M(eds) Termites: Evolution, Sociality, Symbioses, Ecology. Kluwer Academic, Dordrecht, The Netherlands, pp 289–306

Rouland C, Chararas, C, Mora, P & Renoux, J (1988a) Comparaison entre les osidases du termite *Macrotermes mülleri* et celles de son champignon symbiotique *Termitomyces* sp. CRAS Paris, 306:115–120

Rouland, C, Civas, A, Renoux, J and Petek, F (1988b) Purification and properties of cellulases from the termite *Macrotermes mülleri* (Termitidae, Macrotermitinae) and its symbiotic fungus *Termitomyces* sp. Comp Biochem Physiol 91B:449–458

Rouland, C, Civas, A, Renoux, J and Petek, F (1988c) Synergistic activities of the enzymes involved in cellulose degradation, purified from *Macrotermes muelleri* and from its symbiotic fungus *Termitomyces* sp. Comp Biochem Physiol 91B:59–465

Rouland, C, Renoux, J & Petek, F (1988d) Purification and properties of two xylanases from *Macrotermes muelleri* (Termitidae : Macrotermitinae) and its symbiotic fungus *Termitomyces* sp.. Insect Biochem 18:09–715

Rouland C, Diouf M, Brauman A & Neyra M (2002) Phylogenetic relationships in *Termitomyces* based on nucleotide sequences of ITS: A first approach to elucidate the evolutionary history of the symbiosis between fungus-growing termites and their fungi. Mol Phylogenet Evol 22:1–7

Rouland C, Lenoir, F and Lepage M (1991) The role of the symbiotic fungus in the digestive metabolism of several species of fungus-growing termites. Comp Biochem and Physiol 99: 657–663

Roy SB, Gosh AK, Sengupta S & Sengupta S (1994) Development of high-molar-mass cellobiase complex by spontaneous protein-protein interaction in the culture filtrate of *Termitomyces clypeatus*. Folia Microbiol 39:463–470

Sands WA (1956) Some factors affecting the survival of *Odontotermes badius*. Insectes Sociaux 3:531–536

Sengupta S and Sengupta S (1990) β-glucosidase production by the mycelial culture of the mushroom *Termitomyces clypeatus*. Enz Microb Technol 12:309–314

Sieber R, Leuthold RH (1981) Behaviour elements and their meaning in incipient laboratry colonies of the fungus-growing termite *Macrotermes michaelseni* (Isoptera: Macrotermitinae). Insectes Sociaux 28:371–387

Singer R (1986) The Agaricales in modern taxonomy, 4th edition. Koeltz, Koenigstein, Germany

Sinha N and Sengupta S (1995) Simultaneous production of -arabinofuranosidase and xylanase by *Termitomyces clypeatus*. W J of Microbiol and Biotechnol 11: 1-2

Swift MJ, Heal OW, Anderson JM (1979) Decomposition in terrestrial ecosystems. Blackwell Scientific, Oxford

Taprab Y, Okhuma M, Johjima T, Maeda Y, Moriya S, Inoue T, Suwanarit P, Noparatnaraporn N, Kudo T (2002) molecular phylogeny of symbiotic basidiomycetes of fungus-growing termites in Thailand and their relatioship with the host; Biosci Biotechnol Biochem 66:1159–63

Thomas RJ (1987a) Distribution of *Termitomyces* Heim and other fungi in the nests and major workers of *Macrotermes bellicosus* (Smeathman) in Nigeria. Soil Biol Biochem 19: 329–333

Thomas RJ (1987b) Factors affecting the distribution and activity of fungi in the nests of Macroterminae (Isoptera). Soil Biol Biochem 19:343–349

Traniello JF, Leuthold RH (2000) Behavior and ecology of foraging in termites, In: Abe T, Bignell DE, Higashi M (eds) Termites: Evolution, Society, Symbioses, Ecology. Kluwer Academic Publishers, Dordrecht, pp 141–168

Wood, TG (1978) The role of termites in ecosystems. In Brian MV (ed) Production Ecology of Ants and Termites, Cambridge Univ Press, Cambridge, pp 55–80

Wood TG, Thomas RJ (1989) The mutualistic association between Macrotermitinae and *Termitomyces*. In:Wilding N, Collins NM, Hammond PM, Webber JF (eds) Insect-fungus Interection, Academic Press, London, pp 69-92