

# Fungus-Growing Termites Originated in African Rain Forest

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## Summary

Fungus-growing termites (subfamily Macrotermitinae, Isoptera) cultivate fungal crops (genus *Termitomyces*, Basidiomycotina) in gardens inside their colonies. Those fungus gardens are continuously provided with plant substrates, whereas older parts that have been well decomposed by the fungus are consumed (cf. [1, 2]). Fungus-growing termites are found throughout the Old World tropics, in rain forests and savannas, but are ecologically dominant in savannas [3]. Here, we reconstruct the ancestral habitat and geographical origin of fungus-growing termites. We used a statistical model of habitat switching [4] repeated over all phylogenetic trees sampled in a Bayesian analysis of molecular data [5]. Our reconstructions provide strong evidence that termite agriculture originated in African rain forest and that the main radiation leading to the extant genera occurred there. Because extant savanna species are found in most genera, this moreover suggests that the savanna has repeatedly been colonized by fungus-growing termites. Furthermore, at least four independent “out-of-Africa” migrations into Asia, and at least one independent migration to Madagascar, have occurred. Although fungus growing by termites is ecologically most successful under the variable, unfavorable conditions of the savanna, it seems to have evolved under the more constant and favorable conditions of the rain forest.

## Results and Discussion

The agricultural symbiosis between termites (subfamily Macrotermitinae, Isoptera) and fungi (genus *Termitomyces*, Basidiomycotina) is one of the most spectacular examples of mutualistic symbiosis. Recent work has shown that a single transition to agriculture in termites has occurred with no reversions to free-living states [6]. Moreover, the single lineage of domesticated termite fungi has not been able to “escape” to a nonsymbiotic lifestyle after its domestication [6–8]. In other words, the agricultural symbiosis between termites and fungi

is symmetrical in that both partners have a single origin with no reversals to nonsymbiotic states and both are obligatorily dependent on this relationship [1, 6].

Cultivating fungi has allowed fungus-growing termites to become one of the most important decomposer groups in the Old World tropics. Although they are found abundantly in African tropical rain forests [9], their relative contribution to ecosystem decomposition processes in these forests is relatively low (ca. 1%–2% of all C-mineralization, [10]). However, this contribution is much higher in areas of low annual rainfall than in areas of high annual rainfall [11], such that these termites become arguably the predominant decomposer organisms in dry savannas, where they are responsible for ca. 20% of all C-mineralization [3].

*Termitomyces* is a white-rot fungus [12]. White-rot fungi are among the few organisms that can digest lignin. The optimal conditions (high, buffered temperature and high humidity) for white-rot decay are predominantly found in hot, wet habitats [13], such as rain forests. We therefore hypothesize that the symbiosis between macrotermitine termites and fungi evolved in the rain forest, where white-rot fungi are abundant and conditions for their growth are optimal, and that macrotermitine termites later colonized drier savanna habitats, where these fungi are in general less common. We tested this hypothesis with DNA-based Bayesian methods of phylogeny estimation [5] combined with a maximum-likelihood reconstruction of ancestral habitats [4, 14, 15] over all trees sampled in the Bayesian analysis. In this way, our reconstructions are conditional not on any particular phylogenetic hypothesis but instead on the posterior-probability distribution of trees (cf. [16]).

A total of 58 colonies (representing 49 species and 10 of the 11 genera) were sampled from Senegal, Cameroon, Gabon, Kenya, South Africa, Madagascar, India, Sri Lanka, Sabah, Kalimantan, and Thailand (Table S1 in the Supplemental Data available with this article online), a range covering most of the geographical, taxonomic, and ecological diversity of the group. To root the trees, we included representatives from the putative Macrotermitinae sister group, consisting of *Labritermes butelreepeni* and *Foraminitermes valens*, as an outgroup [6]. We estimated the phylogenetic history of the termites by using Bayesian analyses of a fragment of the mitochondrial *cytochrome oxidase I* gene and applying the software MrBayes (version 3.0; [5]). The general time-reversible (GTR) model of sequence evolution with site-specific substitution rates (SSR) was used (see Supplemental Data for full details of methods).

In the Bayesian majority-rule consensus tree (Figure 1), all genera formed monophyletic groups, with the exception of the genus *Odontotermes*, which, however, did form a monophyletic group with the inclusion of the genus *Hypotermes*. The Asiatic samples resolved into four different apical clades within *Macrotermes*, *Microtermes*, *Ancistrotermes*, and *Odontotermes*, indicating that the ancestor of the fungus-growing termites was African and that a total of at least four out-of-Africa migrations have occurred. Within *Microtermes*, the

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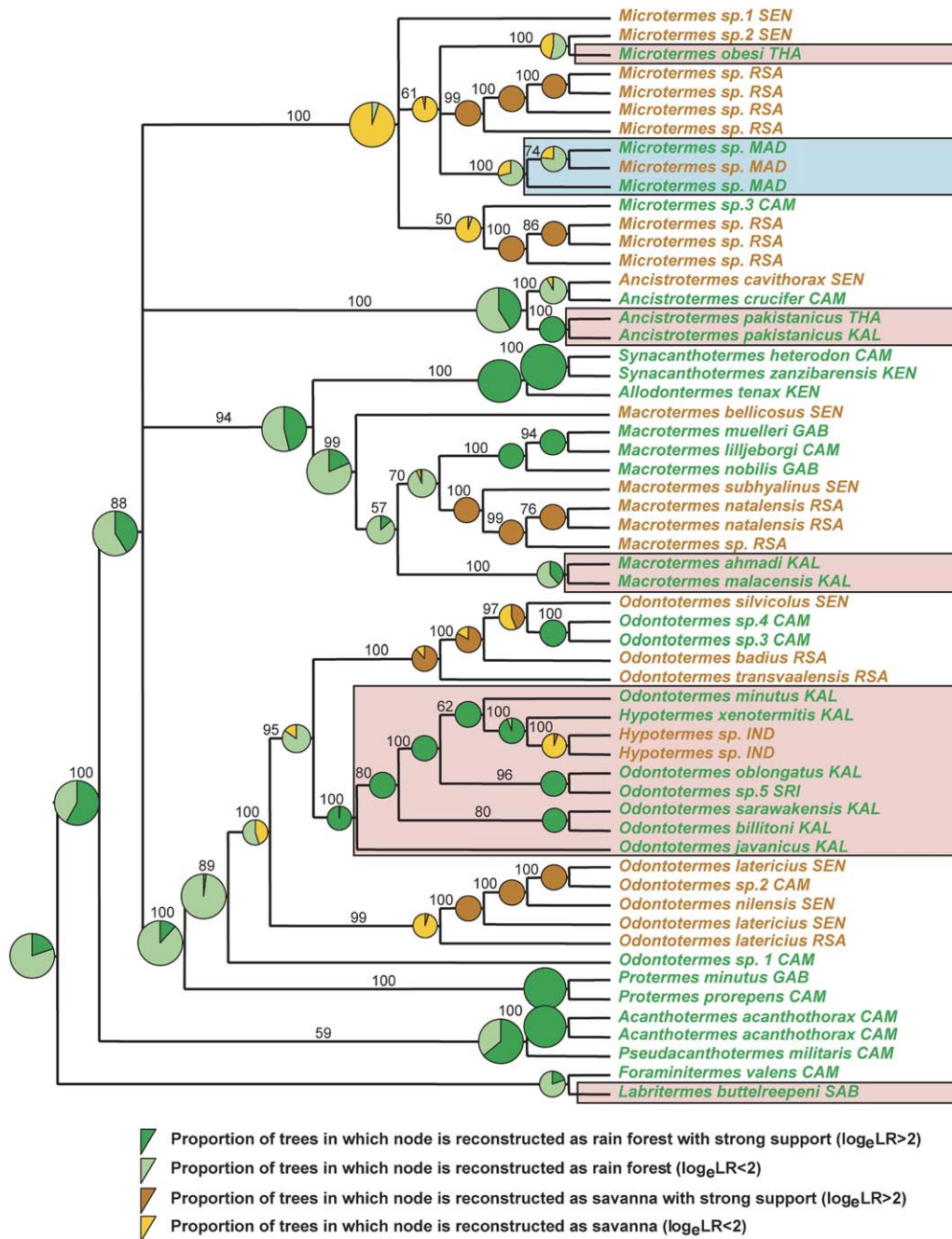


Figure 1. Reconstructions of Ancestral Habitats of Fungus-Growing Termites

Shown is the majority-rule consensus tree of the 28,000 trees sampled in the Bayesian analysis. The habitats of extant taxa are indicated by different colors (green, forest; brown, savanna). The numbers on branches refer to percentage Bayesian posterior probabilities of individual nodes. Pie charts on nodes indicate the frequencies of the sampled trees with the two alternative reconstructions. The two different intensities of green and brown indicate the proportion of significant and nonsignificant reconstructions (dark:  $\log_e LR > 2$ , which corresponds to a relative support of forest over savanna of more than 7.4. Light:  $\log_e LR < 2$ ). The pink boxes indicate Asian clades, and the blue box indicates a Malagasy clade. The following abbreviations are used: SEN, Senegal; CAM, Cameroon; THA, Thailand; RSA, Republic of South Africa; MAD, Madagascar; KAL, Kalimantan; KEN, Kenya; GAB, Gabon; IND, India; SRI, Sri Lanka; and SAB, Sabah.

three Malagasy samples formed a monophyletic group with African sister groups, indicating an African origin of the Malagasy *Microtermes* species as well. Because species of the genera *Ancistrotermes* and *Odontotermes* also occur on Madagascar, a minimum of three migrations to Madagascar has likely occurred.

To reconstruct the ancestral habitat of fungus-growing termites, we first labeled taxa as forest species or savanna species on the basis of their collection locality. Maximum-likelihood reconstruction methods [4, 14, 15] were then used to estimate ancestral habitats with the computer program Mesquite [17]. To ensure that our

reconstructions are conditional not on any particular phylogenetic tree but instead on the posterior-probability distribution of trees (cf. [16]), we performed our analyses over the 28,000 trees sampled (after the burn-in) in the Bayesian analysis. We used equal transition rates between habitats because a model incorporating different rates did not lead to a significant improvement in the fit of the model to the data in comparison to a model with a single rate [4, 15]. To incorporate reconstruction uncertainty, we tested the significance of reconstructed ancestral states for each individual tree by using the likelihood-ratio test and an arbitrary value of two as the cutoff point for a significant difference (following [4] and [18]).

Our reconstructions provide strong evidence that the ancestral habitat of fungus-growing termites was forest (Figure 1). For all 28,000 trees sampled in the Bayesian analysis, the ancestral habitat of fungus-growing termites was reconstructed as forest, and for the majority of trees (58%), the reconstruction was significant (i.e., the  $\log_e$  likelihood ratio between forest and savanna was greater than two, which corresponds to a relative support of forest over savanna of more than 7.4). A hierarchical Bayesian reconstruction of the ancestral character state [19] confirmed this result, with a posterior probability of forest as ancestral habitat ranging from 0.88 to 0.99 depending on the priors chosen for overall rate of change and bias in rates. Furthermore, the ancestral habitat of the next-most-basal nodes, including the ancestors of all genera (except for the *Microtermes* ancestor, which is ambiguous), was reconstructed as forest in all 28,000 trees (Figure 1). The most-basal unambiguous savanna nodes are therefore all derived within genera, indicating that the transition to savanna is a relatively recent event in the evolutionary history of fungus-growing termites and that this transition has occurred repeatedly. These results, of course, assume that there are no basal savanna groups that have subsequently gone extinct. This is impossible to test directly (due to a very sparse fossil record for the group). However, at least during the glacial periods of the Quaternary, savannas would have been much more extensive than forests (cf. [20]). During that time, forest extinction rates are likely to have been higher than savanna ones, thus at least making it somewhat less plausible that entire basal savanna groups would have gone extinct.

A simplified representation of our main findings is depicted in Figure 2. Fungus-growing termites have originated in continental African rain forests and have later repeatedly dispersed into savannas, into Asia, and to Madagascar. Dispersal into adjacent savanna systems has had a filtering effect, reducing the number of genera present but not the number of extant species (Table 1). This latter observation suggests that shifts to savannas in Africa may have led to repeated successful adaptive radiations in those habitats. Our estimated phylogeny and available figures for the number of extant species per genus [21] permit an explicit test of this hypothesis (cf. [22]). There are only three independent contrasts of exclusive rain forest genera versus genera occurring in both habitats (*Acanthotermes* versus *Pseudacanthotermes*, *Synacanthotermes* versus *Allodotermes*, and *Protermes* versus *Odontotermes*;

Figure 2). In line with the hypothesis of an adaptive radiation in all three comparisons, the number of species is lowest for the forest sister group, but this difference is not significant (one-tailed sign test,  $p = 0.12$ ; [23]). However, an alternative explanation for this pattern is that the higher number of savanna species is caused by a species-area effect [24] because total savanna area is much larger than total forest area, particularly in Africa.

Fungal white-rot decay requires temperature-buffered, high-humidity environments, which rain forests provide. In contrast, savannas have variable temperature regimens and intermittently low humidity, which are highly unfavorable conditions for fungal white-rot decay. Fungal decay rates are indeed much lower in dry savannas than in rain forests [25]. Optimal growth conditions for *Termitomyces* are a relative humidity that is near saturation and constant temperatures of about 30°C [26]. The key factor for the relative ecological importance of fungus-growing termites in decomposition processes on the savanna may therefore be the near absence of competition with non-*Termitomyces* fungi. This competition argument can also be applied when comparing fungus-growing termites with wood- and grass-feeding termites because energy consumption by fungus-growing termites, as a share of the total energy consumption by all termites, has been shown to be inversely correlated with climatic humidity in western African savannas [3]. Our reconstructions thus support the hypothesis that the ecological success of fungus-growing termites in savannas is due to the adoption of a highly successful rain-forest process (fungal white-rot decay) by domesticating white-rot fungi. By offering those domesticated fungi a constant supply of growth substrate and humid, highly buffered, rain-forest-like climatic conditions in their nests, termites have been able to export this rain-forest process into the savannas.

This hypothesis is also supported by climatic measurements made within and outside *Macrotermes* mounds in open African savannas [27, 28]. Fungus combs had a temperature of 27°C–28°C (*Macrotermes jeanneli*; [27]) or 30°C (*Macrotermes bellicosus*; [28]) and a relative humidity of ca. 100%. Outside the nest, daily air temperature was 20°C–37°C and surface soil temperature was 21°C–45°C. Conditions within the mound are therefore more similar to rain-forest climates than to savanna climates. Although climatic conditions are also buffered to some degree in nonfungus-growing savanna Termitidae, such complex, highly climatically buffered mounds are unique to fungus-growing termites.

In line with our hypothesis, it has been found that some of the fungal symbionts are shared between forest species and savanna species (e.g., the western African species in the genera *Microtermes* and *Ancistrotermes* all had indistinguishable fungal symbionts; [6]). This shows that it is not the external habitat but the internal habitat that the termites provide for *Termitomyces* that is decisive for where the fungus occurs. The closest-known nonsymbiotic relatives of *Termitomyces* (belonging to the genera *Tephroclybe* and *Lyophyllum*) occur in temperate zones. Those genera are poorly studied in the tropics. To further test our hypothesis, the habitat of the closest nonsymbiotic *Termitomyces* relatives occurring in the tropics needs to be studied.

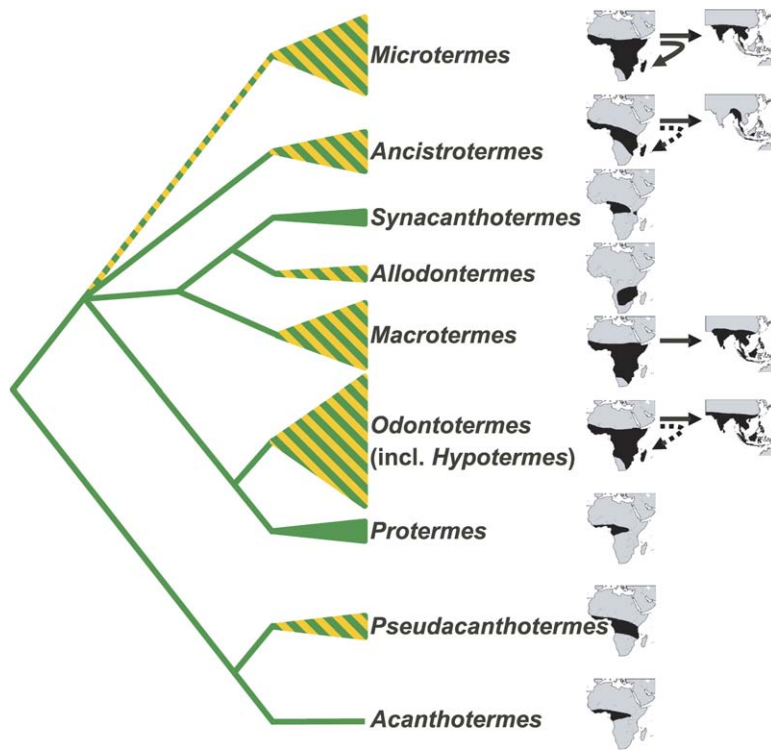


Figure 2. Simplified Representation of the Main Biogeographical Findings for Fungus-Growing Termites

Depicted are phylogenetic relationships among the 10 genera sampled. The surface area of the triangles is proportional to the square root of the number of described species in that genus (from [20] updated with the Termite Distribution Database of The Natural History Museum of London). Colors of lines indicate estimated ancestral habitat (green, forest; striped green-yellow, ambiguous). Colors of triangles indicate the habitat type of extant species found in each respective genus (green, forest species; striped green-yellow, both forest species and savanna species). Note that representatives of the three genera *Ancistrotermes*, *Odontotermes*, and *Microtermes* are found in Madagascar but that for this study we only obtained material of the last genus. The African origin of the Malagasy *Odontotermes* and *Ancistrotermes* is therefore hypothetical, as indicated by a broken arrow.

Our results have some striking parallels with the origin of human agriculture. Human agriculture is believed to have originated in areas to which most numerous and most valuable domesticable organisms are native [29]. From the homelands of domestication, agriculture has later spread around the world, including to much more unfavorable areas. This occurred in two ways: either through the adoption of an agricultural lifestyle by local hunter-gatherers or, far more often, through the replacement of local hunter-gatherers by farmers [29]. The agricultural lifestyle resulted in much higher population densities of both humans and their domesticated organisms than under the ancestral lifestyle, paralleling the ecological success of fungiculture by termites. Like in humans, therefore, in fungus-growing

termites the association of farmers and crops into a mutualistic symbiosis has allowed both partners to exploit unfavorable conditions more effectively than either of their free-living relatives can alone.

Supplemental Data

Supplemental Data, including Supplemental Experimental Procedures and one table, are available with this article online at <http://www.current-biology.com/cgi/content/full/15/9/851/DC1/>.

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Table 1. Summary of Numbers of Macrotermitine Species in African Habitat Types

Genus	Rain Forest		Savanna		
	Guinea-Congo	E. African Coastal	Woodland	Brush Grass	Steppe
<i>Synacanthotermes</i>	2	1			
<i>Odontotermes</i>	10	5	20	10	14
<i>Pseudacanthotermes</i>	2	1	3		
<i>Protermes</i>	3				
<i>Acanthotermes</i>	1				
<i>Allodoterme</i>		2	4		
<i>Ancistrotermes</i>	2	1	6		
<i>Macrotermes</i>	6		9	3	2
<i>Microtermes</i>	6	2	16	5	12
Total genera	8	6	6	3	3
Total species	32	12	58	18	28
Total per major habitat	44		94		

The habitats are listed from left to right in order of increasing dryness. This data is based on The Natural History Museum of London's Termite Distribution Database, which includes undescribed species and species of uncertain taxonomic status.

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## Accession Numbers

The GenBank accession numbers reported in this paper for *Synanthotermes zanzibarensis* (pe203), *Allodoterme tenax* (PE301), *Macrotermes natalensis* (ZA1), *Macrotermes natalensis* (za136), *Macrotermes* sp. (za62), *Odontotermes latericius* (za3), *Odontotermes badius* (ZA7), *Odontotermes transvaalensis* (ZA17), *Microtermes* sp. (ZA49), *Microtermes* sp. (za138), *Microtermes* sp. (za135), *Microtermes* sp. (ZA9), *Microtermes* sp. (ZA10), *Microtermes* sp. (ZA14), *Microtermes* sp. (ZA31), *Hypotermes* sp. (rk1), *Hypotermes* sp. (rk2), *Microtermes obesi* (MO), *Ancistrotermes pakistanicus* (APHyodo), *Ancistrotermes pakistanicus* (APPE), *Microtermes* sp. (pe201), *Microtermes* sp. (pe202), and *Microtermes* sp. (pe302) are, respectively, AY818067–AY818089.