

## Abstracts: Blattodea

### **Bucek *et al.*, 2019, Evolution of Termite Symbiosis Informed by Transcriptome-Based Phylogenies.**

We sequenced genomes and transcriptomes of 55 termite species and reconstructed phylogenetic trees from up to 4,065 orthologous genes of 68 species. We found strong support for a novel sister-group relationship between the bacterial comb-building Sphaerotermittinae and fungus comb-building Macrotermittinae. This key finding indicates that comb building is a derived trait within Termitidae and that the creation of a comb-like “external rumen” involving bacteria or fungi may not have driven the loss of protozoa from ancestral termitids, as previously hypothesized. Instead, associations with gut prokaryotic symbionts, combined with dietary shifts from wood to other plant-based substrates, may have played a more important role in this symbiotic transition.

### **Bourguignon *et al.*, 2017, Mitochondrial Phylogenomics Resolves the Global Spread of Higher Termites, Ecosystem Engineers of the Tropics.**

### **He *et al.*, 2021, Evidence for reduced immune gene diversity and activity during the evolution and activity during the evolution of termites.**

18 cockroach and termite species, including 9 lower termites, 6 higher termites, 2 subsocial wood roaches and 2 solitary roaches were studied via *de novo* assembled transcriptomes. Each immune gene family presented in all species, except antifungal dorsomycin, which was lost in termites and wood roaches. Phylogenetic signal analysis revealed a loss of total immune gene diversity during termite evolution.

Antifungal peptide drosomycin was lost in ancestor of wood roach *Cryptocercus* and termites. C-type lectin (CTL) underwent two contractions in most recent common ancestor (MRCA) of (1) wood roach *Cryptocercus* and termites; and (2) Rhinotermitidae and Termitidae, but together with lysozymes, re-expanded in late branch of higher termites, *i.e.* MRCA of *Promirotermes* and *Dicuspitermes*. Serine protease CLIP contracted in MRCA of Rhinotermitidae and Termitidae. Thioredoxin peroxidase (TPX) and autophagy-related gene (ATG) contracted in Termitidae, while defensin expanded.

In bees, immune gene depletion seems to have preceded evolution of eusociality (Barribeau *et al.*, 2015), indicating immune gene depletion is unrelated with transition to sociality. Although there was

contraction of immune genes in termite evolution, it can be interpreted as an expansion of immune genes in solitary cockroaches (Harrison *et al.*, 2018) followed by returning to a representative level.

### **Meusemann *et al.*, 2020, No Evidence for Single-Copy Immune-Gene Specific Signals of Selection in Termites.**

There are generally two life type in termites: wood-dwelling termites that nest in a single piece of wood that serves as food and shelter, and foraging termites that leave nest and forage outside. Wood-dwelling termites can be further divided into two types: living in drywood or decaying dampwood.

We hypothesized that selective pressure on single-copy immune defense genes (IGs) differs across termites depending on their life type. We tested the hypothesis that wood-dwelling termites show relaxed selection on IGs and fewer signs of positive selection compared to soil-foraging species. Additionally, we tested whether within the wood-dwelling life type, the dampwood termite *Zootermopsis nevadensis* had stronger signs of selection than other wood-dwellers that nest in sound wood.

In order to test for differences in selective forces acting on IGs between wood-dwelling and foraging species, we analyzed a set of 81 previously identified single-copy IGs in eight termite species. Four of the species are wood-dwelling species: *Cryptotermes secundus*, *Incisitermes marginipennis*, *Prorhinotermes simplex*, and *Zootermopsis nevadensis*. The remaining four species are foraging species: *Mastotermes darwiniensis*, *Reticulitermes flavipes*, *Coptotermes sp.* and *Macrotermes natalensis*.

Contrary to our expectation, we did not detect an effect of life type on signs of selection for immune genes. Neither did wood-dwelling species differ from the soil foraging species, nor did the dampwood termite *Zootermopsis nevadensis* differ from the other wood-dwelling termites. There are several alternative explanations: (1) there are life-type-related selection patterns in multi-copy IGs; (2) there are recent selective sweeps in single-copy IGs; (3) evolution of social immunity buffers selection pressure on IGs; (4) evolution of termite IGs is mainly driven by pressure from modulating gut microbiome, which performs, in principle, similar functions including lignocellulose digestion and nitrogen acquisition.

### **Bulmer *et al.*, 2010, Adaptive evolution in subterranean termite antifungal peptides.**

We identified and analysed mRNA sequences of two immune proteins from the subterranean termites *Reticulitermes flavipes* and *Reticulitermes virginicus*. These proteins correspond to two immune proteins described in the distantly related termite genus *Nasutitermes*; termicin, which is a small antifungal peptide, and GGBP2, which functions both as a broad pattern recognition receptor and a direct antifungal effector. A population genetic analysis of nucleotide intraspecific polymorphism and interspecific divergence indicates that a selective sweep has reduced polymorphism in the termicins. Moreover, this selective sweep appears to have been driven by the positive selection of beneficial amino acid changes in the antifungal peptide. In contrast, the pattern of polymorphism and divergence in GGBP2 corresponds

61 to the standard neutral model of evolution.

62 **Sabree *et al.*, 2009, Nitrogen recycling and nutritional provisioning by *Blattabacterium*,**  
63 **the cockroach endosymbiont.**

64 Cockroaches, unlike most terrestrial insects, excrete waste nitrogen within their fat bodies as uric acids,  
65 postulated to be a supplement when dietary nitrogen is limited. The fat bodies of most cockroaches are  
66 inhabited by *Blattabacterium*, which are vertically transmitted, Gram-negative bacteria that have been  
67 hypothesized to participate in uric acid degradation, nitrogen assimilation, and nutrient provisioning. We  
68 have sequenced completely the *Blattabacterium* genome from *Periplaneta americana*. Genomic analysis  
69 confirms that *Blattabacterium* is a member of the Flavobacteriales (Bacteroidetes), with its closest known  
70 relative being the endosymbiont *Sulcia muelleri*, which is found in many sap-feeding insects. Metabolic  
71 reconstruction indicates that it lacks recognizable uricolytic enzymes, but it can recycle nitrogen from  
72 urea and ammonia (uric acid degradation products) into glutamate, using urease and glutamate dehydro-  
73 genase. Subsequently, *Blattabacterium* can produce all of the essential amino acids, various vitamins, and  
74 other required compounds from a limited palette of metabolic substrates. The ancient association with  
75 *Blattabacterium* has allowed cockroaches to subsist successfully on nitrogen-poor diets and to exploit ni-  
76 trogenous wastes, capabilities that are critical to the ecological range and global distribution of cockroach  
77 species.

78 **Neef *et al.*, 2011, Genome Economization in the Endosymbiont of the Wood Roach *Cryp-***  
79 ***tocercus punctulatus* Due to Drastic Loss of Amino Acid Synthesis Capabilities.**

80 Cockroaches (Blattaria: Dictyoptera) harbor the endosymbiont *Blattabacterium* in their abdominal fat  
81 body. The genome of *Blattabacterium* sp. of *Cryptocercus punctulatus* (BCpu) was sequenced and com-  
82 pared with those of the symbionts of *Blattella germanica* and *Periplaneta americana*, BBge and BPam,  
83 respectively. The BCpu genome consists of a chromosome of 605.7 kb and a plasmid of 3.8 kb and is  
84 therefore approximately 31 kb smaller than the other two aforementioned genomes. The size reduction is  
85 due to the loss of 55 genes, 23 of which belong to biosynthetic pathways for amino acids. The pathways for  
86 the production of tryptophan, leucine, isoleucine/threonine/valine, methionine, and cysteine have been  
87 completely lost. Additionally, the genes for the enzymes catalyzing the last steps of arginine and lysine  
88 biosynthesis, argH and lysA, were found to be missing and pseudogenized, respectively. These gene losses  
89 render BCpu auxotrophic for nine amino acids more than those corresponding to BBge and BPam. BCpu  
90 has also lost capacities for sulfate reduction, production of heme groups, as well as genes for several  
91 other unlinked metabolic processes, and genes present in BBge and BPam in duplicates. Amino acids  
92 and cofactors that are not synthesized by BCpu are either produced in abundance by hindgut microbiota  
93 or are provisioned via a copious diet of dampwood colonized by putrefying microbiota, supplying host

94 and *Blattabacterium* symbiont with the necessary nutrients and thus permitting genome economization  
95 of BCpu.

96 **Sabree *et al.*, 2012, Genome Shrinkage and Loss of Nutrient-Providing Potential in the**  
97 **Obligate Symbiont of the Primitive Termite *Mastotermes darwiniensis*.**

98 Termite gut microbes collaborate to fix nitrogen, degrade lignocellulose, and produce nutrients, and the  
99 absence of *Blattabacterium* in nearly all termites suggests that its nutrient-provisioning role has been re-  
100 placed by gut microbes. *Mastotermes darwiniensis* is a basal, extant termite that solely retains *Blattabac-*  
101 *terium*, which would show evidence of relaxed selection if it is being supplanted by the gut microbiome.  
102 This termite-associated *Blattabacterium* genome is 8% smaller than cockroach-associated *Blattabacterium*  
103 genomes and lacks genes underlying vitamin and essential amino acid biosynthesis. Furthermore, the  
104 *Mastotermes darwiniensis* gut microbiome membership is more consistent between individuals and in-  
105 cludes specialized termite gut-associated bacteria, unlike the more variable membership of cockroach gut  
106 microbiomes. The *Mastotermes darwiniensis Blattabacterium* genome may reflect relaxed selection for  
107 some of its encoded functions, and the loss of this endosymbiont in all remaining termite genera may  
108 result from its replacement by a functionally complementary gut microbiota.

109 **Kinjo *et al.*, 2018, Parallel and Gradual Genome Erosion in the *Blattabacterium* En-**  
110 **dosymbionts of *Mastotermes darwiniensis* and *Cryptocercus* Wood Roaches.**

111 Almost all examined cockroaches harbor an obligate intracellular endosymbiont, *Blattabacterium cuenoti*.  
112 On the basis of genome content, *Blattabacterium* has been inferred to recycle nitrogen wastes and provide  
113 amino acids and cofactors for its hosts. Most *Blattabacterium* strains sequenced to date harbor a genome  
114 of 630 kbp, with the exception of the termite *Mastotermes darwiniensis* (590 kbp) and *Cryptocercus*  
115 *punctulatus* (614 kbp), a representative of the sister group of termites. Such genome reduction may have  
116 led to the ultimate loss of *Blattabacterium* in all termites other than *Mastotermes*. In this study, we se-  
117 quenced 11 new *Blattabacterium* genomes from three species of *Cryptocercus* in order to shed light on the  
118 genomic evolution of *Blattabacterium* in termites and *Cryptocercus*. All genomes of *Cryptocercus*-derived  
119 *Blattabacterium* genomes were reduced (614 kbp), except for that associated with *Cryptocercus kyeban-*  
120 *gensis*, which comprised 637 kbp. Phylogenetic analysis of these genomes and their content indicates that  
121 *Blattabacterium* experienced parallel genome reduction in *Mastotermes* and *Cryptocercus*, possibly due to  
122 similar selective forces. We found evidence of ongoing genome reduction in *Blattabacterium* from three  
123 lineages of the *C. punctulatus* species complex, which independently lost one cysteine biosynthetic gene.  
124 We also sequenced the genome of the *Blattabacterium* associated with *Salganea taiwanensis*, a subsocial  
125 xylophagous cockroach that does not vertically transmit gut symbionts via proctodeal trophallaxis. This  
126 genome was 632 kbp, typical of that of nonsocial cockroaches. Overall, our results show that genome

127 reduction occurred on multiple occasions in *Blattabacterium*, and is still ongoing, possibly because of new  
128 associations with gut symbionts in some lineages.

129 **Dietrich *et al.*, 2014, The Cockroach Origin of the Termite Gut Microbiota: Patterns in**  
130 **Bacterial Community Structure Reflect Major Evolutionary Events.**

131 16S profiled gut bacterial communities of 34 termite and cockroach species. Generally, gut bacterial  
132 communities of cockroaches, lower termites and higher termites were clearly separated. Subfamilies of  
133 higher termites formed distinct clusters. Fungus-cultivating Macrotermitinae (higher termites) showed  
134 strong affinity for the cockroaches. Wood-feeding cockroach *Cryptocercus punctulatus*, the closest relative  
135 of termites, was close to lower termites, with which it shares the presence of cellulolytic flagellates. Other  
136 wood-feeding cockroaches *Panesthia angustipennis* and *Salganea esakii* (family Blaberidae), whose gut  
137 microbiota lacks such flagellates, clustered with the omnivorous cockroaches. When mapped onto the  
138 host tree, the changes in community structure coincided with major events in termite evolution, such as  
139 acquisition and loss of cellulolytic protists and the ensuing dietary diversification. UniFrac analysis of  
140 the core microbiota of termites and cockroaches and construction of phylogenetic tree of individual genus  
141 level lineages revealed a general host signal, whereas the branching order often did not match the detailed  
142 phylogeny of the host. It remains unclear whether the lineages in question have been associated with  
143 the ancestral cockroach since the early Cretaceous (cospeciation) or are diet-specific lineages that were  
144 independently acquired from the environment (host selection).

145 **Warnecke *et al.*, 2007, Metagenomic and functional analysis of hindgut microbiota of a**  
146 **wood-feeding higher termite.**

147 Only recently have data supported any direct role for the symbiotic bacteria in the gut of the termite in  
148 cellulose and xylan hydrolysis. Here we use a metagenomic analysis of the bacterial community resident  
149 in the hindgut paunch of a wood-feeding ‘higher’ *Nasutitermes* species (which do not contain cellulose-  
150 fermenting protozoa) to show the presence of a large, diverse set of bacterial genes for cellulose and xylan  
151 hydrolysis. Many of these genes were expressed in vivo or had cellulase activity in vitro, and further  
152 analyses implicate spirochete and fibrobacter species in gut lignocellulose degradation. New insights  
153 into other important symbiotic functions including H<sub>2</sub> metabolism, CO<sub>2</sub>-reductive acetogenesis and N<sub>2</sub>  
154 fixation are also provided by this first system-wide gene analysis of a microbial community specialized  
155 towards plant lignocellulose degradation.

156 **Bourguignon *et al.*, 2014, Rampant Host Switching Shaped the Termite Gut Microbiome.**

157 16S metabarcoding profiled gut bacterial community of 94 termite species (77 higher termites). 211 lin-  
158 eages containing  $\geq 10$  OTUs were processed by phylogenetic analysis. Phylogenetic trees were classified  
159 into 3 categories. Category 1 represents trees in which 30% of termite-derived sequences formed a mono-

160 phyletic group. In category 1, termite-specific lineages are often composed of bacteria associated with  
 161 distinct termite lineages, and are sister groups of bacteria living in invertebrate/vertebrate gut. It is  
 162 hypothesize that the last common ancestors of each termite-specific clade became specialized for termite  
 163 gut environments and eventually became widespread across a large number of termites through both  
 164 parent-to-offspring vertical transmission and horizontal colony-to-colony transfer between termites. Cate-  
 165 gory 2 represents trees containing monophyletic clades composed of ~30% termite-associated bacteria and  
 166 ~10% non-termite-associated bacteria which are often living in guts of other animals. It is hypothesized  
 167 that these bacterial lineages have been specialized for termite gut environments and became widespread  
 168 through vertical transmission and horizontal transmission between termites and other animals. Cate-  
 169 gory 3 represents rest trees in which termite-associated bacteria intersperse with bacteria from other  
 170 environments. Congruence of bacterial and termite phylogeny did not find evidence for strict vertical  
 171 transmission. The results indicate that “mixed-mode” transmission, which combines colony-to-offspring  
 172 vertical transmission with horizontal colony-to-colony transfer, has been the primary driving force shaping  
 173 the gut bacterial community of termites. **Lo Evans, 2006, Phylogenetic diversity of the intracel-  
 174 lular symbiont *Wolbachia* in termites**

175 44 termite populations, representing of a total of 30 species, 14 genera, and 6 families were screened for the  
 176 presence of *Wolbachia*, using PCR assays for the genes 16S rRNA and ftsZ. 12 out of 44 populations were  
 177 found to be infected. **Sabree *et al.*, 2012, Genome Shrinkage and Loss of Nutrient-Providing  
 178 Potential in the Obligate Symbiont of the Primitive Termite *Mastotermes darwiniensis***  
 179 *Mastotermes darwiniensis* is the basal termite harboring *Blattabacterium* endosymbionts, which is present  
 180 in most cockroaches but absent in other termites. *Blattabacterium* endosymbionts of *Mastotermes dar-  
 181 winiensis* has shrunked genome, lossing pathways for synthesis of vitamins and essential amino acids.  
 182 *Mastotermes* gut microbiota contains characteristic termite-associated bacteria. It is proposed that *Mas-  
 183 totermes* gut microbiome replaces functions of *Blattabacterium*, resulting in relaxed selection.

184 **Peterson Scharf, 2016, Lower Termite Associations with Microbes: Synergy, Protection,  
 185 and Interplay.**

186 Grooming and hygienic behavior play an important role in termite immunity. Termites with perturbed  
 187 gut microbiota, by oxygenation or chemical means, display a marked increase in susceptibility to fungal  
 188 pathogens. One biochemical mechanism has been linked to this anti-fungal gut phenomenon in the form  
 189 of symbiont-derived  $\beta$ -1, 3-glucanase activity (most likely protist) that is able to act on fungi and prevent  
 190 their germination. Similarly, the inhibition of this antifungal enzyme activity,  $\beta$ -1, 3-glucanase, results in  
 191 a marked increase in termite susceptibility to a variety of pathogens and is conserved evolutionarily from  
 192 woodroaches to termites. Termites build nests with fecal material. The nests of one species of subterranean

termite, *Coptotermes formosanus*, are commonly laden with symbiotic Actinobacteria demonstrated to have antifungal activity ex vivo in nest walls.

**Koshikawa et al., 2008, Genome size of termites (Insecta, Dictyoptera, Isoptera) and wood roaches (Insecta, Dictyoptera, Cryptocercidae).**

C value of *Hodotermopsis sjostedti* and *Zootermopsis nevadensis* differed by a factor of two, indicating polypoidy.

**Pasari et al., 2019, Genome analysis of *Paenibacillus polymyxa* A18 gives insights into the features associated with its adaptation to the termite gut environment.**

*Paenibacillus polymyxa* A18 was isolated from termite *Odontotermes hainanensis* gut and was identified as a potential cellulase and hemicellulase producer in our previous study. Considering that members belonging to genus *Paenibacillus* are mostly free-living in soil, we investigated here the essential genetic features that helped *P. polymyxa* A18 to survive in gut environment. Genome sequencing and analysis identified 4,608 coding sequences along with several elements of horizontal gene transfer, insertion sequences, transposases and integrated phages, which add to its genetic diversity. Many genes coding for carbohydrate-active enzymes, including the enzymes responsible for woody biomass hydrolysis in termite gut, were identified in *P. polymyxa* A18 genome. Further, a series of proteins conferring resistance to 11 antibiotics and responsible for production of 4 antibiotics were also found to be encoded, indicating selective advantage for growth and colonization in the gut environment. To further identify genomic regions unique to this strain, a BLAST-based comparative analysis with the sequenced genomes of 47 members belonging to genus *Paenibacillus* was carried out. Unique regions coding for nucleic acid modifying enzymes like CRISPR/Cas and Type I Restriction-Modification enzymes were identified in *P. polymyxa* A18 genome suggesting the presence of defense mechanism to combat viral infections in the gut. In addition, genes responsible for the formation of biofilms, such as Type IV pili and adhesins, which might be assisting *P. polymyxa* A18 in colonizing the gut were also identified in its genome. In situ colonization experiment further confirmed the ability of *P. polymyxa* A18 to colonize the gut of termite.

**Korb et al., 2015, A genomic comparison of two termites with different social complexity.**

The two termite genomes represent two fundamental termite life types: the wood-dwelling one-piece nesters and the central place foraging lineages that generally differ in social complexity, feeding ecology, gut symbionts, and developmental plasticity. *Zootermopsis nevadensis* belongs to the former type and *Macrotermes natalensis* to the latter.

Wood-dwelling species nest within a single piece of dead wood that serves both as food and nesting habitat so the termites never leave their nest to forage. This social syndrome is widely considered to be ancestral and associated with high degrees of developmental plasticity for the individual termites. Work-

ers remain totipotent immatures throughout several instars that commonly develop further into sterile soldiers, winged sexuals (alates) that found new nests as primary reproductives, or neotenic reproductives that reproduce within the natal nest. *Zootermopsis nevadensis* dwells in decaying dampwood.

The foraging termite species (also called “multiple piece nesters”) forage for food outside the nest at some point after colony foundation and bring it back to the colony to feed nestmates. They represent more than 85% of the extant termite species. They have true workers and an early separation into distinct developmental pathways. In the apterous line, individuals are unable to develop wings and can thus never disperse as reproductives. They become workers and soldiers, but can in some species also advance to become neotenic reproductives in their own nest. In the nymphal line, however, individuals develop wings and dispersing phenotypes that found new colonies elsewhere. The Macrotermitinae to which *Macrotermes natalensis* belongs are special examples of foraging termites because their colonies are dependent on nutrition provided by a *Termitomyces* symbiont (Basidiomycota: Agaricales). This fungal symbiosis is evolutionarily derived and comes in addition to more fundamental protist (lower termites) and bacterial gut symbionts (all termites), which have played major roles throughout termite evolution. *Macrotermes* species have two (major/minor) worker castes and two (major/minor) soldier castes that may be determined as early as the egg stage (suggested for *Macrotermes michaelsoni*). *Macrotermes* colonies often build conspicuous mounds that may harbor several millions of individuals.

All immune signaling pathways were found. First, *Zootermopsis nevadensis* has 6 gram-negative binding proteins (GNBPs), whereas only four of these were recovered in *Macrotermes natalensis*. These four GNBPs are all termite-specific and some of them were previously shown to be under positive selection in several *Nasutitermes* species, especially in species with arboreal nests. The *Macrotermes* genome seems to lack the insect-typical GNPB duplicate and one GNPB gene that has so far only been found in *Zootermopsis nevadensis*. Second, while AMPs were not enriched in either termite genome, their identities were completely different with *Z. nevadensis* having 2 AMPs and *M. natalensis* having 3 other AMPs. *M. natalensis* has a termite-specific defensin-like gene termicin, a category of genes that seem to have duplicated repeatedly during the radiation of *Nasutitermes* termites. After duplication, one copy seems to often be under strong selection, while the other evolves toward neutrality. Also in the soil-foraging *Reticulitermes* species these genes seem to be under positive selection.

In contrast to other insects where AMP production is normally induced, these genes seem to be constitutively expressed in fungus-growing termites, as has been shown for *Pseudacanthotermes spiniger*, which might be an adaptation to protect the symbiont against competing fungi. Termicin and other defensins were absent in *Z. nevadensis* but this species has GNBPs that are differentially expressed between castes (Terrapon et al., 2014) and may thus serve a similar function in protecting the nest from



259 fungal infections. For the arboreal nesting termite *Nasutitermes corniger* it has been shown that GGBP2  
 260 has (1,3)-glucanase effector activity and functions as an antifungal agent (Bulmer et al., 2009). It is  
 261 incorporated in the nest building material, where it cleaves and releases pathogenic components while  
 262 priming termites for improved antimicrobial defense (Bulmer et al., 2009). Such a defensive strategy is  
 263 likely to be most effective for termites with closed nests, consistent with positive selection on GGBP being  
 264 most pronounced in *Nasutitermes* that live in arboreal nests (Bulmer and Crozier, 2006). Hence, antifungal  
 265 strategies might differ in termites with different habitats; with GGBPs and termicin possibly playing  
 266 complementary roles. This is supported by the fact that GGBPs in subterranean, foraging *Reticulitermes*  
 267 species evolve neutrally while termicin was shown to have been under strong positive selection in these  
 268 species.

269 We can reject the possible alternative hypothesis that different defense strategies are linked to the  
 270 gut symbionts that need different defense strategies to protect the symbiotic partner. As lower termites  
 271 harbor protists as well as bacteria, while higher termites only have bacteria, we would then have expected  
 272 higher termites having more AMPs and lower termites more GGBPs, but this is not the case because  
 273 lower *Reticulitermes* termites have positively selected termicins. If there is an association between nesting  
 274 habit and defense strategy, we expect that GGBPs are under positive selection in other wood-dwelling  
 275 termites, and termicins are selected in soil-foraging termites. Additional genomic data, particularly for  
 276 wood-dwelling termites, would be needed to validate this hypothesis.

277 Reduced numbers of immune defense genes were found in ants and the honeybee (Evans et al., 2006;  
 278 Gadau et al., 2012) but also here there seems to be selection on some of the AMP genes. Similar to  
 279 termicin, positive selection was detected on defensin in ants (Viljakainen and Pamilo, 2008), but this gene  
 280 was not overexpressed after experimental fungal infections of leaf-cutting ant colonies, whereas two other  
 281 AMPs were (Yek et al., 2013). This contrasts with dipterans (*Drosophila* and *Anopheles*) for which no  
 282 evidence was found for positive selection on any AMPs (Sackton et al., 2007; Simard et al., 2007), but  
 283 instead for immune recognition and signaling proteins (Schlenke and Begun, 2003; Jiggins and Kim, 2005;  
 284 Sackton et al., 2007). This provides further support for the hypothesis that social insects have responded  
 285 differently to selection pressure caused by microbial pathogens than solitary insects (Viljakainen and  
 286 Pamilo, 2008).

287 The ancestral termite gut microbiota was derived from a cockroach ancestor, but major subsequent  
 288 changes occurred, most notably when the higher termites evolved (Dietrich et al., 2014). The guts of the  
 289 wood-dwelling termites are dominated by protists that appear to be primarily adapted to break down  
 290 wood (Cleveland, 1923; Brugerolle and Radek, 2006), with complementary roles of bacteria that are often  
 291 symbiotic with the gut-flagellates (Dietrich et al., 2014). The common ancestor of the evolutionarily

derived Termitidae lost these flagellate symbionts so their gut microbiotas became dominated by bacteria, which may have facilitated their dietary diversification (Brune and Ohkuma, 2011; Dietrich et al., 2014). The single origin of fungiculture by the Macrotermitinae led to Termitomyces taking over primary plant decomposition and the gut microbiota shifting phylogenetically and functionally to perform complementary roles (Liu et al., 2013; Dietrich et al., 2014; Otani et al., 2014; Poulsen et al., 2014).

Changes in symbiont associations are tightly associated with termite life styles (for a recent review on termite gut symbionts, see Brune, 2014), but this may hardly induce structural genomic changes in the termite hosts, consistent with the similar gene repertoires for plant biomass decomposition found in the two termite genomes (Poulsen et al., 2014). A comparison of carbohydrate-active enzyme (CAZy) profiles of the two termite species showed a reduction in the absolute number of glycoside hydrolase enzymes (85) in *M. natalensis* compared to *Z. nevadensis* (97) (Table 5), but very similar relative abundances of specific enzyme families (Poulsen et al., 2014). Profile similarities suggest that plant-biomass decomposition genes may be ancestrally conserved across the termites, but additional termite genomes are needed to shed light on this. Such additional genomic work will need to be accompanied by enzyme function validations to test whether differences in absolute numbers reflect changes in the relative importance of termite-derived enzymes.

**Poulsen *et al.*, 2013, Complementary symbiont contributions to plant decomposition in a fungus-farming termite.**

We obtained high-quality annotated draft genomes of the termite *Macrotermes natalensis*, its *Termitomyces* symbiont, and gut metagenomes from workers, soldiers, and a queen. We show that members from 111 of the 128 known glycoside hydrolase families are represented in the symbiosis, that *Termitomyces* has the genomic capacity to handle complex carbohydrates, and that worker gut microbes primarily contribute enzymes for final digestion of oligosaccharides. This apparent division of labor is consistent with the *Macrotermes* gut microbes being most important during the second passage of comb material through the termite gut, after a first gut passage where the crude plant substrate is inoculated with *Termitomyces* asexual spores so that initial fungal growth and polysaccharide decomposition can proceed with high efficiency. Complex conversion of biomass in termite mounds thus appears to be mainly accomplished by complementary cooperation between a domesticated fungal monoculture and a specialized bacterial community. In sharp contrast, the gut microbiota of the queen had highly reduced plant decomposition potential, suggesting that mature reproductives digest fungal material provided by workers rather than plant substrate.

**Ali *et al.*, 2019, Symbiotic cellulolytic bacteria from the gut of the subterranean termite *Psammotermes hypostoma* Desneux and their role in cellulose digestion.**

325 The subterranean termite *Psammotermes hypostoma* Desneux is considered as an important pest that  
 326 could cause severe damage to buildings, furniture, silos of grain and crops or any material containing  
 327 cellulose. This species of termites is widespread in Egypt and Africa. The lower termite's ability to  
 328 digest cellulose depends on the association of symbiotic organisms gut that digest cellulose (flagellates  
 329 and bacteria). In this study, 33 different bacterial isolates were obtained from the gut of the termite  
 330 *P. hypostoma* which were collected using cellulose traps. Strains were grown on carboxymethylcellulose  
 331 (CMC) as a sole source of carbon. Cellulolytic strains were isolated in two different cellulose medium  
 332 (mineral salt medium containing carboxymethylcellulose as the sole carbon source and agar cellulose  
 333 medium). Five isolates showed significant cellulolytic activity identified by a Congo red assay which gives  
 334 clear zone. Based on biochemical tests and sequencing of 16s rRNA genes these isolates were identified as  
 335 *Paenibacillus lactis*, *Lysinibacillus macrolides*, *Stenotrophomonas maltophilia*, *Lysinibacillus fusiformis*  
 336 and *Bacillus cereus*.

### 337 **Peppel *et al.*, 2021, Ancestral predisposition toward a domesticated lifestyle in the** 338 **termite-cultivated fungus *Termitomyces***

339 The events leading toward domestication of *Termitomyces* remain unclear. To address this, we recon-  
 340 structed the lifestyle of the common ancestor of *Termitomyces* using a combination of ecological data  
 341 with a phylogenomic analysis of 21 related non-domesticated species and 25 species of *Termitomyces*.  
 342 We show that the closely related genera *Blastosporella* and *Arthromyces* also contain insect-associated  
 343 species. Furthermore, the genus *Arthromyces* produces asexual spores on the mycelium, which may fa-  
 344 cilitate insect dispersal when growing on aggregated subterranean fecal pellets of a plant-feeding insect.  
 345 The sister-group relationship between *Arthromyces* and *Termitomyces* implies that insect association and  
 346 asexual sporulation, present in both genera, preceded the domestication of *Termitomyces* and did not  
 347 follow domestication as has been proposed previously. Specialization of the common ancestor of these  
 348 two genera on an insect-fecal substrate is further supported by similar carbohydrate-degrading profiles  
 349 between *Arthromyces* and *Termitomyces*. We describe a set of traits that may have predisposed the  
 350 ancestor of *Termitomyces* toward domestication, with each trait found scattered in related taxa outside  
 351 of the termite-domesticated clade. This pattern indicates that the origin of the termite-fungus symbiosis  
 352 may not have required large-scale changes of the fungal partner.

### 353 **Bulmer *et al.*, 2006, Variation in Positive Selection in Termite GNBP and Relish.**

354 We used different models of nucleotide substitution at nonsynonymous (amino acid altering) and syn-  
 355 onymous (silent) sites to compare the different levels and type of selection among three immunity genes  
 356 in 13 Australian termite species (*Nasutitermes*). The immunity genes include two encoding pathogen  
 357 recognition proteins (gram-negative bacterial-binding proteins) that duplicated and diverged before or

soon after the evolution of the termites and a transcription factor (Relish), which induces the production of antimicrobial peptides. A comparison of evolutionary models that assign four unrestricted classes of dN/dS (the ratio of the nonsynonymous to synonymous substitution rate) to different *Nasutitermes* lineages revealed that the occurrence of positive selection ( $dN/dS \geq 1$ ) varies among lineages and the three genes. Positive selection appears to have driven the evolution of all three genes in an ancestral lineage of three subterranean termites. It had previously been suggested that there was a transition along this ancestral lineage to termite morphology and ecology associated with a diet of decayed wood, a diet that may expose termites to elevated levels of fungal and bacterial pathogens. Relish appears to have experienced the highest levels of selective pressure for change among all three genes. Positively selected sites in the molecule are located in regions that are important for its activation, which suggests that amino acid substitutions at these sites are a counter response to pathogen mechanisms that disrupt the activation of Relish.

**Shijenobu *et al.*, 2022, Genomic and transcriptomic analyses of the subterranean termite *Reticulitermes speratus*: Gene duplication facilitates social evolution.**

We report the genome, transcriptome, and methylome of the Japanese subterranean termite *Reticulitermes speratus*. Our analyses highlight the significance of gene duplication in social evolution in this termite. Gene duplication associated with caste-biased gene expression was prevalent in the *R. speratus* genome. The duplicated genes comprised diverse categories related to social functions, including lipocalins (chemical communication), cellulases (wood digestion and social interaction), lysozymes (social immunity), geranylgeranyl diphosphate synthase (social defense), and a novel class of termite lineage-specific genes with unknown functions. Paralogous genes were often observed in tandem in the genome, but their expression patterns were highly variable, exhibiting caste biases. Some of the assayed duplicated genes were expressed in caste-specific organs, such as the accessory glands of the queen ovary and the frontal glands of soldier heads. We propose that gene duplication facilitates social evolution through regulatory diversification, leading to caste-biased expression and subfunctionalization and/or neofunctionalization conferring caste-specialized functions.